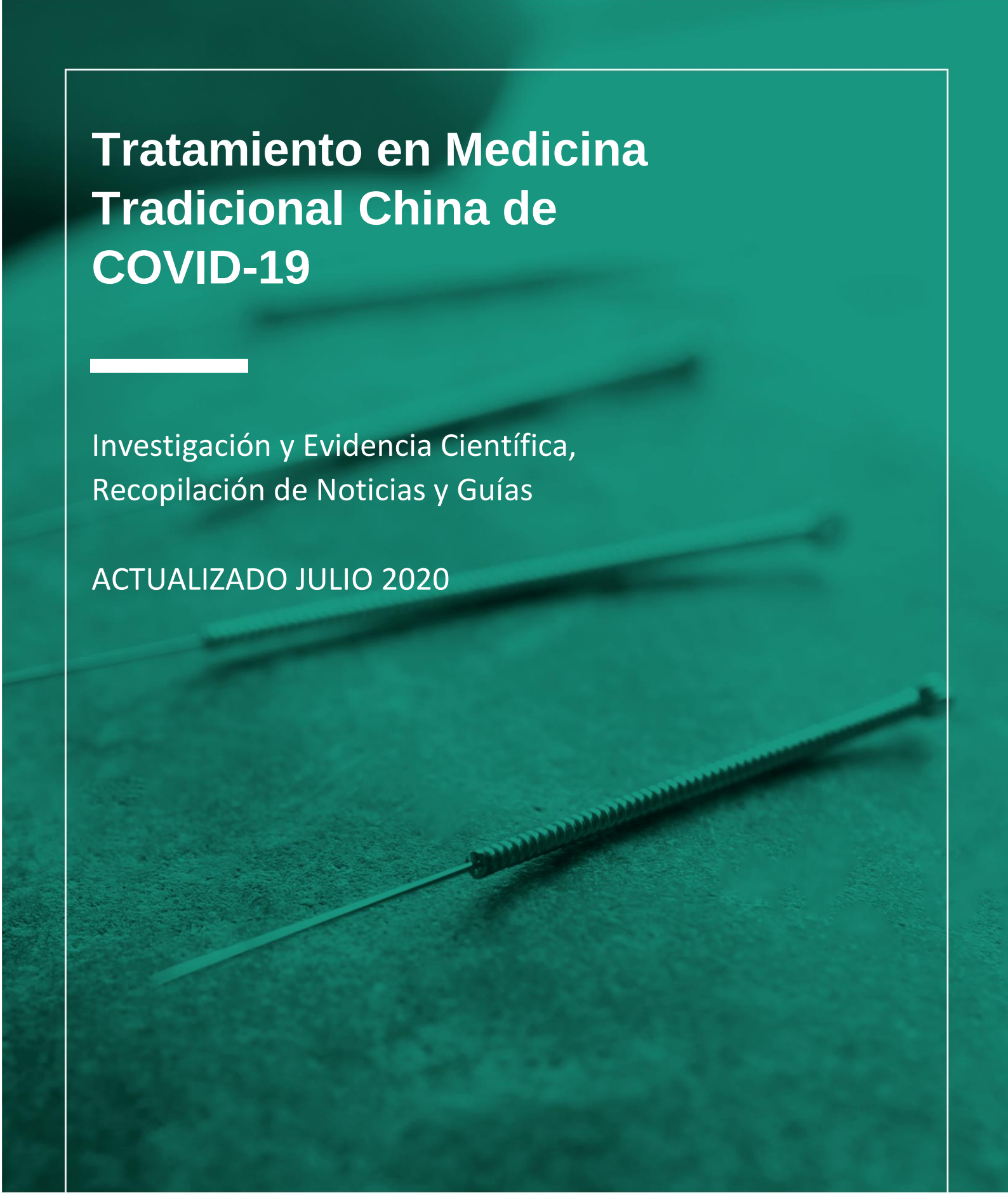
Tratamiento en Medicina Tradicional China de COVID-19

Investigación y Evidencia Científica,
Recopilación de Noticias y Guías

ACTUALIZADO JULIO 2020



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INTRODUCCIÓN

Los datos epidemiológicos existentes indican que el COVID-19 es un brote altamente transmisible de coronavirus. Es probable que la batalla con COVID-19 continúe durante meses a nivel mundial. Por lo tanto, se necesitan más estrategias además de la contención y la mitigación para su llegada a cualquier zona, especialmente aquellos países con infraestructura de salud poco desarrollada, para acortar la estancia hospitalaria y reducir el consumo de recursos sanitarios (Organización Mundial de la Salud, 2020a).

En la actualidad, el COVID-19, al ser un virus recientemente identificado, tiene opciones de tratamiento farmacológico limitadas. La Medicina Tradicional China tiene un buen potencial para complementar las necesidades existentes, pudiendo ser una buena opción adicional considerando las opciones limitadas disponibles para COVID-19 (Chan et al, 2020). En China, la Medicina China se propone como una opción de tratamiento de alto uso según las directrices nacionales y provinciales. Se revisaron las últimas guías clínicas nacionales y provinciales, estudios de cohorte retrospectivos, series de casos sobre el tratamiento de COVID-19 con la Medicina China como tratamiento complementario, entre otros. Los datos disponibles sugieren que Medicina China podría considerarse como una opción terapéutica complementaria en el tratamiento de COVID-19. La estrategia debe enfocarse en ir al origen del problema para acortar la duración de la fiebre, aliviar los síntomas, prevenir la progresión de la enfermedad, reducir la mortalidad y ayudar a la recuperación. La evidencia actual indica que la MTC tiene un beneficio potencial en el alivio sintomático, acortando la duración de la fiebre, revertiendo los cambios radiológicos y acortando la estancia hospitalaria (Lu et al., 2020a; Xia et al., 2020; Yao et al., 2020).

Para el tratamiento del COVID-19 con MTC, la directriz nacional estableció varios periodos; el periodo de observación médica, el período de tratamiento (leve, intermedio, grave y crítico) y el periodo de recuperación. En cada periodo y fase se recomiendan fórmulas fitoterapéuticas de Medicina china y acupuntura. Las directrices provinciales se optimizaron en función de las directrices nacionales para la adaptación local, la mayoría involucraban medidas preventivas y de tratamiento de Medicina china (Chen et al., 2020b; Pang et al., 2020; Zheng et al., 2020).

La utilización de la Medicina china en la gestión del COVID-19 ha sido muy significativa en China (Gao et al., 2020). Los casos confirmados de COVID-19 en Shanghai comenzaron el tratamiento integral de Medicina China y la Medicina Occidental (Yuan et Qiu, 2020). En Guangdong, los gránulos de *Tou-jie-qu-wen* (neumonía n.°1) se evaluaron con una serie de casos y se recomendaron a 30 hospitales designados como el tratamiento estándar de los pacientes con COVID-19 (Administración de Productos Médicos de Guangdong, 2020). En general, más del 85% de los casos confirmados involucraron el uso de MTC a nivel nacional (Wuhan más del 67%) (Le y Liang, 2020; El Consejo de Estado, la República Popular de China, 2020b) y el primer Hospital Módulo designado orientado a MTC en Wuhan funcionó desde el 14 de febrero de 2020 (Wang y Li, 2020).

Son varios los estudios puestos en marcha, a partir del 22 de febrero de 2020, había tres cohortes retrospectivas (Lu et al., 2020a; Xia et al., 2020; Yao et al., 2020), cinco series de casos (Cheng y Li, 2020; Dai et al., 2020 ; Administración de Productos Médicos de Guangdong, 2020; Administración Nacional de Medicina Tradicional China, 2020b; Yong et al., 2020), y dos estudios de caso (Hu et al., 2020; Sun et al., 2020b) sobre la gestión integrada con Medicina China y Medicina Occidental del COVID -19.

A partir del 22 de febrero de 2020, se registraron 24 y 141 estudios de intervención relacionados con COVID-19 en ClinicalTrials.gov y Chinese Clinical Trial Registry, respectivamente, incluyendo la Medicina China. De los 53 ensayos clínicos relacionados con Medicina China, 27 ensayos evalúan los programas de Medicina China y tratamiento integrado.

La acupuntura y moxibustión juegan un papel activo en la prevención y el tratamiento de enfermedades infecciosas, habiendo participado activamente frente al COVID-19, en la prevención y el control, logrando buenos resultados, y estableciéndose las Directrices sobre Intervención de Acupuntura y Moxibustión para el COVID-19 (Guidelines on Acupuncture and Moxibustion Intervention for COVID-19 (second edition)).

En la lucha contra la pandemia de neumonía causada por la infección del COVID-19, el Hospital Provincial de Medicina Tradicional China de Hubei utilizó diligentemente las especialidades de la Medicina China, en cooperación con los departamentos pertinentes para estudiar y formular programas de prevención y tratamiento de la neumonía, estableciendo protocolos de acupuntura y moxibustión para la prevención y el tratamiento del coronavirus. Se puso de manifiesto que la fitoterapia china jugó un papel importante en los resultados positivos del tratamiento. Las fórmulas herbales chinas albergan el potencial único de reducir los síntomas de la fiebre y la tos, limitar la progresión de la enfermedad y mejorar la inmunidad general y, por lo tanto, la capacidad de una persona de generar una respuesta inmune esencial al virus, debiéndose integrar en un plan de tratamiento integral que utilice tanto la medicina occidental como la china para garantizar resultados óptimos para el paciente. Los resultados de la colaboración entre la medicina occidental y la china en Wuhan han sido muy prometedores (Chen et al., 2020).

Son varias las Guías publicadas en China sobre la MTC y el COVID19 y varias las noticias que resaltan los buenos resultados gracias a la integración de la Medicina China y la Medicina Occidental para hacer frente al COVID-19. Una de estas noticias más recientes expone las declaraciones de Yu Yanhong, portavoz del Ministerio de Sanidad Chino y también subdirector de la Administración Nacional de Tradicional Medicina china, indicando que el método puede mejorar rápidamente la condición de los pacientes con síntomas leves, como fiebre, tos, dolor de garganta, debilidad y falta de apetito, también puede ayudar a reducir la duración de la estancia en el hospital. Los expertos chinos señalan buenos resultados de la combinación de medicina china y occidental para el coronavirus y encuentran que la combinación puede ayudar a prevenir el desarrollo de síntomas leves en condiciones graves y críticas, lo que reduce la tasa de mortalidad de la enfermedad. Desde el brote de Covid-19, las autoridades centrales chinas han destacado en muchas ocasiones la aplicación de la medicina tradicional china combinada con la medicina occidental en el tratamiento de pacientes. Según la Administración Nacional de TMTC, los equipos de expertos médicos en 31 regiones a nivel provincial incluyen especialistas en MTC, y la mayoría de las regiones han realizado esquemas de tratamiento de TCM localizados para la enfermedad (https://www.infosalus.com/).

INVESTIGACIÓN Y EVIDENCIA CIENTÍFICA

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RECOPILACIÓN DE NOTICIAS

Muestra de algunas noticias publicadas en referencia al tratamiento de COVID-19 con medicina china ordenadas cronológicamente.

4/02/20

Coronavirus: Chinese researchers claim TCM herbal remedy could “inhibit” Coronavirus
https://www.bioworld.com/articles/432838-coronavirus-chinese-researchers-claim-tcm-herbal-remedy-could-inhibit-2019-ncov?fbclid=IwAR0IW7q4AFzG_ajK4_ArJ15mc568h8Td4MJXlcGpefEDytJUNeIiXAXwJ8Q

12/02/20

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14/02/20

Envían 2200 especialistas en medicina tradicional china a Hubei
https://www.telesurtv.net/news/china-especialistas-medicina-tradicional-hubei-coronavirus-20200214-0027.html

15/02/20

Wuhan abre primer hospital temporal orientado a medicina tradicional china para combatir coronavirus
http://spanish.xinhuanet.com/2020-02/15/c_138784751.htm

17/02/20

Medicina china podría combatir el coronavirus
https://clustersalud.americaeconomia.com/insumos-y-servicios-hospitalarios/medicina-china-podria-combatir-el-coronavirus

18/02/20

Titulares de Xinhua: La medicina tradicional china aporta sabiduría oriental a la lucha contra el coronavirus
http://spanish.xinhuanet.com/2020-02/18/c_138796063.htm?fbclid=IwAR08rgU5SkVg4oDD_u8-2imc0Tl8N21K_E3PTiwTdxGO4r-u0FaXBP2ebAM

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Expertos chinos señalan buenos resultados de la combinación de medicina china y occidental para el coronavirus
https://www.infosalus.com/actualidad/noticia-expertos-senalan-buenos-resultados-combinacion-medicina-china-occidental-tratamiento-20200220124232.html

20/02/20

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https://www.chinadaily.com.cn/a/202002/20/WS5e4e7fafa31012821727915a.html?fbclid=IwAR2tXV9cfQPyhCav1rw11DddwaHZ9NBJYIjnMjR7UZRCnmDjUEpQls4BwQo

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24/02/20

Catalogan efectiva medicina tradicional china para tratar Covid-19 https://www.telesurtv.net/news/china-catalogan-efectiva-medicina-tradicional-tratar-covid-20200224-0042.html

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MTC constituye valioso aporte en lucha contra nuevo coronavirus
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Traditional Chinese medicine used to treat 85% of COVID-19 patients. https://news.cgtn.com/news/2020-02-25/TCM-used-to-treat-85-of-COVID-19-patients-OmQG7PIGWs/index.html

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https://es.theepochtimes.com/fortalecer-el-sistema-inmunologico-con-la-medicina-china-para-prevenir-el-coronavirus-de-wuhan_627994.html

16/03/20

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TCM ready to take on epidemic overseas
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La Medicina China demuestra ser eficaz en el tratamiento del COVID-19
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Combination of TCM, Western medicine highlight of COVID-19 control: oficial
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18/03/20

Cómo ha tratado con éxito el coronavirus COVID-19 en China y Wuhan con Medicina Tradicional China
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20/03/20

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Covid-19: China pushes traditional remedies amid outbreak https://www.bbc.com/news/world-asia-53094603

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Coronavirus: cómo China está impulsando el uso de su medicina tradicional ante la pandemia (y qué se sabe de su eficacia) https://www.bbc.com/mundo/noticias-internacional-53216833

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China to support COVID-19 international research cooperation https://news.cgtn.com/news/2020-07-09/China-to-support-COVID-19-international-research-cooperation-RYHEjVp9de/index.html

GUÍAS

ESTADOS UNIDOS

- Cómo se trata actualmente COVID-19 en China con MTC (Lotus Institute of Integrative Medicine)
- Traditional Chinese Medicine (TCM) in the Prevention and Treatment of COVID-19 (2019-nCoV) & Integration of TCM into Educational Curriculum (Lotus Institute of Integrative Medicine)

R.P.CHINA

- Diagnosis y tratamiento de la neumonía por el nuevo coronavirus en medicina china (Universidad de Medicina China de Tianjin)
- Guía de prevención y tratamiento de Wen Bing del Síndrome de Humedad-Calor de Taiyin Pulmonar (Neumonía por Corona-virus) Universidad de Medicina de Tianjin
- Guidance for CoronaVirus Disease 2019. Prevention, Control, Diagnosis and Management (People's Medical Publishing House)
- Guidelines on Acupuncture and Moxibustion Intervention for COVID-19 (WFAS)
- COVID-19 Diagnosis and Treatment Plan - tentative 7th edition. (Hubei Provincial Hospital of TCM)
- Manual para la prevención y el control de la infección nosocomial en la neumonía por nuevo coronavirus. Primer Hospital Afiliado de la Universidad de Nanchang y Chemical Industry Press (https://www.yourtcm.sg/)

ESPAÑA

- Comprendiendo la amenaza global del Coronavirus COVID-19 (Dr. Nuria Lorite)

Anexo I: ARTÍCULOS INVESTIGACIÓN Y EVIDENCIA CIENTÍFICA (COMPLETOS O ABSTRACTS)

1. Ang L, Lee HW, Kim A, Lee JA, Zhang J, Lee MS. Herbal medicine for treatment of children diagnosed with COVID-19: A review of guidelines. Complement Ther Clin Pract. 2020 May;39:101174. doi: 10.1016/j.ctcp.2020.101174. Epub 2020 Apr 12.

Abstract

This review aimed to summarize and analyze the pattern identification (PI), herbal formulae, and composition of herbs provided by recent guidelines for the treatment of pediatric COVID-19. Seven data sources were reviewed until March 25, 2020. We analyzed the herbal formulae included in the guidelines and performed a network analysis to identify the frequency of herbs recommended in the herbal formulae. All 3 guidelines were provincial guidelines from China. Our results showed that there were 4 stages, 12 PIs, and 13 herbal formulae recommended by the provincial guidelines. These herbal formulae included a total of 56 herbs. Based on our network analysis, Scutellariae Radix was paired with Artemisiae Annuae Herba in one cluster. In another cluster, Armeniacae Semen was paired with Coicis Semen and Ephedrae Herba was paired with Gypsum Fibrosum. This review serves as a reference for the use of traditional medicine in the treatment of pediatric COVID-19.

1. Introduction

As of March 2020, the outbreak of coronavirus disease (COVID-19) has been declared a pandemic, and at least 163 countries across all six continents sustained the transmission of the virus [1,2]. Although there is very limited information about the virus, current evidences show that people can be ill from COVID-19 regardless of their age, gender, ethnicity, and health status. While children are less likely to be infected by the virus as compared to the adults, they are not spared from the disease.

Recent epidemiological reports also showed that the cases reported among children are relatively few and less severe [3]. Mainland China, which has the highest number of cases worldwide has reported that the mean age of pediatric patients with COVID-19 infection was 7 years old. According to the Chinese Center for Disease Control and Prevention (China CDC), there are no fatalities found in children with age ranging from 1 to 9 years old [4]. Regardless of the number of cases reported, children remain vulnerable to COVID-19.

As there is no vaccine or antiviral treatment currently available for COVID-19, traditional medicine, which has been widely used in the past during epidemic outbreaks, is taken into consideration as one of the treatment modalities [5]. Although many countries have issued traditional medicine treatment guidelines on the prevention and treatment of COVID-19, only mainland China has issued the guidelines for children.

This review aimed to systematically summarize and analyze the herbal formulae recommended by all available Chinese guidelines in terms of the composition of herbs, pattern identification (PI) and disease stages in the treatment of pediatric COVID-19.

2. Methods

2.1. Data sources

Seven data sources were searched, until March 25, 2020, to identify available traditional medicine guidelines:

Guidelines International Network (G-I-N) [6].

“Chinese guidelines on Novel Coronavirus” resource by Evidence Aid [7].

- The official government websites of all 31 provinces in Mainland China (including municipal and autonomous regions)
- The Centre for Health Protection of the Hong Kong Special Administrative Region [8].
- Association of Korean Medicine and Korean Association of Traditional Pulmonary Medicine
- Japanese Association for Infectious Diseases and Japanese Respiratory Society [9].

- Taiwan Centers for Disease Control [10].

2.2. Inclusion and exclusion criteria

This study focused on the recommended treatment modalities in traditional medicine for pediatric COVID-19. All herbal formulae recommended by the guidelines for treatment measures were included and those for preventive measures were excluded.

As herbal formulae provided by guidelines from the different provinces were formulated based on regional characteristics, regional folk medicines such as Tibetan medicine, Mongolian medicine, and Miao medicine were excluded. For provincial guidelines that integrate both folk medicine and conventional traditional medicine, only herbal formulae of the latter were included.

2.3. Data extraction and analyses

Data from the included guidelines were extracted based on a predefined data extraction table which included the stages of the disease, pattern identification, clinical symptoms, therapeutic principle, name and composition of herbal formulae, herb dosage, and the province of the provided guideline. The herbal formulae were analyzed based on pattern identification and disease stage. The frequency of herbs recommended in the herbal formulae were also identified by performing a network analysis using Netminer 4.0 (Cyram Inc, Seoul, Korea) which visualized the relationship between the herbs in clusters. In our network analysis, the nodes represented the herbs and their connections represented the relationship of the herbs with each other. The connections were stronger in herbs that were adjacent to each other. The herbs with closer connection as compared to the rest of the network belonged to the same cluster.

2.4. Terminology standardization

We standardized all the terminologies based on the *WHO International Standard Terminologies on Traditional Medicine in Western Pacific Region* [11]. Pattern identification terminology was standardized based on the clinical manifestation provided in the guidelines and *Clinic Terminology of Traditional Chinese Medical Diagnosis and Treatment* [12]. Unnamed herbal formulae were renamed using the *Dictionary of Traditional Chinese Medicine Formula* [13].

3. Results

We only found 3 traditional medicine guidelines from mainland China that provide treatment measures for pediatric COVID-19. Although there were several versions of national diagnosis and treatment guidelines related to traditional medicine issued in mainland China, contents on pediatric treatments were not provided in any of these guidelines. All 3 traditional medicine guidelines included in this review were issued by the provincial government.

The herbal formulae used for treating children diagnosed with COVID 19 were analyzed based on the disease stages and PI. Our results identified 4 stages and 12 PIs based on the provided provincial guidelines. There were 13 herbal formulae recommended by the provincial guidelines, of which 12 herbal formulae were oral decoction prescriptions and one of them was a decoction enema prescription (Table 1). The frequency of the herbs used in the herbal formulae was also calculated. These herbal formulae included a total of 56 herbs, of which 23 had a frequency of use of 3 or more times.

Table 1. Pattern identification and herbal medicines recommendation for pediatric COVID-19.

Table 1
Pattern identification and herbal medicines recommendation for pediatric COVID-19.

Stages	Pattern Identification	Name of herbal formula	Composition of herbal formula
Mild	Seasonal Epidemic Invading the Exterior-defense	Yin Qiao San	△Lonicerae Flos, Forsythiae Fructus, Platycodonis Radix, Menthae Herba, Lophatheri Herba, Schizonepetae Spica, Glycine Semen Preparatum, Arctii Semen, Phragmitis Rhizoma
		Xiang Su San	Cyperi Rhizoma, Perillae Folium, Citri Reticulatae Pericarpium, Glycyrrhizae Radix et Rhizoma, Bupleuri Radix, Cinnamomi Ramulus, Saposhnikoviae Radix, Osterici seu Notopterygii Radix et Rhizoma
Moderate	Dampness-Heat Blocking the Lung	Ma Xing Shi Gan Tang + San Ren Tang	△Ephedrae Herba, Armeniacae Semen Amarum, Glycyrrhizae Radix et Rhizoma, Gypsum Fibrosum, Amomi Fructus Rotundus, Coicis Semen, Pinelliae Rhizoma Praeparatum, Magnoliae Cortex, Talcum, Stachyuri Medulla Helwingiae Medulla, Lophatheri Herba
	Dampness-Heat in the Spleen and Stomach	Buhuan Jin Zhengqi San	△Citri Reticulatae Pericarpium, Atractylodis Rhizoma, Magnoliae Cortex, Glycyrrhizae Radix et Rhizoma, Amomi Tsao-ko Fructus, Pinelliae Rhizoma, Agastachis Herba
Severe	Heat Toxin Blocking the Lung	Xuanbai Chengqi Tang + Ganlu Xiaodu Dan	△Gypsum Fibrosum, Rhei Radix et Rhizoma, Armeniacae Semen Amarum, Trichosanthis Fructus, Talcum, Scutellariae Radix, Artemisiae Scopariae Herba, Acori Tatarinowii Rhizoma, Fritillariae Cirrhosae Bulbus, Akebiae Caulis, Agastachis Herba, Forsythiae Fructus, Amomi Fructus Rotundus, Menthae Herba, Belamcandae Rhizoma
	Intense Heat Toxin with Blockage of Bowel Qi and Dysphagia	Not available	Rhei Radix et Rhizoma (Enema using herbal decoction)
Recovered	Unclear Residual Heat	Liu Junzi Tang + Yu Ping Feng San	△Ginseng Radix, Atractylodis Macrocephalae Rhizoma, Poria Sclerotium, Glycyrrhizae Radix et Rhizoma, Citri Reticulatae Pericarpium, Pinelliae Rhizoma, Saposhnikoviae Radix, Astragali Radix
Not reported	Heat Toxin Fettering the Lung	Ma Xing Shi Gan Tang*	Ephedrae Herba 4 g, Gypsum Fibrosum 20 g, Anemarrhenae Rhizoma 9 g, Armeniacae Semen Amarum 10 g, Coicis Semen 10 g, Phragmitis Rhizoma 10 g, Platycodonis Radix 6 g, Mori Radicis Cortex 10 g, Lonicerae Flos 10 g
	Epidemic Toxin Blocking the Lung	Ma Xing Shi Gan Tang*	Ephedrae Herba 4 g, Gypsum Fibrosum 20 g, Anemarrhenae Rhizoma 9 g, Armeniacae Semen Amarum 10 g, Coicis Semen 10 g, Trichosanthis Fructus 10 g, Rhei Radix et Rhizoma 3 g, Mori Radicis Cortex 10 g, Lepidii seu Descurainiae Semen 6 g, Bubali Cornu 10 g, Pheretima 10 g, Ginseng Radix 6 g
	Wind-Heat Invading the Lung	Yin Qiao San	Lonicerae Flos 15 g, Forsythiae Fructus15 g, Schizonepetae Spica 10 g, Menthae Herba 10 g, Arctii Semen 10 g, Platycodonis Radix 10 g, Scutellariae Radix 10 g, Trichosanthis Pericarpium 15 g, Angelicae Decursivae Radix 15 g, Belamcandae Rhizoma 10 g, Eriobotryae Folium 15 g, Artemisiae Annuae Herba 21 g
	Wind-Heat Blocking the Lung	Ma Xing Shi Gan Tang	Ephedrae Herba 5 g, Armeniacae Semen Amarum 10 g, Gypsum Fibrosum 15 g, Scutellariae Radix 10 g, Trichosanthis Pericarpium 15 g, Angelicae Decursivae Radix 15 g, Belamcandae Rhizoma 10 g, Eriobotryae Folium 15 g, Pumen 20 g, Lepidii seu Descurainiae Semen 10 g, Pheretima 10 g, Artemisiae Annuae Herba 21 g
	Dampness-Heat Fettering the Lung	Qianjin Weijing Tang + Shangjiao Xuanpi Tang	Phragmitis Rhizoma 15 g, Benincasae Pericarpium 15 g, Coicis Semen 15 g, Armeniacae Semen Amarum 10 g, Scutellariae Radix 10 g, Trichosanthis Pericarpium 15 g, Angelicae Decursivae Radix 15 g, Belamcandae Rhizoma 10 g, Eriobotryae Folium 15 g, Curcumae longae Radix 15 g, Lepidii seu Descurainiae Semen 10 g, Artemisiae Annuae Herba 21 g
	Dampness-Heat Fettering the Spleen	San Ren Tang	Armeniacae Semen Amarum 10 g, Amomi Fructus Rotundus 3 g, Coicis Semen 15 g, Pinelliae Rhizoma Praeparatum 10 g, Magnoliae Cortex 15 g, Talcum 10 g, Stachyuri Medulla Helwingiae Medulla 3 g, Agastachis Herba 10 g, Poria Sclerotium 15 g, Arecae Pericarpium 15

In the results of our network analysis, 11 herbs that appeared more than 3 times in the herbal formulae were able to be classified into two different clusters (Fig. 1), where 6 herbs (Angelicae Decursivae Radix, Belamcandae Rhizoma, Eriobotryae Folium, Trichosanthis Pericarpium, Scutellariae Radix, and Artemisiae Annuae Herba) belonged to one cluster and the other 5 (Armeniacae Semen, Lepidii seu Descurainiae Semen, Coicis Semen, Gypsum Fibrosum, and Ephedrae Herba) belonged to the second cluster. In the figure, Scutellariae Radix showed strong connections with Artemisiae Annuae Herba and Belamcandae Rhizoma in one cluster. The herb Armeniacae Semen and Coicis Semen, in addition to Ephedrae Herba and Gypsum Fibrosum, each had strong connections with one another in the other cluster.

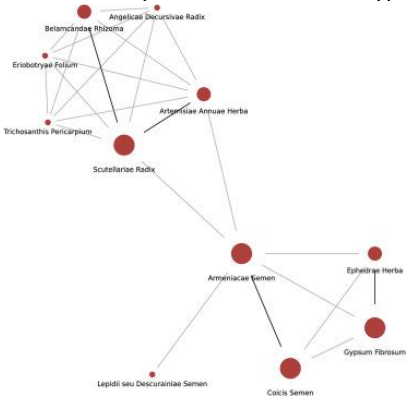

Fig. 1. Network analysis of herbs with frequency of use of 3 or more times.

4. Discussion

In this review, we systematically accessed the herbal treatment recommended by the Chinese provincial guidelines for pediatric COVID-19. According to our results, only 3 provincial guidelines provided herbal treatment recommendations for pediatric COVID-19. A recent case series of 72,314 cases published by the Chinese Center for Disease Control and Prevention showed that children younger than 10 years old accounted for only 1% of cases and another 1% were patients whose age ranged from 10 to 19 years [4]. Besides, recent evaluation of the pediatric cases treated at the Wuhan Children's Hospital, which is the only hospital in Wuhan, China that was assigned by the government for the treatment of pediatric COVID-19, also reported that most infected children presented with milder symptoms as compared to adults [3]. This may be the rationale behind the lack of guidelines issued for the treatment of pediatric COVID-19.

According to our network analysis, Scutellariae Radix, Artemisiae Annuae Herba, and Belamcandae Rhizoma were found to be correlated in one cluster. In the theory of traditional medicine, these three herbs have a heat-clearing effect and are frequently used together in herbal formulae. The herb Scutellariae Radix is approved by the China Food and Drug Administration for the treatment of viral diseases such as influenza, upper respiratory infection, and pneumonia [14]. Baicalin, which is the main bioactive compound derived from Scutellariae Radix, was also reported to have antiviral activity against SARS coronavirus [15]. Similarly, Artemisiae Annuae Herba was reported to have an antiviral compound and showed inhibitory effects on the SARS coronavirus strain [16]. Besides, Belamcandae Rhizoma has also been frequently used for the treatment of inflammation and throat disorder [17].

Additionally, our results also showed that Armeniacae Semen and Coicis Semen were correlated to one another in the second cluster. The herb Armeniacae Semen and Coicis Semen were often prescribed together for the treatment of upper respiratory infection as they both have the effect of nourishing the lungs in traditional medicine [18]. The herb Ephedrae Herba and Gypsum Fibrosum were also shown to have strong connections with one another. Both herbs are the major components of the herbal formula Ma Xin Shi Gan Tang, which is often used for the treatment of common cold. This herbal formula also claimed to antiviral effect that inhibits the entry of influenza virus and have potential in managing seasonal pandemics of influenza infection [19].

Notably, the herb Armeniacae Semen was one of the highest frequencies of use among the herbal formulae recommended for the treatment of pediatric COVID-19. In a nationwide population-based study conducted in Taiwan, Armeniacae Semen was the most frequently prescribed herb for the treatment of pediatric asthma [20]. Besides having antiasthmatic activity, Armeniacae Semen was also reported to inhibit Th_2 cells, which are important for immune responses, reducing hyper-responsiveness in the airway [21]. This further validated the recommendation of Armeniacae Semen for the treatment of respiratory diseases in children.

In particular, the frequently used herbs in the recommended herbal formulae for the treatment of pediatric COVID-19 lack diversity compared to the adults [22]. In the recommendations on adult treatment for COVID-19, the herb Glycyrrhizae Radix et Rhizoma was the herb with the highest frequency of usage [22]. This might be due to the difference in the spectrum of diseases between the children and the adults. The course of diseases in adults was reported to be more severe than children; thus, the herb Glycyrrhizae Radix et Rhizoma, which has both antiviral and

anti-inflammatory qualities were highly used. On the other hand, the herb Armeniacae Semen, which is widely used of respiratory disease, was highly used as the severity of disease is milder in children.
However, there are several limitations to this study. First, this review only summarized the herbal formulae and their herbal compositions recommended by the traditional medicine guidelines. There was no direct evidence on the efficacy of the herbal formulae for the treatment of pediatric COVID-19. Second, we only found 3 provincial traditional medicine guidelines that included pediatric treatments. This information is insufficient for us to provide any specific recommendations or guidance for the treatment of pediatric COVID-19. Third, this review only summarized information from the provincial traditional medicine guidelines available up to March 25, 2020. As guidelines issued on the pandemic COVID-19 are constantly updated, there may be updated information that we failed to retrieve in this review.

In conclusion, this review can only be used as a reference for the traditional medicine treatment of pediatric COVID-19. As the spectrum of disease in children differs from adults, there are many questions that remain unanswered. It is important to define the epidemiology of pediatric COVID-19 with more studies.

Author contributions

Conceptualization: MSL and HWL. Methodology: LA and AK. Data Curation: HWL and AK. Writing – Original Draft: LA and HWL. Writing – Review & Editing: JZ, AK, MSL and JAL. Supervision: MSL.

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2. **Akalın E, Ekici M, Alan Z, Özbir Elevli E, Yaman Bucak A, Aobuliaikemu N, Üresin AY. Traditional Chinese medicine practices used in COVID-19 (Sars-cov 2/Coronavirus-19) treatment in clinic and their effects on the cardiovascular system. Turk Kardiyol Dern Ars. 2020 Jun;48(4):410-424. doi: 10.5543/tkda.2020.03374**

Abstract

Objective: The aim of this study was to evaluate the effectiveness of plants used in the formulations of traditional Chinese medicine (TCM), which were also used in clinical trials to treat patients with the novel coronavirus COVID-19, and to assess their effects on the cardiovascular system.

Methods: A literature review of PubMed, ResearchGate, ScienceDirect, the Cochrane Library, and TCM monographs was conducted and the effects of the plants on the cardiovascular system and the mechanisms of action in COVID-19 treatment were evaluated.

Results: The mechanism of action, cardiovascular effects, and possible toxicity of 10 plants frequently found in TCM formulations that were used in the clinical treatment of COVID-19 were examined.

Conclusion: TCM formulations that had been originally developed for earlier viral diseases have been used in COVID-19 treatment. Despite the effectiveness seen in laboratory and animal studies with the most commonly used plants in these formulations, the clinical studies are currently insufficient according to

standard operating procedures. More clinical studies are needed to understand the safe clinical use of traditional plants.

Objective: The aim of this study was to evaluate the effectiveness of plants used in the formulations of traditional Chinese medicine (TCM), which were also used in clinical trials to treat patients with the novel coronavirus COVID-19, and to assess their effects on the cardiovascular system.
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Results: The mechanism of action, cardiovascular effects, and possible toxicity of 10 plants frequently found in TCM formulations that were used in the clinical treatment of COVID-19 were examined.
Conclusion: TCM formulations that had been originally developed for earlier viral diseases have been used in COVID-19 treatment. Despite the effectiveness seen in laboratory and animal studies with the most commonly used plants in these formulations, the clinical studies are currently insufficient according to standard operating procedures. More clinical studies are needed to understand the safe clinical use of traditional plants.
More than 100 clinical studies, some complete and some ongoing, have been initiated to study treatments for the current coronavirus pandemic, which has infected more than 80,000 and killed more than 3,000 in China. Studies of potential approaches to this virus, COVID-19, have primarily evaluated the effects of standard and new antiviral medications on this novel coronavirus, in both single and combination drug therapies. A number of protocols are being tested by doctors with the aim of treating symptoms of the disease in addition to antiviral medications. The types of treatments that have been tested in China to treat COVID-19 are provided in Figure 1.[1] Among the treatments used in these clinical trials are remedies from traditional Chinese medicine (TCM), most of which are plant-based formulations. Approximately 20% of COVID-19 patient-oriented clinical trials have examined TCM treatments. The fight against COVID-19 can be summarized in 2 strategies: prevention and treatment. The search for a vaccine is at the forefront of prevention, and work is progressing in earnest to this end. Aside from isolation, contact avoidance, and disinfection, another hotly debated approach to disease prevention is fortification of the immune system and preventing the entry of the virus into the body using various plantbased blends, supplement products and vitamins, or other methods. The appropriateness of strengthening the immune system, a very complex system, as a treatment for a viral infection that is still not fully understood is open to discussion. A considerable number of those infected with the virus show no symptoms and there are unpredictable risks associated with using immunity-strengthening products on such individuals. The antiviral activity of some aromatic compounds has been proven in a laboratory setting. However, there remains insufficient proof of their safety in clinical usage. We must take into consideration any potential harmful effects, such as irritation, especially of the lungs, resulting from improper use of aromatherapeutic treatment. In theory, while administration in drop form seems feasible, protective usefulness against the virus has not been clinically proven.[2] The presence of source plants in pharmacopoeias and monographs does not necessarily indicate the safe clinical use of these plant products or dietary supplements. The word “pharmacopoeia” is derived from the Ancient Greek words for the practice of making healing medicines. Today, pharmacopoeias have been defined as “documents containing the physiochemical properties of all active ingredients and inactive ingredients used in the production of medicines, along with methods for their qualitative and quantitative analysis, and including national and international rules that must be adhered to, both legally and scientifically.”[3] Monographs have been defined as “the portion of a pharmacopoeia that describes chemically/biologically active and inactive ingredients, plant-based drugs and botanical preparations, along with the description, contents, properties (such as appearance, solubility, melting and boiling points), identification analyses, dosage, handling and storage conditions, and impurities of the finished products. The quality control analyses of the products contained in the monographs primarily include analytic methods conforming to the necessary standards, microbiological purity, dissolution, stability and similar test protocols.”[4] Regardless of how many sections are devoted to therapeutic information, such as the description and identification parameters of plant products alongside traditional or scientifically verified usage in pharmacodynamic and pharmacokinetic studies, the limits of

preclinical and clinical safety, toxicity, contraindications, special warnings and precautions, unwanted side-effects, dosage effect, and dose-drug interactions, inclusion in extended monographs of international institutions and countries does not necessarily translate to safety in clinical use.[3,5] The European Pharmacopoeia, which is Turkey's official pharmacopoeia, contains the contributions of 37 countries, including Turkey.[3] The pharmacopoeia and its printed supplements have only the necessary sections covering the protocols necessary for standardization, such as identification, description, and analytic procedures. In Turkey, monographs with the specific aim of standardizing medicinal plant use are being prepared for plants widely used in the country.[5] Founded in 2004, the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency shares data on herbal substances with European Union member countries as well as observer countries, such as Turkey. Thus far, the HMPC has approved 84 of 169 Turkish monographs. However, because the majority of the usages they describe are traditional methods, it emphasizes the need for clinical investigation.[4] Traditional Chinese medicine (TCM) TCM is a system with a history spanning more than 2500 years and with foundations in the Chinese civilization's first schools of philosophy: Taoism, Confucianism and Buddhism. TCM adheres to the principle of restoring balance in the body as a whole during treatment and applies a number of different methods according to a differentiation of syndromes. TCM emphasizes a holistic approach to diagnosis and treatment. Alongside medicines made from plant, animal, and mineral materials, TCM utilizes a number of different physical techniques, including oil massage, tui na massage, scraping massage, acupuncture, heat therapy, and qi gong and related forms of massage therapy.[6] The classic TCM diagnostic procedures of looking, listening/smelling, asking, and touching are still used by today's practitioners of TCM alongside modern procedures.[7] More than 32,000 plants are found growing naturally in China. According to the most recent data, 11,200 species of plants are used there medicinally. Plants make up 87% of the sources of medicines used in TCM.[8] The Chinese Pharmacopoeia is reviewed every 5 years; the 10th edition, printed in 2015, is in use today. This most recent pharmacopoeia consists of 4 volumes. The first contains information on plants and drugs, plant oils and extracts, mixture preparations in the form of formula recipes and single-plant preparations, and features 2165 monographs. The second volume contains monographs related to chemicals, antibiotics, biochemical medications, and radioactive drugs. The third volume is dedicated to biological products, and the fourth consists of monographs with topics such as general principles on the preparation of medicine, test procedures, guides, reference materials and relevant test solutions, reagent guidelines, and pharmaceutical additives. The Chinese Pharmacopoeia is available in both Chinese and English in digital and print form. The aim of this study was to evaluate plants found in mixtures used in TCM treatments in the context of evidence-based medicine and to analyze the effects of these plants on the cardiovascular system.

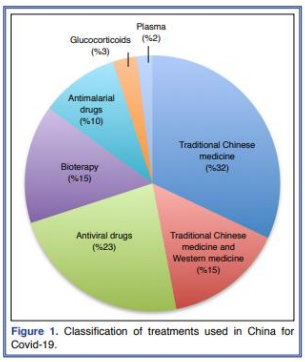

Figure 1. Classification of treatments used in China for Covid-19.

METHODS

A search of PubMed, ResearchGate, ScienceDirect, the Cochrane Library, and TCM monographs was conducted to retrieve studies related to plants used in mixtures used in TCM formulations published between 1990 and 2020. The 10 plants used most often in the examined TCM mixtures were selected for this study based on the effective role in the mixture and the mechanism of action. The information provided in the various literature sources about these 10 plants was compiled and organized for this study, and includes the scientific name, the Turkish name (omitted in cases where the plant does not have a Turkish name), the English name, the Chinese name, the parts used, active substances, potential pharmacologic mechanism of action

against COVID-19, the pharmacologic mechanism of action with respect to effects on the cardiovascular system, interactions with cardiovascular medications, and toxicity. The compounds listed as responsible for effects are shown in bold.

RESULTS

Clinical studies There are relatively few detailed reports of TCM treatments and/or studies conducted in line with the criteria of good clinical practices. In summary, TCM methods are generally used in the form of plant products given as supplements alongside conventional Western treatments.

In data released by the National Administration of Traditional Chinese Medicine, 214 COVID-19 patients in 4 provincial hospitals were given an additional treatment of the Qingfei Paidu Decoction for 3 days. In all, 60% of the patients showed a significant improvement, and 30% saw a reduction of symptoms. This prescription was recommended for patients in mild, serious, and even critical condition.[9]

Another report published by the same administration stated that the Qingfei Paidu Decoction was in wide use in 28 cities with good results. A total of 1262 patients, 57 of whom were on the verge of being seriously ill, were treated with the Qingfei Paidu Decoction in 66 of China's major cities. Cure rates reached 99.28% and 1253 patients were discharged from the hospital. Not a single patient experienced an increase in severity from mild/normal to critical condition. The results suggest that the preparation may prevent the illness from reaching a critical stage.[10]

In a study conducted by Wang et al.,[11] the Qingfei Paidu Decoction was used to treat 98 newly diagnosed COVID-19 patients in China's Sichuan Province and the lab test indicators were compared with the pretreatment results after 3, 6, and 9 days of treatment. After the third day of treatment, lymphocyte percentages, aspartate aminotransferase, and alanine aminotransferase, and D-dimer lab indices had returned to normal in more than 70% of the patients ($p < 0.01$) After 6 days, C-reactive protein and erythrocyte sedimentation rate laboratory indices had normalized in more than 80% of the patients ($p<0.01$) Following 9 days of treatment, lab indices had returned to normal in more than 90% of the patients. CT results showed normalization in 79 patients after six days of treatment.

Of 101 COVID-19 patients with pneumonia at the Wuhan Iron and Steel Corporation General Hospital, which is affiliated with the Wuhan Science and Technology Medical College, 63 were given Lianhua Qingwen granules orally in addition to standard treatments (nutritional supportive therapy, symptomatic therapy, antiviral and antibiotic drug therapy) and defined as the treatment group, while 38 patients in a control group were treated only with conventional procedures. The duration of primary symptoms (fever, cough, fatigue, shortness of breath) and rates of symptom loss were compared between the 2 groups after 10 days. The treatment group demonstrated symptom reduction rates of 86.7%, 55.6%, and 82.5% for fever, cough, and fatigue, respectively. These rates were significantly higher than those of the control group (respectively, 67.7%, 30.6%, 58.6%; $p<0.05$). The Lianhua Qingwen treatment group also saw fever reduction after 6 days, while the controls had a fever reduction period of 7 days. There was a 68.2% reduction in labored breathing in the Lianhua Qingwen group, which was a significant increase compared with that of the control group (20%; p $p<0.05$). The condition of 4 patients (6.4%) in the treatment group and 6 patients (15.8%) in the control group worsened. No serious side effects were observed in the Lianhua Qingwen group. As a result, it was found that a combination therapy of Western medicine and Lianhua Qingwen granules may significantly reduce fever, cough, fatigue, shortness of breath and other symptoms in cases where pneumonia is suspected.[12] In another study analyzing the clinical effectiveness of conventional treatment versus conventional treatment combined with Lianhua Qingwen granules, Cheng et al.[13] analyzed 2 groups of 51 patients in a Lianhua Qingwen treatment group and a control group and compared the duration and rate of symptom reduction for primary symptoms (fever, fatigue, cough), as well as other symptoms, such as the presence of phlegm, shortness of breath, chest tightness and loss of appetite, as well as BT results of each group after 7 days of treatment. The rate of reduction of the primary symptoms of fever, fatigue, and cough in the treatment group was 83.7%, 61.3%, and 62.2%, respectively, while it was 61%, 34.3%, and 35.9% in the control group. The differences between the groups were statistically significant ($p<0.05$). The rate of the symptoms of phlegm, shortness of breath, chest tightness, and loss of appetite was 55%, 61.5%, 54.6%, and 34.8% in the treatment

group, while it was 15.8%, 14.3%, 15.8%, and 7.7% in the control group, respectively. The condition of 4 patients (7.8%) in the treatment group and 11 (21.6%) in the control declined from moderate to severe. The BT results of 28 patients (54.9%) from the treatment group and 23 (45.1%) in the control group returned to normal. The results offer evidence of Lianhua Qingwen granules as a potential treatment option in the treatment of COVID-19.[13]

In a retrospective clinical study that included 42 patients in the city of Wuhan diagnosed with COVID-19, a combined treatment using Lianhua Qingwen was demonstrated to significantly reduce clinical symptoms such as fever, cough, phlegm, and shortness of breath. The duration of symptoms was reduced by 1.5 days on average, and it had a positive effect on fatigue, muscle aches, nasal congestion, and headache.[14] In addition, another study evaluated traditional Chinese medicine data based on treatment plans published by the State and Provincial Health Committee and the National Administration of Traditional Chinese Medicine from the outbreak of COVID-19 in Wuhan through February 19, 2020. The study found that 84 plant mixtures and 230 plant-based drugs had been used and analyzed the frequency of use (Table 1).[15–20] Plants used Given the vast number of plants found in the research evaluated in this study, and in accordance with TCM principles that some may not be directly responsible for the mixture's effect, it was decided that this review would be limited to 10 plants and exclude auxiliary plants considered not responsible for the primary effect. Criteria regarding the usage frequency of the mixtures and plants, the results in COVID-19 treatment, effects on the cardiovascular system, and the known mechanism of action were also included. The most commonly used plants were Glycyrrhiza uralensis (216), Scutellaria baicalensis (188), Forsythia suspensa (153), Lonicera japonica (134), Pogostemon cablin (115), and Atractylodes macrocephala (88). Astragalus membranaceus, Bupleurum chinense, Isatis tinctoria, and Salvia miltiorrhiza were also included in the study due to the fulfillment of other criteria; however, the rate of use was not analyzed. Of the more than 300 scientific sources evaluated, those that contained information other than chemical contents; known antiviral, immunomodulator, anti-inflammatory, and cardiovascular effects; and side effects and toxicity were excluded from the study. This study provides the scientific name, the Chinese name, the English name, and the Turkish name of the plants examined; however, many of the species do not have a Turkish name. Only "çivitotu" (Isatis tinctoria) grows naturally in Turkey. Lonicera japonica, Japon hanımeli, Japanese honeysuckle (Jin yin hua).

Drug name: Lonicera japonicae flos Chemical compounds: The main compounds are the organic acids (chlorogenic acid, caffeic acid, cinnamic acid, ferulic acid), flavonoids (sinarozit, luteolin, lonicerin, chrysin, apigenin, rutin, quercetin, astragalin, hyperozit), iridoids (loganin, loganic acid, 8-epiloganic acid, secologanin, secoxiloganin, dimetiksecologanosit, centaurosit, sverosit), saponins (a-hederein, lonicerocid A-E hederagenin), oleanolic acid, and ursolic acid.[21] Pharmacological action mechanisms Anti-inflammatory effect: In an in vitro study, extract containing chlorogenic and caffeic acids showed an effect against inflammation and oxidation caused by free radicals. It inhibited nitric oxide (NO) production, tumor necrosis factor alpha (TNF-α), and secretion of interleukin (IL) IL-1B, IL-6, IL-8, and increased IL10 secretion.[21] Antiviral effect: In vitro, a compound of secoxiloganin has been reported to have antiviral effects against the H1N1 (Influenza A) virus and inhibit H1N1 replication.[22] In vivo, the fruit extract of the plant demonstrated an inhibitory effect against HIV, adenovirus, herpes simplex type 1, and herpes simplex type 2.[23] Effect on the cardiovascular system: The results of the application of an extract of pure polysaccharides obtained from the flowers of the plant to streptozosininduced rats for 42 days were analyzed. Total cholesterol, triglyceride, very low-density lipoprotein, and low-density lipoprotein levels decreased, while highdensity lipoprotein levels increased.[24] Drug interaction: Not reported. Toxicology: With use as an injection, hypersensitivity reactions may suddenly appear. Although hypothermia was observed after an injection in animal studies, the injection was generally recorded as safe.[21] Forsythia suspense, Weeping forsythia, (Lian qiao) Drug name: Forsythiae fructus Chemical compounds: Phenylethanoid glycosides, lignans (pinoresinol, phillygenin, phillyrin [forsythin], phorsytiasid), flavonoids, terpenoids, alkaloids, chlorogenic acid, and caffeic acid are main compounds.[25] Pharmacological action mechanisms Antiviral effect: In an in vivo study, it was determined that when the compound of phillyrin (forsythin), which is obtained from the fruit of the plant, was applied to H1N1-infected mice, tissue damage and lung damage were reduced and survival was prolonged. Furthermore,

the IL-6 level, virus load, and influenza hemagglutinin level decreased.[26] Effect on the cardiovascular system: When fractions obtained from the fruit of the plant were applied to mice with streptozosin-induced diabetes for 4 weeks, an ethyl acetate fraction was more inhibited in the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase fractions compared with n-butanol and chloroform fractions. Serum total cholesterol, hepatic total cholesterol, and total triglyceride levels decreased.[27] Drug interaction: Not reported. Toxicity: Thus far, no toxicity has been reported. Water and ethanol extracts obtained from the leaves of the plant did not cause acute toxicity in studies with mice.[28] Glycyrrhiza uralensis, Çin meyankökü, Chinese licorice (Gan cao) Drug name: Liquiritiae radix Chemical compounds: It contains triterpenic saponins (glycyrrhizin, glycyrrhic acid, glyciric acid, glycyrrhetinic acid) and flavonoids (liquritin, liquiritigenin, isoliquiritigenin, licoricidin).[29] Pharmacological action mechanisms Anti-inflammatory effect: It has been demonstrated that glycyrrhizin suppresses proinflammatory cyclooxygenase 2 (COX-2), inducible NO synthase, and TNF-α, and it was found to inhibit the phosphorylation and secretion of protein kinase C and HMGP 1 in an in vivo study.[30] Antiviral effect: It was noted that glycyrrhizin reduced the production of chemokine ligand 10, IL-6 and chemokine ligand 5, and suppressed Avian influenza H5N1-induced apoptosis.[31] It has been observed that 18p-glycyrrhetinic acid effectively inhibits HIV-1 and virus antigen p24 accumulation, and protects cells from the cytopathogenic effect of the virus.[32]

Immunomodulatory effect: In an in vivo study on mice, glycyrrhizin was found to prevent airway resistance and increases in the number of eosinophils, improve IL-4, IL-5, and IL-13 levels in the bronchoalveolar lavage fluid and increase the level of interferon gamma (IFN-γ).[33] Effect on cardiovascular system: In an in vivo study, isoliquiritigenin reduced the severity of reperfusion-induced arrhythmias and myocardial infarction by improving the activation of the Janus kinase 2-signal transducer and activator of transcription 3 (JAKSTAT3) pathway.[34] In another study, positive effects were reported regarding protection against heart disease such as ischemic damage due to the strong inhibition of K+ channels.[35] Drug interaction: It can reduce the effectiveness of antihypertensive drugs. When taken with the thiazide group diuretics, it may pose a risk of hypokalemia and interact with digitalis glycosides.[36] Toxicity: Severe hypertension and hypokalemic metabolic alkalosis can be observed with long-term use of the root of the plant.[37] Isatis tinctoria (sin. Isatis indigotica), Çivitotu, Indigo woad, (Song lan) Drug name: Isatidis radix Chemical compounds: The root extract contains alkaloids (isatindigoside A, isatindigoside B, isatindigobisindoloside A, isatindigobisindoloside B), polysaccharides, terpenic compounds, aldehydes (furfural), nitriles (2-Pentenenitrile) and isothiocyanates (3-Butyl).[38] Pharmacological action mechanisms Anti-inflammatory effect: According to an in vitro study, the alkaloids in the root extract had a COX inhibitory effect and reduced the release of proinflammatory cytokines.[38] Antiviral effect: An in vitro study indicated that polysaccharides in the root extract of the plant inhibited viral duplication and adsorption of the virus to cell surfaces.[38] Effect on the cardiovascular system: Not reported Drug interaction: Not reported. Toxicity: Not reported.

Pogostemon cablin, Paçuli (Tefârik), Patchouli (Guang huo xiang) Drug name: Pogostemonis herba Chemical compounds: It includes essential oils (pogostemon, trans-caryophyllene), phenylethanoids (isoacteoside, acteoside and krenatoside) flavonoids (apigenin), phytosterols (daucosterol, β-sitosterol, stigmasterol), glycosides, terpenic compounds and organic acids (succinic acid).[39] Pharmacological action mechanisms Antiviral effect: In an in vitro study, phenylethanoids (neurosinidase) showed a neuraminidase inhibitory effect and reduced the replication and infectiousness of the influenza virus.[40] Anti-inflammatory effect: According to the results of an in vitro study, pogostone, eugenol, and rosmarinic acid substances in the essential oil decreased the release of proinflammatory cytokines by inhibiting the nuclear factor kappa B (NF-κB) pathway.[41] Effect on the cardiovascular system: In an in vivo study, it was stated that the alpha-bulnesene substance in its content may increase platelet aggregation time as a result of an inhibitory effect on the platelet activating factor.[42] Drug interaction: Theoretically, it can increase the coagulation time by increasing the effectiveness of anticoagulant and antiaggregant drugs.[42] Toxicity: Not reported. Atractylodes macrocephala, Large head (Bai zhu) Drug name: Atractylodis macrocephalae rhizoma Chemical compounds: Its rhizome extracts include polysaccharides, steroids (β-sitosterol, stigmasterol), flavonoids, flavonoid glycosides, benzoquinones, phenylpropanoids (caffeic acid, ferulic acid, 2-hydroxiferulic acid),

sesquiterpenoids (atractilenolide, biatractilenolide, biepiasterolid), polyacetylenes, and coumarins.[43] Pharmacological action mechanisms Anti-inflammatory effect: In an in vitro study, the terpenic atractilenolide substance in the plant content decreased the release of proinflammatory cytokines (TNF-a, IL-1β and IL-6) by inhibiting the NF-κB pathway.[44]

Immunomodulatory effect: An in vivo study of mice indicated that polysaccharides increased T lymphocyte activity and IFN-γ release.[45] Effect on the cardiovascular system: According to an in vivo study, the lactones in the extract can prolong bleeding time by inhibiting ADP-dependent platelet aggregation.[46] Drug interaction: Theoretically, it could increase coagulation time by increasing the effectiveness of anticoagulant and antiaggregant drugs.[46] Toxicity: It has been stated that an aqueous extract given to mice at doses of 20 and 100 mg/kg in the form of an intraperitoneal injection did not cause toxicity.[43] Scutellaria baicalensis, Baykal kasidesi, Baical skullcap (Huang qin) Drug name: Scutellariae radix Chemical compounds: Flavonoids (baicalin, baicalein, wogonoside, wogonin), diterpenoids, polysaccharides, β-carotene, and β-sitosterols are the main compounds.[47] Pharmacological action mechanisms Antiviral effect: Baicalin has demonstrated an antiviral effect through HIV-1 inhibition. Baicalin acts by binding to selected chemokines and interfering with the capacity of cellular receptors. For HIV-1 infection, these 2 mechanisms have co-receptor effects on essential elements. Baicalin causes HIV-1 reverse transcriptase inhibition by binding near the active site of the enzyme to which the viral RNA will bind.[48] In cell studies, the wogonin compound, which belongs to the flavonoid group of the plant, has an inhibitory effect on influenza A and B viruses via type 1 IFN and the adenosine monophosphate activated protein kinase pathway.[49] Effect on cardiovascular system: It has been noted that after the spontaneous application of the active ingredient of baicalin, found in the flavone structure obtained from the plant, to hypertensive rats for 14 days, there was a decrease in systolic blood pressure and vascular tension of the aorta. When the mechanism of the active substance of baical for an antihypertensive effect was examined, the activation of ATP-dependent K+ channels and intracellular Ca+2 channels in vascular smooth muscles had a vasorelaxant effect.[50]

Drug interaction: Theoretically may interact with warfarin.[51] Toxicity: The plant has been used safely in TCM for centuries. However, some studies have shown that it can also have toxic effects. Despite the absence of a pronounced allergic reaction in oral preparations, some patients have had stomach discomfort and diarrhea. Hypothermia, muscle pain, and leukopenia were noted when high-dose injectable preparations were administered to humans. Nonetheless, it is widely used in clinical practice due to its low toxicity.[47] There have been cases of hepatotoxicity in which the related mechanisms cannot be explained. It was observed that jaundice and other symptoms appeared in patients within 6–24 weeks and that enzyme cases typically affect the hepatocellular level. After cessation of plant use, recovery was rapid. Case series with acute liver injuries that began with jaundice 1–3 months after the use of the plant were recorded.[52] Astragalus membranaceus, Çin geveni, Milkvetch, (Huang qi) Drug name: Astragali radix Chemical compounds: It contains triterpene saponins (astragalosides I-VII and acetyl derivatives, agroastragalosides I-VI, astramembranins), isoflavones (formononetin and calycosin), polysaccharides (astragaloglucans).[29] Pharmacological action mechanisms Anti-inflammatory effect: In an in vitro study, astragaloside IV significantly inhibited the production of NO, proinflammatory cytokines IL-1β, and TNF-α, depending on the dose.[53] Astragaloside IV lowered the serum levels of monocyte chemoattractant protein-1 and TNF-α in mouse lungs and also significantly suppressed mRNA levels of MCR-1, TNF-α, IL-6, and TLR4. It has been observed to reduce neutrophil infiltration and activation.[54] Immunomodulatory effect: Astrogaloside II increased T and B lymphocyte proliferation in vitro. It also significantly increased CD25 and CD69 expression.[55] Astragaloside I, II, III, and IV showed marked IL-2 inducing activity.[56] Antiviral effect: Published studies have reported that astragaloside IV has anti-human adenovirus type 3 (HAdV-3) capacity, and the underlying mechanisms are inhibition of HAdV-3 replication and HAdV-3- induced apoptosis.[57] It also reduced eosinophil and lymphocyte infiltration. It was observed that it modulates Th1/2 immune balance and activates peroxisome proliferator-activated receptors.[58] Effect on cardiovascular system: In rats with isoprenaline-induced myocardial ischemia, it has been noted that astragaloside IV showed a calcium antagonistic effect, inhibiting calcium overload due to ischemia and hypoxia.[59] In an in vivo study, astragaloside IV induced phosphorylation of JAK and STAT3, increasing the

activity of the vascular endothelial growth factor regulated by STAT3. Therefore, it has been shown to promote angiogenesis and alleviate heart failure.[60] Drug interaction: Not reported. Toxicity: Not reported. Bupleurum chinense, Çin şeytanayağı, Chinese thorowax (Bei chai hu) Drug name: Bupleuri radix Chemical compounds: The plant contains triterpene saponins (saikosaponins, saikogenins), polysaccharides (bupleurans) and phytosterols. Pharmacological action mechanisms Anti-inflammatory effect: In an in vitro study, saikosaponins in the extract were shown to have anti-inflammatory effects by reducing COX-2, NO synthase, TNF-α, IL-1β, IL-6, NF-κB, and mitogen-activated protein kinase levels and increasing the IL-10 level.[61] Antiviral effect: Saikosaponins exhibited antiviral activity by reducing the replication of influenza A virus strains, NF-κB, and caspase-3-dependent virus ribonucleoprotein nuclear transport, and proinflammatory cytokine production.[62] Immunomodulatory effect: An in vivo study revealed that saikosaponins had increased the proliferation of T lymphocytes, IL-4, and IL-10 levels, as well as decrease IFN-γ and TNF-α levels.[63] Effect on the cardiovascular system: Not reported. Drug interaction: Not reported. Toxicity: Acute or accumulative chronic hepatotoxicity and other digestive system diseases have been reported due to the use of Radix Bupleuri ex tract and TCM formulas containing this plant extract at high doses.[64] Salvia miltiorrhiza, Çin Adaçayı, Danshen/Chinese sage, (Dan shen) Drug name: Radix Salviae miltiorrhizae Chemical compounds: Diterpenoids (tanshinone [Tan] IIA and Tan IIB, cryptotansinone), phenolic acids (salvianolic acid, caffeic acid, isoferulic acid), fatty acids, salvianen, luteolin, and glycosides are the main compounds.[29] Pharmacological action mechanisms Anti-inflammatory effect: Salvianolic acid B and the plant's aqueous ethanol extract inhibited TNF-α-induced NF-κB activation in human aortic endothelial cells.[65] Antiviral effect: In an in vitro study, Salvia miltiorrhiza root extract inhibited viral entry and RNA synthesis, and lightened the apoptotic process in cells infected with enterovirus.[66] Immunomodulatory effect: In a study on mice, the plant extract reduced serum immunoglobulin E production at different doses and increased cell-mediated immunity. It inhibited the production of oxygen radicals in the liver and spleen and NO production in the liver. It also increased the host resistance to Listeria monocytogenes by increasing the number of peripheral monocyte and natural killer cells and reduced IL1β production.[67] Effect on cardiovascular system: In an in vivo study, a Tan IIA compound was found to decrease the mean arterial pressure, have a vasodilator effect, and increase the amount of periarteriolar NO. Tan IIA prevented the reduction of endothelial nitric oxide synthase (eNOS) due to hypertension and increased eNOS expression.[68] Drug interactions: It has been noted that Chinese sage increased bleeding time and decreased thromboxane formation and platelet aggregation when used with warfarin.[69] Toxicity: It has been observed that the injection given to patients in China may have caused some negative results, such as headache, flushing, dizziness, skin itching, thrombocytopenia, and abnormal liver function.[70]

Preparations	Plants
Lian Hua Qing Wen[71]	*Lonicera japonica Thunb.* *Ephedra sinica Stapf* *Prunus armeniaca L.* *Gypsum Fibrosum* *Isatis tinctoria L. / Isatis indigotica Fortune* *Dryopteris crassirhizoma* *Houttuynia cordata Thunb.* *Pogostemon cablin (Blanco) Benth.* *Rheum palmatum L.* *Rhodiola rosea L.* *Mentha haplocalyx* *Glycyrrhiza uralensis Fisch.*
Tan Re Qing[72]	*Scutellaria baicalensis Georgi* *Ursus arctos Linnaeus* *Capra hircus Linnaeus* *Lonicera japonica Thunb.* *Forsythia suspensa (Thunb.) Vahl*
Shuang Huang Lian[73]	*Lonicera japonica Thunb.* *Scutellaria baicalensis Georgi* *Forsythia suspensa (Thunb.) Vahl*
Qing Fei Jie Du Tang[74]	*Ephedra sinica Stapf* *Glycyrrhiza uralensis Fisch.* *Prunus armeniaca L.* *Gypsum Fibrosum* *Cinnamomum cassia (L.) J.Presl* *Alisma plantago-aquatica subsp. orientale* *Polyporus umbellatus* *Atractylodes macrocephala Koidz.* *Wolfiporia extensa (Ilieck) Ginns* *Bupleurum chinense DC.* *Scutellaria barbata D.Don* *Zingiber officinale Roscoe* *Aster tataricus L.f.* *Tussilago farfara L.* *Belamcanda chinensis (L.) DC.* *Dioscorea batatas Decne.* *Citrus × aurantium L.* *Citrus reticulata Blanco* *Agastache rugosa (Fisch. & C.A.Mey.) Kuntze*
Xue Bi Jing[75]	*Carthamus tinctorius L.* *Paeonia veitchii Lynch* *Ligusticum striatum DC.* *Salvia miltiorrhiza Bunge* *Angelica sinensis (Oliv.) Diels*

Table 2. Classification of plants used in the treatment of COVID-19 according to their effect and mechanisms of action

Effect	Mechanism of action	Plants
Antiviral effect	Inhibition of viral replication and adsorption of the virus to cell surfaces.[22,38,40,48,57,62,66]	*Forsythia suspensa* *Lonicera japonica* *Scutellaria baicalensis* *Glycyrrhiza uralensis* *Astragalus membranaceus* *Bupleurum chinense* *Isatis tinctoria* *Pogostemon cablin* *Salvia miltiorrhiza*
Anti-inflammatory effect	Cyclooxygenase-inhibitory effect, reduction of tumor necrosis factor and proinflammatory cytokine release, inhibition of the Nuclear factor kappa B pathway,[30,38,41,44,61] inhibition of nitric oxide production,[21,53] inhibition of neutrophil infiltration and activation.[54]	*Lonicera japonica* *Glycyrrhiza uralensis* *Astragalus membranaceus* *Atractylodes macrocephala* *Bupleurum chinense* *Isatis tinctoria* *Pogostemon cablin* *Salvia miltiorrhiza*
Immunomodulatory effect	Increased phagocytic activity and interferon-γ release of T lymphocytes.[45,55] Increased cell-mediated immunity[67] and interleukin 2-inducing activity.[54]	*Astragalus membranaceus* *Atractylodes macrocephala* *Bupleurum chinense* *Salvia miltiorrhiza*

Table 3. **Effects of plants used in the treatment of COVID-19 on the cardiovascular system**

Effect	Mechanism of action	Plants
Anticholesterol effect	Lowering total cholesterol, LDL and VLDL, increasing HDL.(24) HMG-CoA reductase inhibition, decrease in serum and hepatic total triglyceride levels.[27]	*Lonicera japonica* *Forsythia suspensa*
Antiplatelet effect	Inhibition of platelet activating factor.[42,46,69]	*Pogostemon cablin* *Atractylodes macrocephala* *Salvia miltiorrhiza*
Vasodilator effect Antihypertensive effect	Activation of intracellular Ca^{+2} channels in vascular smooth muscles, reduction of systolic blood pressure and vascular tension in the aorta.[50] eNOS expression, reducing arterial pressure, increasing nitric oxide production.[68]	*Scutellaria baicalensis* *Salvia miltiorrhiza*
Calcium antagonistic effect	Inhibition of calcium overload due to ischemia and hypoxia, improving the activity of myocardial calcium pumps.[59]	*Astragalus membranaceus*
Antiarrhythmic effect	Activation of JAK-STAT3 pathway, inhibition of K^+ channels.[34,35]	*Glycyrrhiza uralensis*

DISCUSSION

The extract mixtures chosen for this study are derived from plants trialed in their respective traditional medicine, generally to prevent and improve symptoms of other respiratory tract diseases (such as H1N1 and severe acute respiratory syndrome). In other words, these are not novel formulas for the treatment of COVID-19.[1] The plant ingredients found in the mixtures investigated have been generally shown to exhibit immunomodulator, antiviral, and anti-inflammatory activity in in vitro and in vivo clinical studies. Each plant mixture has been provided with a dedicated formula and name and is administered via oral or parenteral routes. Generally, the plants are in use because they demonstrate multiple actions. There are anti-inflammatory and antiviral effects (Lonicera japonica, Isatis tinctoria, Pogostemon cablin), anti-inflammatory and immunomodulatory effects (Atractylodes macrocephala), or only antiviral effects (Forsythia suspensa, Scutellaria baicalensis), just as there are species demonstrating 3 of the mentioned properties (Glycyrrhiza uralensis, Astragalus membranaceus, Bupleurum chinense, Salvia miltiorrhiza). Table 2 lists the plants used in the treatment of COVID-19 categorized according to their activity and the mechanism of action. Taking into account the most recent data on the effect of the virus on the function of the epithelial tissue of blood vessels, we also studied the effect of the plants on the cardiovascular system. Of these plants, 8 (Forsythia suspensa, Glycyrrhiza uralensis, Scutellaria baicalensis, Astragalus membranaceus, Lonicera japonica, Pogostemon cablin, Atractylodes macrocephala, Salvia miltiorrhiza) were chosen because of the various effects they are shown to have on the cardiovascular system. The cardiovascular effects are provided in Table 3. Damage to the endothelial tissues on the inside of blood vessels is one of the effects of COVID-19 infection. Some phy tochemicals have demonstrated a reparative action on damaged endothelial function in a laboratory setting. Of compounds from the plants used in TCM treatments in our study, the phenolic acids and Tan II found in Salvia miltiorrhiza, polysaccharides found in Astragalus membranaceus and baicalein found in Scutellaria baicalensis, in particular are thought to have a positive effect on endothelial dysfunction through their anti-inflammatory and antioxidant activities.[29,71,72] Aside from the demonstrated positive effect of their mechanisms of action on the cardiovascular or hemostatic systems, potential drug interactions may lead to undesirable side effects associated with the use of some plants. St. John's wort (Hypericum perforatum L.), ginseng (Panax ginseng C.A. Mey), ginkgo (Ginkgo biloba L.), garlic (Allium sativum L.), grapefruit (Citrus paradisi Macfad), and licorice root (Glycyrrhiza glabra L.) are the plants with the most common instances of such interactions. Interactions may be observed between plants used in the treatment of COVID-19 and some cardiovascular drugs. Especially, Glycyrrhiza uralensis may interact with antihypertensive drugs, thiazide diuretics and digitalis glycosides.[36] Salvia miltiorrhiza, Atractylodes macrocephala, Pogostemon cablin and Scutel laria baicalensis may interact with anticoagulant drugs and warfarin.[42,46,69] Among the plants in use, the species Scutellaria baicalensis and Bupleurum chinense must be considered carefully due to their potential for liver toxicity. Possible side effects of the species Lonicera japonica and Scutellaria baicalensis in

preparations used for injection should be evaluated, as well as hypertension and hypokalemia linked with the use of Glycyrrhiza uralensis. Although there may not be work specific to the effects on COVID-19, the plants in some TCM formulations have been tested in clinical studies for other viral infections and some have had positive results. However, debate is ongoing whether these applications are in line with evidence-based medical principles. The World Health Organization (WHO) has stated concerns about clinical studies performed on traditional Chinese plant extracts, including difficulty in accepting their results due to a lack of a sufficient number of subjects and appropriate protocol, and the need for future clinical studies to be updated and developed in line with the appropriate standards.[16] Sackett,[73] a major contributor to the development of the concept of the clinical study around 50 years ago, said that evidence-based medicine was not restricted to randomized trials and meta-analysis. We cannot act on randomized trials and meta analyses alone in the midst of the current pandemic, because there is not enough time for many extensive studies while there are thousands of patients in need of urgent treatment. This means that a greater number of safety studies and clinical studies meeting good clinical practice guidelines are needed in order for these plants to reach the level of evidence necessary to be used for treatment. As a case in point, after reports were published putting forward artemisin, derived from the plant Artemisia annua, as a compound possessing preventive and curative properties for COVID-19, the WHO released a statement saying that while it supports traditional remedies, there was insufficient evidence to support claims to prevent or treat COVID-19.[74] Besides the need for more clinical studies in accordance with good clinical practice standards, the biggest problems seen with such treatment recommendations are generally a lack or abandonment of treatment using standard methods, and interruption in the course of treatment. As specified in the studies from China, the tests were given supplementary to normal treatment. Another critical point that must be taken into account is interactions with other medications. The mechanisms of action of the plants and/ or mixtures administered must be well understood in order to elucidate these issues. Another important problem is the safety of the administered product. Just as interaction problems may result from substances found in the plant used, they may also result from the methods of preparation and administration. For this reason, we have provided the side effects associated with a portion of the aforementioned plants. Plant products may interact with drugs used for cardiovascular diseases, such as warfarin, digitalis, aspirin, alpha blockers, calcium channel blockers, diuretics, statins, antihypertensives, steroids, cardiac glycosides, and anticoagulants. Among the most common unwanted side effects of these plants, most notably bleeding and arrhythmia, are effects with other serious implications, such as hypokalemia, rising or falling blood pressure, and increased heart rate.[75] It is impossible to say how the many botanical products used during the COVID-19 pandemic will affect a virus whose mechanism of infection is of confidence among those who use them and have a negative effect on the spread of the pandemic. People who use these products or methods may feel like they cannot get infected or fall ill, which may lead them to act recklessly in terms protecting themselves and others from infection. As a result, although the plants found in these formulations have been observed to have effects in laboratory or animal studies, the existing clinical studies in accordance with standard procedures are insufficient. More clinical studies are necessary in order to be able to use these plants safely at a clinical level. Peer-review: Externally peer-reviewed. Conflict-of-interest: None. Authorship contributions: Concept: E.A.; Design: E.A., M.E., Z.A., E.Ö.E.; Supervision: E.A., A.Y.Ü.; Materials: E.A., M.E., Z.A., E.Ö.E., A.Y., N.A.; Data: E.A., M.E., Z.A., E.Ö.E., A.Y., N.A.; Analysis: E.A., A.Y.Ü.; Literature search: E.A., M.E., Z.A., E.Ö.E., A.Y., N.A.; Writing: E.A., M.E., Z.A., E.Ö.E., A.Y., N.A.; Critical revision: E.A., A.Y.Ü.

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3. **Bian YQ, Ma J, Ren Y, Zhang YL, Qiao YJ. Discovery of intervention effect of Chinese herbal formulas on COVID-19 pulmonary fibrosis treated by VEGFR and FGFR inhibitors Zhongguo Zhong Yao Za Zhi. 2020 Apr;45(7):1481-1487. doi: 10.19540/j.cnki.cjcmm.20200315.401.**

Abstract

Since February 2020, a large number of patients infected with new coronavirus has been cured and discharged with the controlling of epidemic. Pulmonary fibrosis, which may be one of the sequela caused by COVID-19, not only brings dyspnea and deterioration of lung function, but also affects patients' life because of its high mortality and poor prognosis. Vascular endothelial growth factor receptor(VEGFR) and fibroblast growth factor receptor(FGFR) can inhibit the proliferation, activation and migration of fibroblasts by regulating the signal transduction pathway involved in the process of pulmonary fibrosis. Chinese herbal formulas pose a good therapeutic effect on pulmonary fibrosis. Present study explores the intervention effect on pulmonary fibrosis of traditional Chinese medicine(TCM) by screening the potential inhibitors of VEGFR and FGFR. The docking models of VEGFR and FGFR were established to obtain the potential active ingredients which were filtered by the docking score. According to 2 prescriptions in the Protocol for the diagnosis and treatment of coronavirus disease 2019(7th edition)and 9 prescriptions in Traditional Chinese medicine prescriptions for treating blight, 959 and 1 047 potential ingredients were obtained as the inhibitors of VEGFR and FGFR respectively with the screening thres-hold set as eighty percent of the docking score of the initial ligands. The potential herbs were then filtered by the components with a hit rate higher than 30%, such as Scutellariae Radix, Adenophorae Radix, Pinelliae Rhizoma, Coicis Semen, etc. To discuss the rule of TCM in the treatment of pulmonary fibrosis, the networks of TCM-channel tropism and TCM-efficacy of the potential herbs was constructed. The potential herbs for treating pulmonary fibrosis mostly belong to lung(degree=14) and spleen(degree value=8), and the efficacy is focused on reinforcing deficiency(degree=9). Qiyin Prescription and Buzhong Yiqi Decoction contain the largest number of the potential herbs. The main symptom of COVID-19 is damp-heat stagnating in the lung, which always causes impairment of body fluid and Qi. Clinical observation shows that patients in the recovery period are mostly at the status that the remaining virus toxicity is not exhausted while the vital Qi have not recovered. The results of this study are expected to provide references for clinical medication in preventing and treating pulmonary fibrosis caused by COVID-19.

4. **Cao P, Wu S, Wu T, Deng Y, Zhang Q, Wang K, Zhang Y. The important role of polysaccharides from a traditional Chinese medicine-Lung Cleansing and Detoxifying Decoction against the COVID-19 pandemic. Carbohydr Polym. 2020 Jul 15;240:116346. doi: 10.1016/j.carbpol.2020.116346. Epub 2020 Apr 22.**

Abstract

The new coronavirus pneumonia, named COVID-19 by the World Health Organization, has become a pandemic. It is highly pathogenic and reproduces quickly. There are currently no specific drugs to prevent the reproduction and spread of COVID-19. Some traditional Chinese medicines, especially the Lung Cleansing and Detoxifying Decoction (Qing Fei Pai Du Tang), have shown therapeutic effects on mild and ordinary COVID-19 patients. Polysaccharides are important ingredients in this decoction. This review summarizes the potential pharmacological activities of polysaccharides isolated by hot water extraction from Lung Cleansing and Detoxifying Decoction, which is consistent with its production method, to provide the theoretical basis for ongoing research on its application.

1. Introduction

Since the outbreak of a large number of a new coronavirus infection in Wuhan, China in December 2019, the WHO has pronounced this novel coronavirus pneumonia epidemic to be a Public Health Emergency of International Concern (PHEIC), and named this infectious disease as “COVID-19” (Wu, Zhao et al., 2020). By mid-March 2020, more than 80,000 patients had been diagnosed with the disease in China, with 3000 deaths. The Chinese government has initiated a joint prevention and control initiative to prevent the spread of this COVID-19 epidemic. This coronavirus has spread to 44 countries on all continents except Antarctica, and transmission of COVID-19 in Italy, Iran and the Republic of Korea has brought the total number of infected cases in these three countries to nearly 35,000 as of March 14, 2020, from less than two percent that number just three weeks before. The situation is becoming worse daily, although in mid-March 2020, the number of new cases in China dropped noticeably.

Despite world-wide intense scientific effort, there is as yet no drug showing significant clinical effects on COVID-19 (Cao et al., 2020). However, traditional Chinese medicine has been playing a critical role in the prevention, treatment and rehabilitation of the COVID-19 (Ren, Zhang, & Wang, 2020). According to recent data collected by the National Administration of Traditional Chinese Medicine, a Traditional Chinese Medicine (TCM) named “Lung Cleansing and Detoxifying Decoction (Qing Fei Pai Du Tang)”, of which the main components are carbohydrate polymers, has shown notable therapeutic effects on COVID-19 (Liu et al., 2020). Specifically, 214 confirmed cases in four provinces were administered with this drug for three treatment courses between January 27 and February 5, 2020, with more than 60 % of patients showing obvious improvement in symptoms and computed tomography (CT) manifestation and the remaining 30 % being stable without deterioration (Ren et al., 2020). As a result, the Lung Cleansing and Detoxifying Decoction was deployed in four mobile “Fangcang” hospitals in the epicenter city, Wuhan, which were temporary hospitals to quarantine mild cases. This decoction showed satisfactory efficacy, with nearly all the patients recovering from the symptoms of fever, fatigue and cough, according to the data collected in 66 designated medical institutions in 10 provinces (Stated by National Administration of Traditional Chinese Medicine, http://www.satcm.gov.cn).

As a result, the latest version of “Diagnosis and treatment of novel coronavirus pneumonia” (新型冠状病毒肺炎诊疗方案) statement issued by the National Health Commission of the People’s Republic of China (http://www.nhc.gov.cn, the sixth and seventh editions accessed 2020-02-19 and 2020-03-04, respectively), the Lung Cleansing and Detoxifying Decoction is recommended for all COVID-19 patients, especially in combination with western medical treatment. Even though this TCM has been used clinically for a long time in improving symptoms of fever, cough and fatigue as well as lung condition, and recently manifested definite therapeutic effect on COVID-19 patients (Ren et al., 2020; K. Zhang, 2020), its active ingredients remain unknown. This is probably because this TCM decoction has 21 herbal components which is derived from several classic recipes in a traditional Chinese medicine work. The complex constituents of Lung Cleansing and Detoxifying Decoction makes it a hard work to deeply explore its active ingredient in a short time.

It is generally acknowledged that polysaccharides are the main active ingredients of TCM decoction (Cao et al., 2018; Yu, Shen, Song, & Xie, 2018). Polysaccharides generally have a rather low toxicity, and contain hundreds or even thousands of monosaccharide units (Delattre, Fenoradosoa, & Michaud, 2011). These polar macromolecular compounds are usually readily soluble in water. By employing the principle of "similar miscibility", polar macromolecular polysaccharides are extracted in boiled water. The present review summarizes the polysaccharides isolated by hot water extraction, which is consistent with the preparation method of Lung Cleansing and Detoxifying Decoction. So, we suppose that the polysaccharides are important activate ingredients in Lung Cleansing and Detoxifying Decoction and polysaccharides may play a vital role in treating COVID-19 patients.

2. The bioactive polysaccharides in Lung Cleansing and Detoxifying Decoction

Among the raw materials of Lung Cleansing and Detoxifying Decoction, the majority of their polysaccharide components have been elucidated. The structures of the following polysaccharides were well established (Fig. 1): Bupleurum polysaccharide (Zhao, Li, Yue, Zhang, & Dou, 2012), Glycyrrhiza glabra polysaccharide (Mutaillifu et al., 2020), Scutellaria baicalensis polysaccharide (Cui et al., 2019a), Poria cocos polysaccharide (Lu, Cheng, Lin, & Chang, 2010), almond polysaccharide (Bouaziz, Koubaa, Ellouz Ghorbel, & Ellouz Chaabouni, 2017), Pinelliae Rhizoma polysaccharide (Hu et al., 2019), Chinese yam polysaccharide (Zhao, Kan, Li, & Chen, 2005), ginger polysaccharide (Chen, Chen, Wang, & Kan, 2020), Rhizoma alismatis polysaccharide (Zhao, Zhang, Li, Dong, & Liu, 2015), Polyporus umbellatus polysaccharide (He, Zhang, Zhang, Linhardt, & Sun, 2016), Aster tataricus polysaccharide (Zhang et al., 2012), Ephedra polysaccharide (Kuang, Xia, Liang et al., 2011), Rhizoma Atractylodis polysaccharide (Liang,

Bioactivities	Polysaccharides	Proposed structure	Composition	Molecular Weight	Mechanisms	References
Immunomodulatory activity	Glycyrrhiza polysaccharide	β-1,3-linked d-galactose residues; α-1,4-linked d-glucose	Not determined	10kD	Promoting the maturation, differentiation and reproduction of immune cells such as lymphocytes and macrophages, as well as activating the reticuloendothelial system	Cheng et al. (2008)
	Bupleurum polysaccharide	Not determined	Ara: Gal: Glc: Rha = 6.35: 3.15: 1.47: 1	2000kD	Enhance phagocytic functions of murine peritoneal macrophages	Cheng et al. (2010)
	Ephedra polysaccharides	Not determined	ESP-B4: Xyl (1.5 %), Ara (6.8 %), Glc (1.5 %), Rha (3.0 %), Man (1.5 %), Gal (8.3 %), GlcUA (2.3 %) and GalUA (75.2 %)	>2000 × 106Da for ESP-B4	Inhibition on splenocyte proliferation	Kuang, Xia, Yang et al. (2011)
	Poria cocos polysaccharide	(1→3)-β-d-glucan possessing 9–10 branches of (1→6) linked β-d-glucopyranosyl groups and internal (1→6)-β-d-linkages	Man (92 %), Gal (6.2 %), and Ara (1.3 %)	8kD	Enhance both cellular and humoral immunity in mice; Activate T cells; Activate NF-κB/Rel and iNOS expression by upregulating p38 kinase in murine macrophages	Lee et al. (2004), Ma et al. (2010), Tian et al. (2019)
	Chinese yam polysaccharide	1, 3-linked-glc, 1-linked-gal and 1, 6-linked-gal glycosidic bonds	Glu: Gal = 1.52:1	16619Da	Chinese yam polysaccharide nanoparticles: Promote lymphocyte proliferation and trigger the transformation of T lymphocytes into Th cells	Luo et al. (2017, 2016)
	Polyporus umbellatus polysaccharide	(1→ 6, 1→ 4)-linked β-d-glucopyranosyl backbone, substituted at O-3 position of (1→6)-linked β-d-glucopyranosyl by (1→3)-linked β-d-glucopyranosyl branches	β-Glucans (>90 % D-glucose)	2.27kD	Activator of B cells, macrophages and dendritic cells; Promote the activation and maturation of murine bone-derived dendritic cells via TLR4	Dai et al. (2012), Huang et al. (2019), Li et al. (2010)
	Rhizoma Atractylodis polysaccharide	1, 3-linked-D Galp and 1, 6- linked-D Galp residues	Glu (60.67 %), Man (14.99 %), Rha (10.61 %), Ara (8.83 %) and Gal (4.90 %)	1.87kD	Promotes productions of NO, ROS and cytokines via an interaction network including NF- κB and JAk-STAT signaling pathways	Xu et al. (2020)

	Ephedra polysaccharide	Not determined	ESP-B4: Xyl (1.5 %), Ara (6.8 %), Glc (1.5 %), Rha (3.0 %), Man (1.5 %), Gal (8.3 %), GlcUA (2.3 %) and GalUA (75.2 %)	>2000 × 106Da for ESP-B4	Regulate Factor-1/Smad2 signaling pathway; Inhibit the TLR4 signaling pathway	Liang et al. (2018), Wang et al. (2016)
	Scutellaria baicalensis polysaccharide	Not determined	Man: Rib: GlcUA: Glu: Xyl: Ara = 2.14:3.61:1:2.86:5.98:36.39	456kD	Suppress NF-κB signaling and NLRP3 inflammasome activation	Cui et al. (2019b)
Anti-inflammatory activity	Bupleurum polysaccharide	(1→5)-linked Ara, (1→4)-linked Gal and (1→3)-linked Gal residues with occasionally branches at O-6	Ara: Gal: Glc = 2.1:2.5:1	29kDa	Inhibit P-selectin-mediated recruitment of neutrophils rolling along the CHO-P cells	Sun et al. (2010), Tong et al. (2014, 2018)
	Poria cocos polysaccharide	neutral 1,6-branched 1,3- a-d-galactan	myo-inositol, sorbitol, fucose, galactosamine, glucosamine, galactose, glucose and mannose	610.7, 40.7, 7.9, 1.6, and 0.3 kDa	Suppress IP-10	Lu et al. (2010)
	Bupleurum polysaccharide	Not determined	51.20 % total carbohydrate and 48.47 % uronic acid; Gal: Ara: Glc: Rha: Man = 13.43: 11.57: 4.02: 1.02: 1.0	Not determined	Reduce the content of MDA in serum and bronchoalveolar lavage fluid (BALF) and enhancing the SOD, in acute lung injury model	Xie et al. (2012)
	Ephedra polysaccharide	Not determined	Not determined	Not determined	Increase the SOD activity and reduce the production of MDA	Fan et al. (2015)
	Glycyrrhiza polysaccharide	Not determined	Glu: Gal: Ara = 23.4: 25.18: 8.32(GUPs-1), 14: 25.67: 17.54(GUPs-2), 1.13: 22.04: 31.44(GUPs-3)	10160(GUPs-1), 11680(GUPs-2) and 13360(GUPs-3) Da	Fe2+-chelating activity; scavenge hydroxyl radicals, superoxide radical and DPPH radical	Zhang et al. (2015b)
Anti-oxidative activity	Polyporus umbellatus polysaccharide	Backbone: 1→6, 1→3-linked; side chains: →6)-β-d-Glcp-(1→, →3)-β-d-GlcpA-(1→, →3)-β-d-Glcp-(1→, →4)-β-d-Glcp-(1→, β-d-Glcp-(1→, →4)-β-d-GlcpA-(1→ and →3,6)-β-d-Glcp-(1→	glucose and 8.5 % uronic acid	PUP80S1: 8.8kDa	Absorb oxygen radical and scavenge 2,2-diphenyl-1-picrylhydrazyl radical	He, Zhang, Zhang et al. (2016)
		Backbone: (1→6)-β-d-glucopyranosyl; side chains: β-d-Glcp, (1→3)-β-d-Glcp, (1→3)-β-D-GlcpA, (1→4)-β-D-Glcp and (1→4)-β-D-GlcpA	glucose and 22.3 % glucuronic acid	PUP60S2: 14.4 kD		He, Zhang, Wang et al. (2016)

	Poria cocos polysaccharide	Not determined	Man: Glu: Gal: Ara = 0.92: 86.88: 0.18: 12.01	21.5kDa	Relieve ox-LDL-induced oxidative stress via the ERK/Nrf2/HO-1 signaling pathway; Reduce DPPH radical and hydroxyl radical	Wang et al. (2016), Zhao et al. (2020)
	Rhizoma Atractylodis polysaccharide	Not determined	Rha: Xyl: Ara: Glu: Man: Gal = 1: 1.3: 1.5: 1.8: 2.1: 3.2	19.6kD	Reduce NOS, NO and MDA activity or contents, increase SOD and GSH-Px activities	Han et al. (2016)
		Sugar resides: →1,4)-α-d-Glcp-(1→; →2,3,4)-α-d-Manp-(1→; →1,4,6)-α-d-Galp-(1→	Man: Glu: Gal = 4.96: 92.24: 2.80	GP1: 6128Da		
	Ginger polysaccharide	Sugar resides: →1,4)-α-d-Glcp-(1→; →2,3,4)-α-d-Manp-(1→; →1,4,6)-α-d-Galp-(1→; →3)-β-l-Arap-(1→	Ara: Man: Glu: Gal = 4.78: 16.70: 61.77: 16.75	GP2: 12619Da	Eliminate DPPH free radical	Wang, Wei et al. (2018)
	Chinese yam polysaccharide	1,3-linked-glc, 1-linked-gal and 1,6-linked-gal glycosidic bonds	Glu: Gal = 1.52: 1	16619Da	Remove superoxide anion	Yang et al. (2015)
	Bupleurum polysaccharide	Not determined	Man: Rha: GlcA: GalA: Glc: Gal: Xyl: Ara = 2.93: 2.62: 1.00: 4.57: 15.11: 23.28: 1.46: 25.34	2,917731, 281670 and 2707 Da	Firmicutes/Bacteriodetes ↓ Rikenellaceae↑ Ruminococcus↑ Oscillospira↑ Roseburia↑	Feng et al. (2019)
	Chinese yam polysaccharide	Not determined	Not determined	Not determined	Bifidobacteria↑ Lactobacilli↑ Enterococcus ↓ Clostridium perfringens↓	Zhang, Liang et al. (2019)
Maintain intestinal homeostasis	Poria cocos polysaccharide	Not determined	Not determined	4486kDa, 403kDa	Lachnospiracea↑ Alloprevotella↑ Parabacteroides↑ Clostridium IV↑ Ruminococcus↑ Bacteroides↑ Megamonas↓ Proteus↓	Sun et al. (2019)
	Poria cocos polysaccharide	Not determined	Not determined	Not determined	Akkermansia muciniphila↑ Alistipes massiliensis↓ Robinsoniella peoriensis↓ Helicobacter hepaticus↓ Lactococcus↑ Lactobacillus↑ Faecalibacterium spp.↑ Bacteroides spp.↑	Khan et al. (2018)
Antibacterial activity	Chinese yam polysaccharide	1,3-linked-glc, 1-linked-gal and 1,6-linked-gal glycosidic bonds	Glu: Gal = 1.52: 1	16619Da	Inhibit E. coli (MIC 2.5 mg/mL)	Yang et al. (2015)
	Poria cocos polysaccharide	Not determined	Glu: Gal = 40.9: 1	20.5kDa	Inhibit Staphylococcus aureus and Escherichia coli growth	Li et al. (2018), Wang, Zhang et al. (2018)
Antitussive activity	Asarum polysaccharides	Not determined	Not determined	Not determined	Decrease cough sensitivity and suppress airway inflammation	US20160339054A1 US20180271896A1

Zhu, & Bai, 2011**), *Fructus aurantii* polysaccharide (Shu et al., 2020), tangerine peel polysaccharide (Chen et al., 2016). However, there is no report relating to the identification of polysaccharides extracted from *Asarum*, *Cassia Twig*, *Flos Farfarae*, *Belamcanda chinensis* or *Agastache rugosa* (Table 1).**

Fig. 1. The processed raw materials of "Lung Cleansing and Detoxifying Decoction" in treating COVID-19. (The images are collected from Internet, with no conflict of interest).

Based on the raw TCM materials and clinical evidence of Lung Cleansing and Detoxifying Decoction, we give an overview of its potential bioactive polysaccharides in treating COVID-19, the biological benefits of which appear to involve immunomodulatory activity, anti-inflammatory activity, anti-oxidative activity and regulation of gut microbiota balance.

2.1. Immunomodulatory activity

Since the outbreak of COVID-19, it has been realized that an effective body immune response plays an important role in the elimination of the virus and the prognosis of the disease (Chen, Zhou et al., 2020). Viruses are intracellular parasitic non-cellular microorganisms, and cellular immunity plays a leading role in eliminating viral infections (Li et al., 2020). From the perspective of general viral infections and individual immune responses, Immunomodulatory drugs have thus attracted attention because artificial passive immunity induced by administrating immunomodulatory drugs can rapidly enhance cellular immunity and may help fight viral infection. For example, clinical studies have found that the immunomodulatory thymosin drugs can increase the therapeutic effect of viral infections including hepatitis C virus (HCV) and rotavirus (Ciancio et al., 2012). The immunomodulatory effects of macrolides are also beneficial in pneumonia or chronic pulmonary inflammatory syndromes, decreasing disease severity and mortality (Kovaleva et al., 2012). Immune cells such as macrophages, neutrophils, monocytes, lymphocytes and NK cells are the main targets of coupling between immunostimulatory polysaccharides and specific proteins (Altan-Bonnet & Mukherjee, 2019). Polysaccharides with immunostimulatory activity can directly or indirectly interact with the host immune system, initiating a series of molecular interactions, leading to the activation of the immune system (Xie, Hao et al., 2016).

An immunomodulatory activity is one of the most significant properties of polysaccharides (Wang et al., 2020; Wu, Feng et al., 2020). In the Lung Cleansing and Detoxifying Decoction, there are a number of immunomodulatory polysaccharides, as follows. Glycyrrhiza polysaccharide could activate the immune system by promoting the maturation, differentiation and reproduction of immune cells such as lymphocytes and macrophages, as well as activating the reticuloendothelial system (Ayeka, Bian, Githaiga, & Zhao, 2017; Cheng, Wan, Wang, Jin, & Xu, 2008; He et al., 2011; Hong, Wu, Ma, Liu, & He, 2009; Yang & Yu, 1990). The immunomodulatory effects of Bupleurum polysaccharides had been demonstrated by enhancing phagocytic functions of murine peritoneal macrophages including phagocytosis of apoptotic thymocytes, chicken red blood cells, and IgG-opsonized sheep red blood cells (Cheng et al., 2010; Jiang et al.,

2012; Matsumoto, Guo, Ikejima, & Yamada, 2003). Of note, polysaccharides in water extract of Bupleurum manifested anti-complementary activity, whereas the ethanol extract of that didn't show any activity (Xie et al., 2012). Ephedra polysaccharides isolated by hot water extraction contained four homogeneous fractions, of which ESP-B4 exhibited the highest bioactivity, which might be ascribed to its higher content of GalA and branches (Kuang, Xia, Yang, Wang, & Wang, 2011). Poria cocos polysaccharide, an oral drug approved by the Chinese Food and Drug Administration (CFDA) for treating hepatitis, cancers and other diseases (Li, Ma, & Zhang, 2019), was able to enhance both cellular and humoral immunity in mice (Lee et al., 2004; Ma, Chang, Chang, & Wu, 2010; Tian, Liu, Pu, & Bao, 2019), and thus showed potential as an adjuvant in vaccination (Zhang, Cheng et al., 2019). Chinese yam polysaccharides could be efficacious for immunomodulatory functions and immune enhancement, and also acted as adjuvants in developing vaccines (Luo et al., 2017, 2016). Polyporus umbellatus polysaccharide was a potent activator of B cells, macrophages and dendritic cells, manifesting significant ability to enhance innate immune function (Dai et al., 2012; Huang, Li, Chen, Liu, & Wang, 2019), probably via the activation of the TLR-4 signaling pathway (Li & Xu, 2011; Li, Xu, & Chen, 2010). Rhizoma Atractylodis polysaccharide promoted productions of NO, ROS and cytokines, enhancing immune response and immune function via a pivotal interaction network including NF-κB and JAk-STAT signaling pathways (Xu, Fang, Wang, Zhang, & Hu, 2020). In general, the relation between immunomodulatory activity of polysaccharides and their structures remains obscure. Some studies speculated that the complete structure, high molecular weight and basic structure-oligosaccharide unit were highly desired for the immunomodulatory action polysaccharides (Yan et al., 2003). Based on the sugar composition analysis, some investigations suggested that polysaccharides composed of glucan were known to stimulate the immune system (Kuang, Xia, Yang et al., 2011).

2.2. Anti-inflammatory activity

According to a recent paper in Lancet, the levels of inflammatory factors in the plasma of critical patients, such as IL-2, IL-7, IL-10, GCSF, IP10, MCP1, MIP1A and TNF-α, are all higher than those without intensive care, suggesting that the occurrence of this "cytokine storm" is closely related to the severity of COVID-19 patients (Huang et al., 2020). A cytokine storm, a term initially proposed in 1993 (Ferrara, 1993), is considered to be an important signal for the transformation from ordinary patients to progress to severe and critically ill, and it is also the main cause of acute respiratory distress syndrome (ARDS) and sepsis, which are the leading causes of COVID-19 death (W. Zhang, 2020). A direct suppression on the lung inflammatory response seems warranted as the cytokine storm may be relieved after inflammatory therapy. Previous studies had demonstrated the benefits of anti-inflammatory agents in lung diseases. Drugs that target inflammation have been shown to slow the decline in lung function and improve survival (Konstan et al., 2011; VanDevanter et al., 2012). Ibuprofen, a commonly used anti-inflammatory drug, is recommended for the long-term treatment of airway inflammation in cystic fibrosis lung disease (Flume et al., 2007).

An anti-inflammatory activity is very common in various sources of polysaccharides (Bezerra et al., 2018; Gao et al., 2019; Kang et al., 2011). It was reported that polysaccharides consisting of Gal, Glc, GalA and Rha revealed potent anti-inflammatory activity (Capek et al., 1988). The anti-inflammatory polysaccharides in the Lung Cleansing and Detoxifying Decoction may play crucial roles in suppressing the cytokine storm, thus effectively treating mild COVID-19 patients and blocking the conversion from mild cases to severe stage. As an example, an acid component of Ephedra polysaccharide, ESP-B4, possessed obvious protective effects on pulmonary inflammation and rheumatoid arthritis by reducing the production of TNF-α, IL-6, IL-8 and MMP-9 (Liang et al., 2018) and inhibiting the TLR4 signaling pathway (Wang et al., 2016), respectively. A polysaccharide from Scutellaria baicalensis might be a drug candidate in treating colitis via suppressing NF-κB signaling and NLRP3 inflammasome activation (Cui et al., 2019b). Bupleurum polysaccharide could significantly relieve lung injury in an acute pneumonia model by inhibiting P-selectin-mediated recruitment of neutrophils rolling along the CHO-P cells (Sun et al., 2010; Tong et al., 2014, 2018). A Poria cocos polysaccharide could suppress the production of IP-10, the marker of interferon (IFN)-c-induced inflammation, in a dose-dependent manner, suggesting its anti-inflammatory potential (Lu et al., 2010).

Pachyman, a kind of Poria cocos polysaccharide, showed antinephritic effect in rats with nephritis, probably via the inhibition of inflammation caused by C3 deposition in the glomeruli (Chihara, Hamuro, Maeda, Arai, & Fukuoka, 1970; Hattori et al., 1992).

2.3. Anti-oxidative activity

An anti-oxidative activity is an important pharmacological action in polysaccharides (Li et al., 2018; Mzoughi et al., 2018; Raguraman et al., 2019). Oxidative stress and inflammation can act together to form a positive feedback cycle (Mittal, Siddiqui, Tran, Reddy, & Malik, 2014). Under normal circumstances, the production and elimination of reactive oxygen species (ROS) in the body maintains an oxidation-antioxidation balance, which plays an important role in regulating signal pathway transduction and cell proliferation (Cao et al., 2019; Forrester, Kikuchi, Hernandes, Xu, & Griendling, 2018). When the balance is broken by inflammatory factors, the body will produce an oxidative stress response, leading to cell oxidative damage and development of multi-system diseases (Kruk, Aboul-Enein, Kladna, & Bowser, 2019; Sies, 2015). Oxidative stress in turn activates multiple signaling pathways to induce inflammation, such as activation of NF-κB and NOD-like receptor protein 3 (NLRP3), further promoting the maturation of pro-inflammatory factors (Ahmad & Ahsan, 2020). Vitamin C, a powerful antioxidant, has been demonstrated to play a role in lowing the incidence of pneumonia in several controlled trial with human subjects (Hemila, 2017).

A large body of researches have suggested that many polysaccharides possess anti-oxidative properties (Yu et al., 2018), which may be critical to their multiple pharmacological activities. Bupleurum polysaccharides had been demonstrated to exert definite protective effects in murine lung-injury models (Cheng et al., 2012; Xie et al., 2012). Specifically, this kind of polysaccharide could alleviate the degree of acute lung injury by reducing the amount of malondialdehyde (MDA) in serum and bronchoalveolar lavage fluid and enhancing superoxide dismutase (SOD) activity (Xie et al., 2012). Ephedra polysaccharide could significantly increase the activity of SOD and reduce the production of MDA, thereby protecting the liver from free radical and lipid peroxidation damage on hyperlipidemic mice (Fan et al., 2015). Glycyrrhiza polysaccharide had significant ability to scavenge hydroxyl, superoxide radical and DPPH radicals in vitro, and also enhanced the SOD, CAT, GSH-Px and TAOC activities in vivo (Hong et al., 2009; Zhang, Yu, Liang, & Chen, 2015). It was shown that glycyrrhiza polysaccharide with lower molecular weight and higher ratio of glucose exhibited more effective antioxidant activities at the same concentration (Zhang, Yu, Liang, & Chen, 2015). This phenomenon might be explained as a high molecular weight polysaccharide possesses a high viscosity, which may have great influence on its bioactivities (Chen, Lu, Cheng, & Wang, 2005). Two kinds of antioxidative polysaccharides, named PUP60S2 and PUP80S1, were isolated from Polyporus umbellatus, among which PUP60S2 showed higher antioxidative activity (He, Zhang, Zhang et al., 2016; He, Zhang, Wang et al., 2016). This might be ascribed to more uronic acid residues and a higher level of branching of PUP60S2 when they have similar structures, as demonstrated that higher degree of polysaccharide branching is beneficial for exerting antioxidant activity (Zhao et al., 2014). Poria cocos polysaccharide could significantly relieve ox-LDL-induced oxidative stress via the ERK/Nrf2/HO-1 signaling pathway in vascular smooth muscle cells (Tang et al., 2014; Zhao et al., 2020). It was demonstrated that Poria cocos polysaccharides extracted by different methods manifested different antioxidative properties, with microwave-assisted extraction possessing best antioxidant activity (Wang et al., 2016). Rhizoma Atractylodis polysaccharide acted as a potent antioxidant by reducing NOS activity, increasing SOD and GSH-Px activities, and decreasing NO and MDA contents in mice (Han et al., 2016; Liang et al., 2011). Ginger polysaccharides also showed high oxidation resistance in several studies (Chen, Yuan, Wang, Qi, & Cheng, 2019; Wang, Wei et al., 2018).

2.4. Regulation of population balance of gut microbiota

The composition of intestinal microbiota is closely related to human health and plays a vital role in maintaining physiological balance. Intestinal microbiota acts as a protective mediator during pneumococcal pneumonia by enhancing primary alveolar macrophage function (Schuijt et al., 2016). It has also been demonstrated that modulating gut microbiota can reduce ventilator-associated pneumonia and enteritis (Bradley et al., 2019).

Probiotic bacteria such as Bifidobacterium and Lactobacillus can stimulate the immune system and reduce serum lipids, while increased amounts of pathogens like Enterococcus and Clostridium perfringens may cause diseases (Buffie & Pamer, 2013; Gerritsen, Smidt, Rijkers, & de Vos, 2011). The risk of heterotopic intestinal flora is increased if the intestinal mucosal immune barrier is in a vulnerable state, when microbiota dysbiosis makes patients prone towards secondary bacterial infections (Gao, Chen, & Fang, 2020). COVID-19 patients show intestinal microbial dysbiosis with decreased levels of certain probiotic microbiota, including Lactobacillus and Bifidobacterium. Even though there is currently no direct clinical evidence proving that modulation of gut microbiota has a therapeutic role in treating COVID-19, we suggest that modulating gut microbiota might be a new therapeutic strategy or at least an adjuvant therapeutic choice (Konig & Brummer, 2020). The latest version of "Diagnosis and treatment of novel coronavirus pneumonia", published by National Health Commission of the People's Republic of China, suggests using intestinal microbiological regulators to maintain the intestinal microecological balance in severe and critical cases.

Regulating gut microbiota is a major focus of current polysaccharide research. In a study on the effect of Bupleurum polysaccharide on diabetic nephropathy in mice, the ratio of phyla Firmicutes/Bacteriodetes, which has been widely regarded as the marker of gut microbiota homeostasis, was elevated in diabetic mice but could be reversed by a supplementation of Bupleurum polysaccharide (Feng et al., 2019). In addition, the abundances of Rikenellaceae, Ruminococcus and Oscillospira were also increased after polysaccharide treatment, among which Ruminococcus is a probiotic that is found dominantly in healthy gut (Ma et al., 2018), and Oscillospira may relieve inflammation by utilizing host glycans as growth substrates (Konikoff & Gophna, 2016). Butyrate is an essential energy source that can influence microbial environment and protect the host against the pathogenic bacteria (Cani, 2018). It is noteworthy that Roseburia, a bacterium producing butyrate (Delzenne, Cani, Everard, Neyrinck, & Bindels, 2015), was also elevated after polysaccharide intervention. These results suggest that Bupleurum polysaccharide may alleviate gut microbiota dysbiosis by increasing the relative abundance of beneficial bacteria, including proliferating butyrate-producing ones.

It has been found that polysaccharides from Chinese yam enriched beneficial intestinal bacteria and inhibited the growth of bacterial pathogens in the cecum of SD rats (Kong et al., 2009). The effect of Chinese yam polysaccharides on intestinal microbiota was also evaluated in a model of antibiotic-associated diarrhea (Zhang, Liang et al., 2019). In this experiment, the effect of yam polysaccharides on fecal microbiota was assessed by the colony-count technique, and the results suggested that these polysaccharides elevated the richness and diversity of bacterial communities. In addition, administrating yam polysaccharides increased probiotic Bifidobacteria and Lactobacilli by 47 % and 21 %, and decreased pathogen Enterococcus and Clostridium perfringens by 8 % and 27 %, respectively, compared with a model group (Turroni et al., 2014; Wagley et al., 2019).

Poria cocos polysaccharide is also a prebiotic in ob/ob mice, significantly reducing glucose- and lipid-metabolism disorders and reducing inflammation through modulating the gut microbiota (Sun, Wang, Ma, Bao, & Liu, 2019). It altered the bacterial contents of 20 genera, with six elevated SCFAs-production bacteria (Lachnospiracea, Alloprevotella, Parabacteroides, Clostridum IV, Ruminococcus and Bacteroides) reaching significant statistical differences. Further, Poria cocos polysaccharide significantly reduced the abundance of the pro-inflammatory bacteria Megamonas and Proteus, by 120- and 101-fold, respectively (Byndloss et al., 2017). These results demonstrate that the beneficial effects of Poria cocos polysaccharide is dependent on modulating gut microbiota composition, which is shown to be causative by a fecal transplantation test (Sun et al., 2019).

In another study, normal C57BL/6 mice were intragastrically administered with Poria cocos polysaccharide for 15 consecutive days, followed by 16S rRNA gene sequencing of their feces (Khan et al., 2018). Poria cocos polysaccharide significantly promoted the growth of Akkermansia muciniphila, a beneficial bacterium that enhances host immunity (de Vos, 2017), whereas the abundances of pro-inflammatory bacteria, including Alistipes massiliensis, Robinsoniella peoriensis and Helicobacter hepaticus, were reduced after

polysaccharide gavage (Halfvarson et al., 2017). In addition, Poria cocos polysaccharide enriched lactic acid-producing (LAP) genera, namely Lactococcus and Lactobacillus, both of which have been shown to possess anti-inflammatory and immunoenhancement properties (Castillo, de Moreno de LeBlanc, Galdeano, & Perdigon, 2013; Luerce et al., 2014). Moreover, Faecalibacterium spp. and Bacteroides spp., the SCFA producing bacteria, were particularly promoted with polysaccharide treatment (Chang et al., 2015; Rios-Covian et al., 2016).

2.5. Other effects

Anti-virus and anti-bacterial activities are also important properties of polysaccharides. Considering that there is no evidence demonstrating anti-viral activity of these polysaccharides helps to eliminate coronavirus, and no antivirus drug has been demonstrated to cure COVID-19 till now. Thus, it is reasonable to suppose that the antiviral activity of polysaccharides in the Lung Cleansing and Detoxifying Decoction does not play a significant role in its therapeutic action.

Secondary bacterial co-infection is common in COVID-19 infected patients, which may lead to serious outcomes (MacIntyre et al., 2018). Antibiotic treatment is therefore necessary in a portion of patients infected by the bacteria. Apart from the predominant bioactivities mentioned above, some polysaccharides also display antibacterial activity (He, Yang, Yang, & Yu, 2010), which might be helpful to some extent in treating the COVID-19 pandemic. Glycyrrhiza polysaccharide displayed antimicrobial activity, inhibiting the growth of B. cereus, S. aureus, E. Aerogens and E. coli (Harish & Jyoti, 2019). The purified Chinese yam polysaccharide showed an inhibitory activity against Escherichia coli, with a minimum inhibitory concentration (MIC) of 2.5 mg/mL (Yang, Wang, Li, & Yu, 2015). A recent study found that Poria cocos polysaccharide could inhibit the growth of Staphylococcus aureus and Escherichia coli (Wang, Zhang et al., 2018).

There is a lack of studies on the structure and activity of Asarum polysaccharide, but it has been reported in two patents (US20160339054A1 and US20180271896A1) that the total polysaccharides extract from Asarum exerted excellent antitussive activity. This suggests that Asarum polysaccharides can decrease cough sensitivity and suppress airway inflammation. It is reasonable to suppose that the Asarum polysaccharides in the Lung Cleansing and Detoxifying Decoction play an important role in relieving cough symptoms, which are prevalent in COVID-19 patients.

3. Summary and future prospects

Most COVID-19 infected individuals are diagnosed as in the mild or ordinary stages. There is not at present any conventional drug that can cure this disease. However, according to the data collected by National Health Commission of the People's Republic of China, clinical practice in Chinese hospitals has reported that TCMs have definite therapeutic actions at the early stage of disease (Liu et al., 2020), while common antiviral drugs such as oseltamivir, Arbidol and Lopinavir/Ritonavir failed to cure these patients (Cao et al., 2020). As the main component of the medical practice, TCM has been used for more than 5000 years in China in treating human diseases (Li & Kan, 2017). It is a natural chemical library which leads to synergistic actions through multiple mechanisms. Modern medical research has demonstrated that polysaccharides are one of the main active ingredients of TCM (Liu, Sun, Liu, & Yu, 2012; Zheng et al., 2019). In recent decades, polysaccharides extracted from medicinal plants are attracting increasing attention due to their significant bioactivities, such as antioxidant activity, anti-viral activity and immunomodulatory activities (Xie, Jin et al., 2016). In addition, they are non-toxic and rarely show side effects, making them suitable as medicinal candidates. This review summarizes the underlying actions of TCM in view of polysaccharides. It would appear that the immunomodulatory, anti-inflammatory, anti-oxidative and regulating gut balance activities of these polysaccharides play the most important roles in the treatment of COVID-19 infected patients.

In addition to drug therapy, effort is now going toward developing novel vaccines to slow the COVID-19 pandemic. In the development of a novel vaccine, adjuvants are a vital component because they boost and accelerate the innate immune response (Xia et al., 2018). Producing vaccines with polysaccharides as the

adjuvants is an innovative strategy. In recent years, the pharmaceutical industry has made an effort to discover active ingredients from natural products for clinical use, due to their low toxicity. Polysaccharide-based vaccines have the potential to be adjuvants. For instance, Pinus massoniana pollen polysaccharide improved the effects of various vaccines, by acting as an immune adjuvant (Guo et al., 2014). Bacterial capsular polysaccharides vaccines had shown significant effects in the prevention of pneumonia and epidemic meningitis (Yu et al., 2018). Streptococcus pneumoniae polysaccharide vaccine and Haemophilus influenzae Hib polysaccharide vaccine have been successfully applied to prevent encephalitis. From a clinical view, TCM polysaccharides are very promising to act as new adjuvants in the formulations of vaccines, due to their universal immunomodulation and promotion effects, safety and biocompatibility. These data have proven the adjuvant benefits of the active polysaccharides from TCM, which may represent an attractive source of the vaccine development against COVID-19.

There is no doubt that the bioactive TCM polysaccharides are going to take important place in the fight against COVID-19 world-wide. However, each TCM prescription is a complex system and has multiple targets and links in curing disease, but that also makes it difficult to illustrate its function mechanism clearly and completely in a short time. More research on TCM polysaccharides should be undertaken to clarify the regulatory mechanisms, assess the possible side effects and conduct standard clinical trials. The insights provided in this review may help mitigate the COVID-19 pandemic.

CRediT authorship contribution statement

Peng Cao: Writing - original draft, Conceptualization. Sanlan Wu: Writing - review & editing. Tingting Wu: Visualization, Investigation. Yahui Deng: Investigation. Qilin Zhang: Investigation. Kaiping Wang: Validation. Yu Zhang: Supervision.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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COVID-19: An Update on the Epidemiological, Clinical, Preventive and Therapeutic Evidence and Guidelines of Integrative Chinese–Western Medicine for the Management of 2019 Novel Coronavirus Disease

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Abstract: As of 22 February 2020, more than 77662 cases of confirmed COVID-19 have been documented globally with over 2360 deaths. Common presentations of confirmed cases include fever, fatigue, dry cough, upper airway congestion, sputum production, shortness of breath, myalgia/arthralgia with lymphopenia, prolonged prothrombin time, elevated C-reactive protein, and elevated lactate dehydrogenase. The reported severe/critical case ratio is approximately 7–10% and median time to intensive care admission is 9.5–10.5 days with mortality of around 1–2% varied geographically. Similar to outbreaks of other newly identified virus, there is no proven regimen from conventional medicine and most reports managed the patients with lopinavir/ritonavir, ribavirin, beta-interferon, glucocorticoid and supportive treatment with remdesivir undergoing clinical trial. In China, Chinese medicine is proposed as a treatment option by national and provincial guidelines with substantial utili- zation. We reviewed the latest national and provincial clinical guidelines, retrospective co- hort studies, and case series regarding the treatment of COVID-19 by add-on Chinese medicine. We have also reviewed the clinical evidence generated from SARS and H1N1 management with hypothesized mechanisms and latest in silico findings to identify candidate Chinese medicines for the consideration of possible trials and management. Given the paucity of strongly evidence-based regimens, the available data suggest that Chinese medicine could be considered as an adjunctive therapeutic option in the management of COVID-19.

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Abstract

Background: Coronavirus disease 2019 (COVID-19) is a respiratory illness that can spread from person to person. The virus that causes COVID-19 is a novel coronavirus that was first identified during an investigation into an outbreak in Wuhan, China. The clinical spectrum of SARS-CoV-2 infection appears to be wide, encompassing asymptomatic infection, mild upper respiratory tract illness, and severe viral pneumonia with respiratory failure and even death, with many patients being hospitalised with pneumonia. In China and East Asia, Chinese medicine has been widely used to treat diverse diseases for thousands of years. As an important means of treatment now, Chinese medicine plays a significant role in the treatment of respiratory diseases in China. The aim of this study is to assess the efficacy and safety of Chinese medicine for COVID-19.

Methods: We will search the following sources for the identification of trials: The Cochrane Library, PubMed, EMBASE, Chinese Biomedical Literature Database (CBM), Chinese National Knowledge Infrastructure Database (CNKI), Chinese Science and Technique Journals Database (VIP), and the Wanfang Database. All the above databases will be searched from the available date of inception until the latest issue. No language or

publication restriction will be used. Randomized controlled trials will be included if they recruited participants with COVID-19 for assessing the effect of Chinese medicine vs control (placebo, no treatment, and other therapeutic agents). Primary outcomes will include chest CT and nucleic acid detection of respiratory samples. Two authors will independently scan the articles searched, extract the data from articles included, and assess the risk of bias by Cochrane tool of risk of bias. Disagreements will be resolved by consensus or the involvement of a third party. All analysis will be performed based on the Cochrane Handbook for Systematic Reviews of Interventions. Dichotomous variables will be reported as risk ratio or odds ratio with 95% confidence intervals (CIs) and continuous variables will be summarized as mean difference or standard mean difference with 95% CIs.

Results and conclusion: The available evidence of the treatment of COVID-19 with traditional Chinese medicine will be summarized, and evaluation of the efficacy and the adverse effects of these treatments will be made. This review will be disseminated in print by peer-review.

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Abstract

Qingfei Paidu decoction (QFPD), a multi-component herbal formula, has been widely used to treat COVID-19 in China. However, its active compounds and mechanisms of action are still unknown. Firstly, we divided QFPD into five functional units (FUs) according to the compatibility theory of traditional Chinese medicine. The corresponding common targets of the five FUs were all significantly enriched in Go Ontology (oxidoreductase activity, lipid metabolic process, homeostatic process, etc.), KEGG pathways (steroid biosynthesis, PPAR signaling pathway, adipocytokine signaling pathway, etc.), TTD diseases (chronic inflammatory diseases, asthma, chronic obstructive pulmonary Disease, etc.), miRNA (MIR183), kinase (CDK7) and TF (LXR). QFPD contained 257 specific targets in addition to HCoV, pneumonia and ACE2 co-expression proteins. Then, network topology analysis of the five components-target-pathway-disease networks yielded 67 active ingredients. In addition, ADMET estimations showed that 20 compounds passed the stringent lead-like criteria and in silico drug-likeness test with high gastrointestinal absorption and the median lethal dose (LD50 > 1600 mg/kg). Moreover, 4 specific ingredients (M3, S1, X2 and O2) and 5 common ingredients (MS1, MX16, SX1, WO1 and XO1) of QFPD presented good molecular docking score for 2019-nCov structure and non-structure proteins. Finally, drug perturbation of COVID-19 network robustness showed that all five FUs may protect COVID-19 independently, and target 8 specifically expressed drug-attacked nodes which were related to the bacterial and viral responses, immune system, signaling transduction, etc. In conclusion, our new FUNP analysis showed that QFPD had a protection effect on COVID-19 by regulating a complex molecular network with safety and efficacy. Part of the mechanism was associated with the regulation of anti-viral, anti-inflammatory activity and metabolic programming.

1. Introduction

2019-novel coronavirus (2019-nCov) outbreak took place in December 2019 and continues to spread around the world. By April 3, 2020, more than 1 million patients have been diagnosed with corona virus disease 2019 (COVID-19) [1]. The virus has a long incubation period, is highly contagious, and is generally susceptible to all types of people, which has a huge negative impact on people's health, economic development, and social stability [2]. However, there is still a lack of effective clinical drugs or vaccine to control the virus. Traditional Chinese medicine has a good effect on viral infectious pneumonia and has shown a certain effect in the treatment of SARS. On February 7, 2020, the China Health Commission and the Administration of Traditional Chinese Medicine jointly issued a notice recommending formula Qingfei Paidu decoction (QFPD, *Herba Ephedrae, Radix Glycyrrhizae, Semen Armeniacae Amarum, Gypsum Fibrosum, Ramulus Cinnamomi, Rhizoma Alismatis, Polyporus Umbellatus, Rhizoma Atractylodis Macrocephalae, Poria, Radix Bupleuri, Radix Scutellariae, Rhizome Pinelliae Preparata, Rhizoma Zingiberis Recens, Radix Asteris, Flos*

Farfarae, Rhizoma Belamcandae, Herba Asari, Rhizoma Dioscoreae, Fructus Aurantii Immaturus, Pericarpium Citri Reticulatae, Herba Pogostemonis) for the treatment of COVID-19 according to clinical treatment and efficacy. QFPD is a compound prescription in TCM including Ma Xing Shi Gan decoction (MSXG), She Gan Ma Huang decoction (SGMH), Xiao Chai Hu (XCH), and Wu Ling San (WLS), which was first discovered in the classic Treatise on Exogenous Febrile Disease (Shanghan Lun). MXSG (*Herba Ephedrae, Radix Glycyrrhizae, Semen Armeniacae Amarum, Gypsum Fibrosum*) has been used for the treatment of the common cold, fever, and influenza virus infections via damaging the viral surface structure and inhibiting viral entry [3]. SGMH (*Herba Ephedrae, Rhizome Pinelliae Preparata, Rhizoma Zingiberis Recens, Radix Asteris, Flos Farfarae, Rhizoma Belamcandae, Herba Asari*) is a classical prescription for the treatment of flu-like symptoms, asthma, inflammation, tonsillitis and sore throat [4]. XCH (*Radix Glycyrrhizae, Radix Bupleuri, Radix Scutellariae, Rhizome Pinelliae Preparata, Rhizoma Zingiberis Recens*) possesses antiviral [5] and various anticarcinogenic properties [6]. WLS (*Ramulus Cinnamomi, Rhizoma Alismatis, Polyporus Umbellatus, Rhizoma Atractylodis Macrocephalae, Poria*), a famous Chinese prescription for nephritic syndrome, can improve kidney excretion function and inhibit inflammatory response [7]. These researches indicate that MXSG, SGMH, XCH and WLS may be functional units of formula QFPD. Previous studies have focused on the mechanism of compound prescription based on a single traditional Chinese medicine. However, it may not reflect functional compatibility mechanism of traditional Chinese medicine. Therefore, it is worthy of comparing the similarities and differences of different QFPD functional units in the treatment of COVID-19, including MXSG, SGMH, XCH, WLS and Others.

QFPD contains a total of 21 traditional Chinese medicines, and it is difficult to elucidate the complex mechanism of QFPD on COVID-19 by traditional pharmacological methods due to the multi-components and multi-targets of the formula. Network pharmacology, a new method in recent years, can integrate interactions of drugs, targets, pathways and diseases into a biological network system [8]. Therefore, more and more TCM researchers have begun to use network pharmacology to explore the material basis of TCM, and to reveal the overall comprehensive effects of multi-path, multi-component and multi-target of TCM prescription and its treatment of diseases [9,10]. More importantly, previous study reported that disease conditions can be more fragile than health systems against various perturbations for the un-optimized system [11]. So the formula may be more effective for COVID-19 disease via the stronger effects on the reduction of the robustness of the COVID-19 disease network [12].

In our study, since MSXG, SGMH, XCH and WLS have been independently used for the treatment of viral infectious pneumonia, this study firstly screened out major effective compounds from five functional units respectively. Then we offered a new understanding of the functional units mechanism of QFPD against COVID-19 by a novel functional units of network pharmacology (FUNP) approach and formula perturbation analysis, and provided a combination strategy to explore mechanisms of inter-ingredients interactions from a holistic perspective.

2. Materials and methods

2.1. Data preparation

Compounds of the main herb in formula MSXG, SGMH, XCH, WLS and Others were searched in TCMSP [13], and screened based on drug-likeness (DL) ≥0.18 [14] and oral bioavailability (OB) ≥30 % [15]. Then, the corresponding Pubchem CIDs of the compounds were retrieved from the Pubchem database [16]. Finally, BATMAN-TCM [17], an bioinformatics analysis tool for studying TCM's molecular mechanisms, was used to identify potential target genes of the active components (uploaded by Pubchem CIDs). To make the results more credible, we set the cutoff score ≥ 30 as the standard. Finally, to discovery the co-differentially presented targets in the five formulae, we conducted pan-formula analysis using Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/).

2.2. Functional and pathway enrichment analyses of QFPD targets

To better understand the functional involvements of MSXG, SGMH, XCH, WLS and Others targets, bioinformatics analyses of multiple formulae targets were first performed, including Gene Ontology (GO) function term, KEGG biological pathway and OMIM/TTD disease enrichment analyses. Then, kinase, microRNA and transcriptional factor (TF) enrichment analyses of the five formulae targets were conducted using the tool WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt) [18] and the bubble and chord plot

map were drawn with the R language ggplot2 and GOplot installation package. P-values were adjusted for multiple testing by Benjamini-Hochberg adjustment.

2.3. Construction of PPI network and MCODE modules analysis

To further explore the pharmacological mechanisms, five PPI networks were built including: MSXG, SGMH, XCH, WLS and Others targets PPI network. Specifically, the five kinds of target proteins were respectively uploaded to Metascape to build PPI networks, with the species limited to "Homo sapiens". Next, MCODE analysis [19], a method for finding densely connected modules in PPI networks, was carried out by Cytoscape 3.2.1 (http://www.cytoscape.org/) [20]. Finally, KEGG (Kyoto Encyclopedia of Genes and Genomes) signaling pathway enrichment analysis was further conducted on the identified functional modules of MSXG, SGMH, XCH, WLS and Others targets PPI networks, respectively.

2.4. Network construction

Based on the five formulae's active components, BATMAN-TCM was used to set up five networks of components-target-pathway-disease (MSXG, SGMH, XCH, WLS and Others). To emphasize the important elements of the five networks, we only exhibited the hub targets according to the default criteria (targets with no fewer than 6, 5, 8, 7 and 4 linking compounds for MSXG, SGMH, XCH, WLS and Others, respectively). Finally, these important linking compounds of MSXG, SGMH, XCH, WLS and Others networks were obtained for further analysis.

2.5. ADMET evaluation of the predicted active compounds

Based on the SwissADME database [21], the physicochemical properties of the active components was predicted, including molecular weight (MW), rotatable bonds count, H-bond acceptors and donors count, TPSA and leadlikeness violations. Second, pharmacokinetic properties was predicted through pkCSM database [22], which contained the absorption (Caco-2 cell permeability, HIA and skin permeability), distribution (VDss, unbound fraction, blood-brain barrier and central nervous system permeability), excretion (total clearance and renal OCT2 substrate) and toxicity (AMES toxicity, maximum tolerated dose, hERG I inhibitor, hERG II inhibitor, oral rat acute toxicity (LD50), hepatotoxicity, skin sensitisation, and minnow toxicity).

2.6. Molecular docking

To facilitate drug discovery against COVID-19, we used COVID-19 Docking Server (https://ncov.schanglab.org.cn/index.php) [23] to predict the binding modes between 12 COVID-19 targets and the 20 lead-likeness of QFPD. Specifically, the 10 nonstructural and 2 structural proteins of 2019-nCov were collected (Mpro, PLpro, nsp12 [RdRp with RNA], nsp12 [RdRp without RNA], nsp13 [Helicase ADP site], nsp13 [Helicase NCB site], nsp14 [ExoN], nsp14 [N7-MTase], nsp15 [endoribonuclease], nsp16 [2'-O-MTase], N protein NCB site and E protein [ion channel]); and the corresponding Protein Data Bank (PDB)codes were 6LU7, 4OW0, 3H5Y (with RNA), 3H5Y (without RNA), 6JYT (ADP site), 6JYT (NCB site), 5C8S (ExoN),5C8S (N7-MTase), 2RHB, 2XYR, 4KYJ, and 5 × 29, respectively. Finally, Discovery Studio software elucidated the 14 best docking results between compounds and the COVID-19 target proteins.

2.7. ACE2 and CD147 expression across tissues and co-expression genes

To understand the expression and distribution of ACE2 and CD147 across tissues, a radar plot including 53 tissues was performed through COXPRESdb [24]. And the top 200 co-expression genes of ACE2 and CD147 ($P < 1E-16$) were obtained, respectively. Then, text mining method from the literature was used to screen for pneumonia-associated genes through COREMINE (http://www.coremine.com/). In addition, co-expression genes of ACE2 in colonic epithelial cells [25] and HCoV-associated host proteins with references [26] were obtained. Finally, we performed UpsetView analysis (http://www.ehbio.com/ImageGP/) between these five sets of proteins and QFPD targets.

2.8. Validation of drug positioning for QFPD against COVID-19 via the robustness of disease network

Since QFPD effects on COVID-19 via multi-component and multi-target, we evaluate the potential efficacy of QFPD through TCMATCOV platform, which uses the quantitative evaluation algorithm of multi-target drugs to disturb the disease network. Specifically, the disturbing effect of drugs on diseases is simulated by deleting disease network nodes. The disturbance rate of drugs is calculated by comparing the changes of network topology characteristics before and after drug intervention, which is used to evaluate the intervention effect of drugs on diseases. Firstly, COVID-19 disease network was constructed based on

specific cytokines of COVID-19 [27] and differentially expressed genes of SARS (GSE36969, GSE51387, GSE68820). Then, this platform uses four kinds of network topology characteristics to evaluate the robustness of COVID-19 network, including network average connectivity, network average shortest path, connectivity centrality and compactness centrality. And the five formulae (MSXG, SGMH, XCH, WLS and Others) disturbance scores are calculated according to the changes before and after drug intervention. Finally, the disturbance effect of the five formulae on the COVID-19 network was compared with null models with the total score of the disturbance, and the higher the value is, the higher the damage degree of drugs to the stability of the network is [12]. We take Banxia tianma baizhu decoction (BXTM) as negative control; and another efficient formula Yi du bi fei decoction (YDBF) as positive control.

3. Result

3.1. Prediction of active components and potential targets of QFPD

Firstly, the DL ≥ 0.18 and OB ≥ 30 %.s were set as the standard to screen the chemical components obtained through online database TCMSP. Specifically, a total of 175 effective components of QFPD were screened from the TCM database, including 82 species of MSXG, 35 species of SGMH, 105 species of XCH, 21 species of WLS and 32 species of Others (Table 1). Among these effective components, 89 (50.86 %) components exited in more than two formulae; CID5280343 and CID5280794 were owned by MXSG, Others, SGMH and XCH; CID12303645 was owned by MXSG, Others, WLS and XCH (Fig. 1A). Secondly, a total of 300 targets of QFPD were screened from the BATMAN-TCM database, including 192 targets of MSXG, 201 targets of SGMH, 221 targets of XCH, 96 targets of WLS and 99 targets of Others. Among these proteins, 21 (7%) targets exited in five formulae (Fig. 1B).

Table 1. Effective components of QFPD.

Formula	N	PubChem_Cid
MSXG	82	CID10090416,CID10542808,CID10881804,CID11267805,CID114829,CID11558452,CID11602329,CID11975273,CID120074,CID12303645,CID124049,CID124052,CID13965473,CID14604077,CID14604078,CID14604081,CID15228663,CID15380912,CID162412,CID177149,CID193679,CID197678,CID23724664,CID25015742,CID268208,CID336327,CID354368,CID3764,CID439246,CID440833,CID442411,CID44257530,CID480774,CID480780,CID480787,CID480859,CID480873,CID49856081,CID503731,CID503737,CID5280343,CID5280378,CID5280448,CID5280544,CID5280794,CID5280863,CID5281619,CID5281654,CID5281789,CID5282768,CID5282805,CID5312521,CID5316900,CID5317300,CID5317478,CID5317479,CID5317480,CID5317652,CID5317768,CID5317777,CID5318437,CID5318585,CID5318679,CID5318869,CID5318998,CID5318999,CID5319013,CID5320083,CID5460988,CID5481234,CID5481948,CID5481949,CID5997,CID636883,CID637112,CID64971,CID6918970,CID73205,CID928837,CID9927807,CID15840593,CID15228662
SGMH	35	CID1135,CID1174,CID3026,CID5789,CID6782,CID6998,CID8437,CID8679,CID13625,CID117158,CID159225,CID185,CID10019512,CID11438306,CID11869417,CID11870462,CID12315507,CID162350,CID16726037,CID222284,CID389888,CID3902,CID440833,CID5280343,CID5280445,CID5280544,CID5280794,CID5280863,CID5281331,CID5281605,CID5281616,CID5281628,CID5281654,CID5281779,CID5282768,CID5315890,CID5316876,CID5320945,CID5484202,CID5488781,CID5491637,CID64959,CID64982,CID676152,CID71307581,CID72307,CID13688752
XCH	105	CID10090416,CID10542808,CID10881804,CID11267805,CID11438306,CID114829,CID11558452,CID11602329,CID117443,CID12303645,CID124049,CID124052,CID124211,CID13965473,CID14135323,CID14604077,CID14604078,CID14604081,CID15228662,CID15228663,CID15380912,CID156992,CID158311,CID15840593,CID159029,CID161271,CID162412,CID177149,CID185034,CID193679,CID197678,CID222284,CID23724664,CID25015742,CID25721350,CID268208,CID336327,CID354368,CID373261,CID3764,CID389001,CID389888,CID439246,CID442411,CID44257530,CID44258628,CID480774,CID480780,CID480787,CID480859,CID480873,CID49856081,CID503731,CID503737,CID5280343,CID5280378,CID5280442,CID5280448,CID5280794,CID5280863,CID5281605,CID5281619,CID5281654,CID5281674,CID5281703,CID5281789,CID5282768,CID5312521,CID5316900,CID5317300,CID5317478,CID5317479,CID5317480,CID5317652,CID5317768,CID5317777,CID5318437,CID5318585,CID5318679,CID5318869,CID5318998,CID5318999,CID5319013,CID5319042,CID5319252,CID5320083,CID5320315,CID5320399,CID5321865,CID5322059,CID5322078,CID5460988,CID5481234,CID5481948,CID5481949,CID5484202,CID636883,CID637112,CID64959,CID64971,CID64982,CID73205,CID821279,CID928837,CID9927807
WLS	21	CID10181133,CID10743008,CID12303645,CID14036811,CID15225964,CID15226717,CID15976101,CID182232,CID222284,CID44575602,CID5283628,CID5471851,CID5471852,CID56668247,CID6436630,CID712316,CID73402,CID9064,CID9805290,CID14448075,CID14236575
Others	32	CID10212,CID11824478,CID122159,CID12303645,CID14057197,CID145659,CID17897,CID33934,CID373261,CID40429858,CID42607889,CID439246,CID442834,CID443024,CID5280343,CID5280445,CID5280794,CID5281326,CID5281617,CID5281781,CID5319406,CID5320621,CID5495928,CID5997,CID631170,CID632135,CID676152,CID712316,CID72344,CID79730,CID1149877,CID45359875

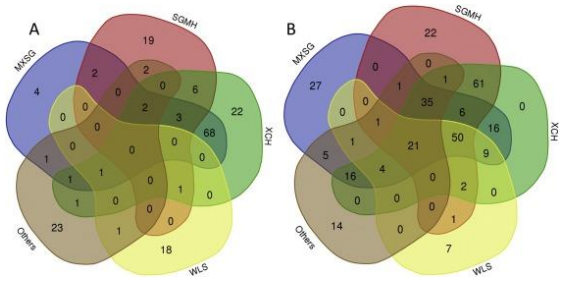


Fig. 1. Venn diagram of the five formulae' active compounds and targets. A: compounds, B: targets.

3.2. Functional and pathway enrichment analyses of QFPD targets

As shown in Fig. 2, the 11 enriched GO terms of the targets in all five formulae were found, such as oxidoreductase activity, lipid metabolic process, lipid binding, small molecule metabolic process, homeostatic process, signal transducer activity and cell proliferation. Furthermore, the results of pathway enrichment analysis showed that the 7 KEGG pathways were significantly related to more than 4 formula groups, including dteroid biosynthesis, adipocytokine signaling pathway, neuroactive ligand-receptor interaction, steroid hormone biosynthesis, PPAR signaling pathway, arginine and proline metabolism and ABC transporters (Fig. 3A). In addition, TTD analysis showed that the 8 diseases were significantly association with more than 3 formula groups, such as acne, Behcet'S disease, benign prostate hyperplasia, intrahepatic cholestasis and brain injury (Fig. 3B). However, the five formulae contained their specific (MSXG, SGMH, XCH, WLS and Others) GO, KEGG and TTD terms. For example, neurological system process and beta-Alanine metabolism terms were specific for MXSG; membrane organization and parasitic infections of the eye terms for SGMH; circadian rhythm and hypertension terms for XCH; nucleic acid binding transcription factor activity, ovarian steroidogenesis and chronic inflammatory diseases terms for WLS; fat digestion and absorption terms for Others.

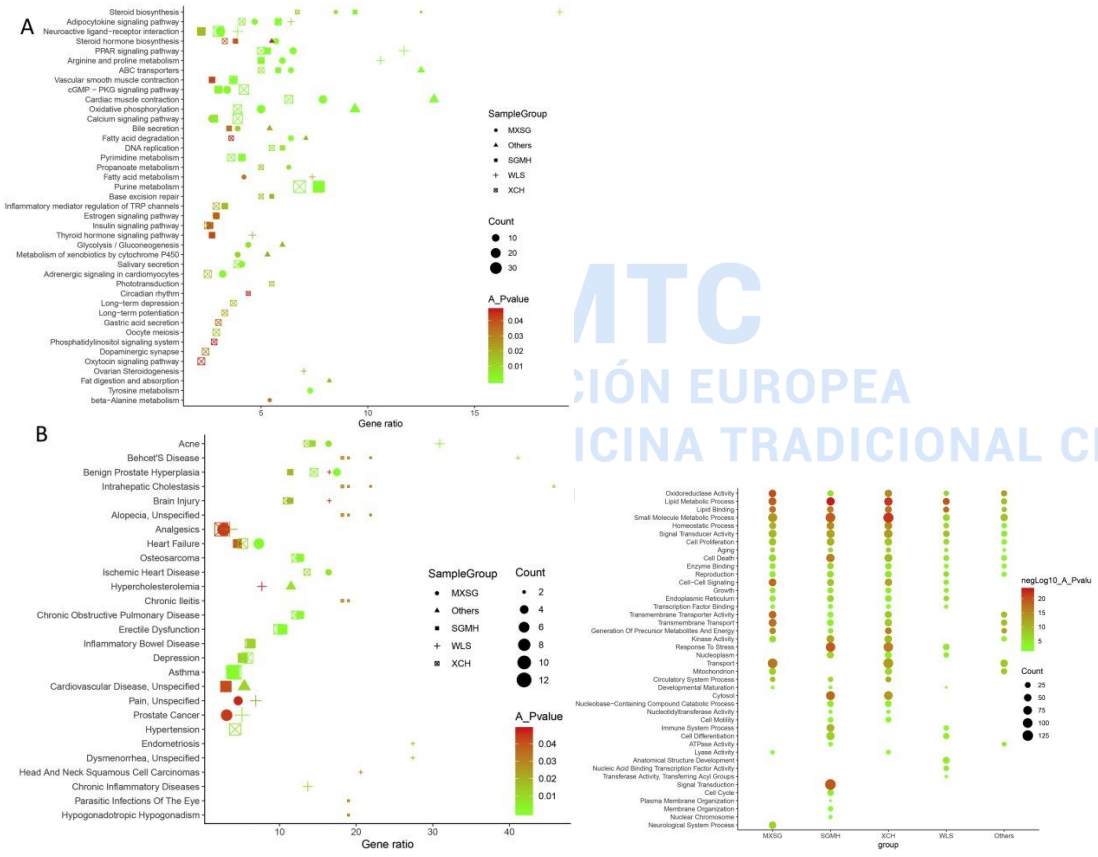


Fig. 2. Bubble plot of the GO analysis of the five formulae' targets.

Fig. 3. Bubble plot of the KEGG/TTD analysis of the five formulae' targets. A: KEGG, B: TTD.

In the prediction of miRNAs in QFPD targets, MIR-183 and MIR-130A/B/301 were the highest linking terms to bind the five formulae targets and formulae Others was the highest group to bind miRNAs (Fig. 4A). In addition, kinase prediction revealed CDK7 were significantly enriched in formulae MSXG, SGMH, XCH, WLS and Others (Fig. 4B). Finally, TF analysis showed that LXR was the highest linking TF to bind the four formulae targets and formulae WLS was the highest group to bind TFs (Fig. 4C).

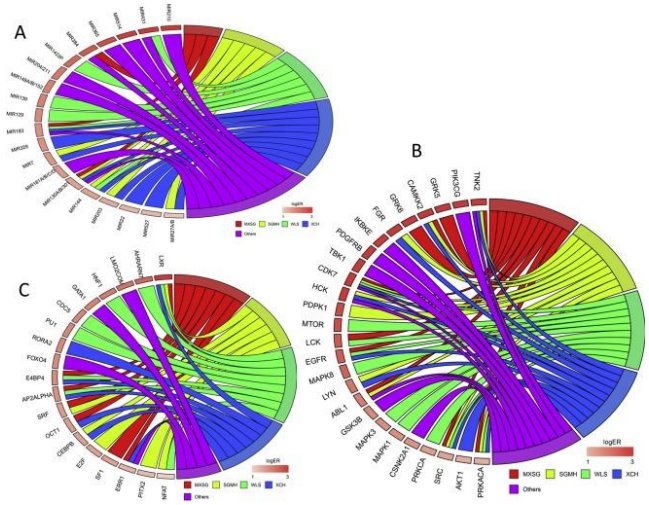


Fig. 4. The miRNA, kinase and TF analysis of the five formulae' targets by WebGestalt. Chord plot showing the five formulae' targets present in the represented enriched miRNA, kinase and TF terms. Outer ring shows miRNA/kinase/TF term and log2 enrichment ratio (left) or five formulae grouping (right). Chords connect miRNA/kinase/TF term with formulae groups. A: miRNA, B: kinase, C: TF.

3.3. Construction of PPI network and MCODE modules analysis

To further explore the functional relationship among five formulae, PPI networks were constructed through Metascape, and visual composition carried out by Cytoscape. Firstly, the potential 192 target genes of MXSG were analyzed by PPI network, and the results showed that there were 144 nodes and 510 edges, which represented the interaction between protein and function. The MXSG PPI network function module was confirmed by the MCODE plug-in and a list of the corresponding meaningful modules presented (Fig. 5A). 3 modules scores were > 2.5. Module 1 (score: 5.769) consisted of 13 nodes and the seed gene was COX7A1; Module 2 (score: 4.429) consisted of 14 nodes and the seed gene was ALDH1A1; module 3 (score: 5.0) consisted of 11 nodes and the seed gene was CNR2. KEGG pathway enrichment analysis showed that MXSG modules were enriched in neuroactive ligand-receptor interaction, calcium signaling pathway, inflammatory mediator regulation of TRP channels, et.al.

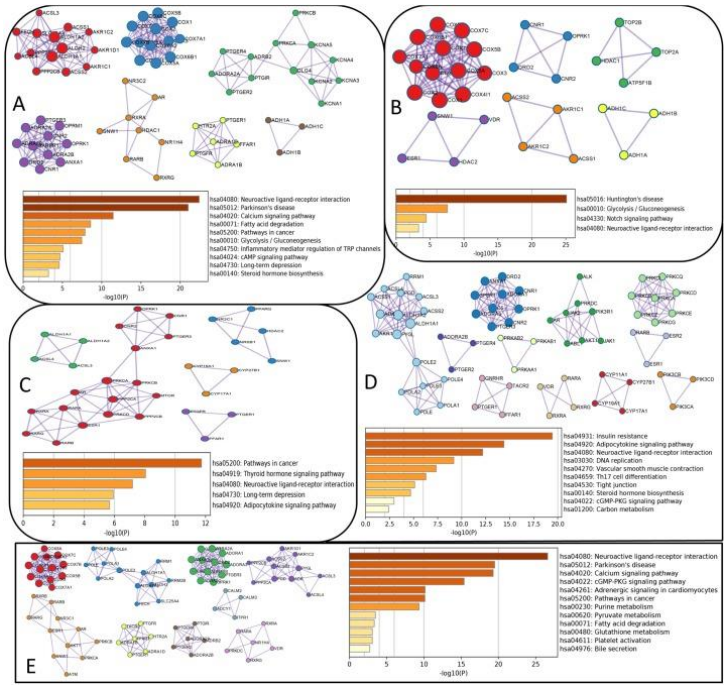


Fig. 5. KEGG analysis of MCODE modules. MCODE analysis was performed after the construction of the five formulae' targets PPI; then, KEGG analysis was conducted on the MCODE modules. A: MXSG, B: Others, C: WLS, D: SGMH, E: XCH.

Secondly, the potential 99 target genes of Others were analyzed by PPI network, and the results showed that there were 77 nodes and 194 edges. Only 1 module score were > 2.5 (Fig. 5B). Module 1 (score: 5.769) consisted of 13 nodes and the seed gene was COX7A1. KEGG pathway enrichment analysis showed that Others modules were enriched in huntington's disease, glycolysis / gluconeogenesis, Notch signaling pathway, et.al.

Thirdly, the potential 96 target genes of WLS were analyzed by PPI network, and the results showed that there were 60 nodes and 143 edges. Only 1 modules score were > 2.5 (Fig. 5C). Module 1 (score: 2.706) consisted of 17 nodes and the seed gene was CNR2. KEGG pathway enrichment analysis showed that WLS modules were enriched in thyroid hormone signaling pathway, adipocytokine signaling pathway, neuroactive ligand-receptor interaction, et.al.

Fourthly, the potential 201 target genes of SGMH were analyzed by PPI network, and the results showed that there were 153 nodes and 505 edges. 3 modules scores were > 2.5 (Fig. 5D). Module 1 (score: 3.529) consisted of 17 nodes and the seed gene was ACSS1; Module 2 (score: 4.5) consisted of 7 nodes and the seed gene was CNR2; module 3 (score: 3.5) consisted of 8 nodes and the seed gene was PRKCG. KEGG pathway enrichment analysis showed that SGMH modules were enriched in insulin resistance, adipocytokine signaling pathway, Th17 cell differentiation, et.al.

At last, the potential 221 target genes of XCH were analyzed by PPI network, and the results showed that there were 166 nodes and 643 edges. 5 modules scores were > 2.5 (Fig. 5E). Module 1 (score: 5.769) consisted of 13 nodes and the seed gene was COX7A1; Module 2 (score: 2.769) consisted of 13 nodes and the seed gene was RRM1; module 3 (score: 5.5) consisted of 12 nodes and the seed gene was CNR2; module 4 (score: 2.909) consisted of 11 nodes and the seed gene was ACSS1; module 5 (score: 3.0) consisted of 7 nodes and the seed gene was FFAR1. KEGG pathway enrichment analysis showed that XCH modules were enriched in calcium signaling pathway, cGMP-PKG signaling pathway, neuroactive ligand-receptor interaction, et.al.

3.4. Network construction

After using the BATMAN-TCM, we constructed five ingredients-target-pathway-disease networks, including MSXG, SGMH, XCH, WLS and Others. In order to emphasize the important network elements, we showed the networks that exhibit those targets with larger than 6, 5, 8, 7 and 4 linking compounds for MSXG, SGMH, XCH, WLS and Others, respectively (Fig. 6). MSXG network contained 31 key components, 50 proteins and 17 pathways; SGMH network contained 15 key components, 20 proteins and 8 pathways; WLS network contained 18 key components, 9 proteins and 4 pathways; XCH network contained 32 key components, 15

proteins and 12 pathways; Others network contained 10 key components, 13 proteins and 3 pathways. To find the potential drugs of formulae QFPD for COVID-19, a total of 67 hub components were used for ADMET analysis.

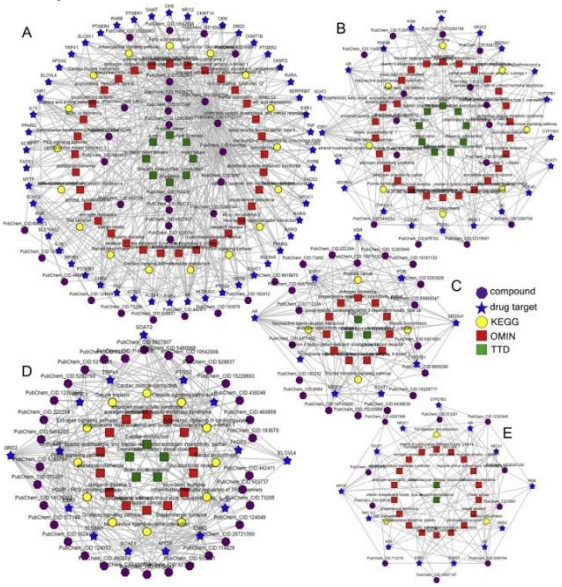
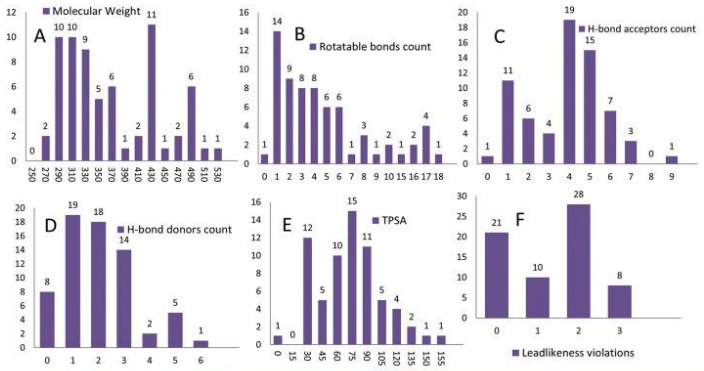


Fig. 6. The component-target-pathway-disease network. Purple polygons: PubChem ID of QFPD compounds; blue pentagrams: QFPD targets; yellow circles: KEGG pathway; red square: Therapeutic Target Database (TTD) disease term, green square: Online Mendelian Inheritance in Man (OMIN) disease term. A: MXSG, B: SGMH, C: WLS, D: XCH, E: Others. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. ADMET evaluation of the 67 key compounds

Since in silico ADMET prediction can help early drug design and evaluation, ADMET properties of the 67 key compounds were predicted by SwissADME and pkCSM. Chemical properties including molecular weight (MW), rotatable bonds count, H-bond acceptors and donors count, TPSA and leadlikeness violations were calculated by SwissADME and shown as Fig. 8A. It is worth mentioning that 21 (31.34 %) compounds passed the stringent lead-like criteria (250 g/mol ≤ MW ≤ 350 g/mol, XLOGP ≤ 3.5 and rotatable bonds ≤ 7), which are excellent candidates for drug discovery (Fig. 7A). And these lead-likeness compounds were further predicted by pkCSM, with the exception of S3 (low gastrointestinal absorption)



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	Absorption			Distribution				Excretion		Toxicity							
	Caco2	HIA	Skin	VDss	FU	BBB	CNS	TC	OCT2	AMES	MTDD	hERG I	hERG II	LD50	HT	SS	MT
M3	1.78	96.42	-2.62	0.65	0.05	-0.04	-1.74	0.80	No	No	-0.25	No	Yes	2.44	No	No	0.09
M5	1.25	95.19	-2.82	0.07	0.04	0.32	-1.84	0.27	No	Yes	-0.10	No	No	2.51	No	No	0.66
S1	0.88	99.83	-2.49	-0.03	0.16	-0.39	-2.05	0.77	Yes	No	-0.59	No	No	2.09	No	No	1.85
S5	1.28	95.96	-2.82	-0.15	0.05	-0.50	-3.04	0.17	No	No	0.24	No	No	2.47	No	No	1.06
W5	-0.28	68.83	-2.74	1.03	0.24	-1.05	-3.30	0.18	No	No	0.44	No	No	2.43	No	No	3.59
W11	-0.28	68.83	-2.74	1.03	0.24	-1.05	-3.30	0.18	No	No	0.44	No	No	2.43	No	No	3.59
X1	1.20	93.08	-2.84	0.32	0.12	-0.05	-2.22	-0.02	No	No	-0.62	No	No	2.17	No	No	1.29
X2	1.08	94.97	-2.74	0.01	0.10	-0.97	-2.31	0.03	No	No	-0.10	No	No	2.28	No	No	2.48
X4	1.30	93.90	-2.82	-0.07	0.03	0.07	-2.04	0.19	No	Yes	0.14	No	No	2.17	No	No	0.35
O1	1.08	93.54	-2.80	-0.26	0.04	-0.09	-2.16	0.17	No	Yes	0.79	No	No	2.19	No	No	1.08
O2	0.32	88.41	-2.74	0.79	0.13	-0.77	-3.07	0.48	No	No	-0.03	No	No	1.88	No	No	3.09
XO1	-0.09	74.69	-2.74	0.38	0.11	-0.83	-3.14	-0.01	No	No	0.01	No	No	2.03	No	No	2.97
MS1	-0.25	56.71	-2.74	1.82	0.43	-0.91	-3.21	-0.07	No	No	0.45	No	No	2.39	No	No	5.18
MX16	1.16	92.26	-2.75	0.31	0.03	-0.10	-2.12	0.27	No	Yes	0.15	No	No	2.08	No	No	1.31
MX17	1.14	94.33	-2.77	0.22	0.10	0.38	-2.07	0.07	No	No	-0.35	No	No	2.37	No	No	1.21
MX8	1.14	94.33	-2.77	0.22	0.10	0.38	-2.07	0.07	No	No	-0.35	No	No	2.37	No	No	1.21
MXO1	1.03	91.31	-2.74	-0.02	0.06	-0.58	-2.22	0.06	No	No	-0.18	No	No	1.79	No	No	2.14
SO1	0.29	70.28	-2.74	0.75	0.12	-0.72	-2.98	0.04	No	No	0.25	No	No	2.04	No	No	2.31
SX1	0.11	56.45	-3.14	0.03	0.12	-0.20	-2.57	0.29	No	No	-0.40	No	Yes	1.62	No	No	2.00
WO1	0.92	64.71	-2.74	1.64	0.32	-0.73	-3.20	-0.08	No	No	0.35	No	No	2.26	No	No	4.69

Fig. 7. Chemical properties statistics of hub components in the formulae. A: Molecular weight, B: rotatable bond count, C: H-bond acceptors count, D: H-bond donors count, E: topological polar surface area (TPSA), F: leadlikeness violations, G: pharmacokinetic and toxicity evaluated parameters of 20 leadlikeness compounds by pkCSM; green = good, yellow = tolerable, red = bad. Caco2: Caco-2 Permeability,HIA: Intestinal Absorption (Human), Skin: Skin Permeability, VDss: volume of distribution, FU: Fraction Unbound (Human), BBB: Blood Brain Barrier permeability, CNS: Central Nervous System permeability,TC: Total Clearance, OCT2: Renal Organic Cation Transporter 2, AMES: AMES toxicity, MTDD: Maximum Tolerated Dose (Human), hERG I/II: hERG I and II Inhibitors, LD50: Oral Rat Acute Toxicity (LD50), HT: Hepatotoxicity, SS: Skin Sensitisation, MT: Minnow toxicity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

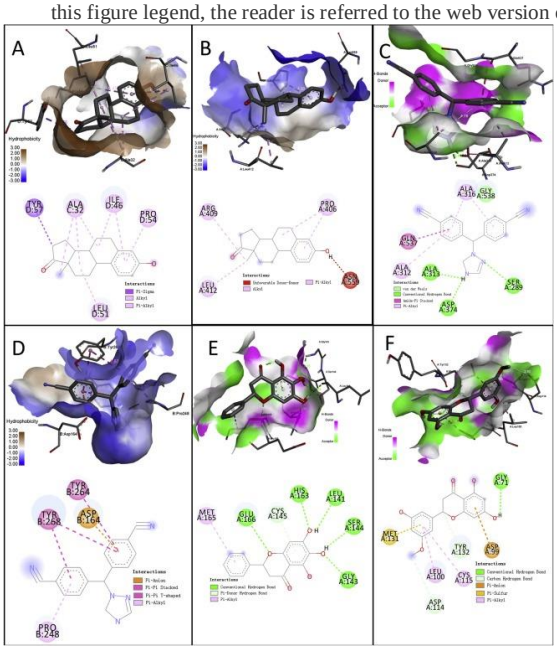

Fig. 8. Schematic (3D and 2D) representation that molecular model of specific compounds of each formulae with COVID-19 proteins. A: M3 and E protein [ion channel], B: M3 and nsp13 [Helicase NCB site], C: S1 and nsp13 [Helicase ADP site], D: S1 and PLpro, E: X2 and Mpro, F: O2 and Mpro. M: MXSG, S: SGMH, X: XCH, O: Others.

Regarding the absorption parameters, all 20 compounds (Table 2) presented a promising oral availability including the optimal Caco-2 cell permeability, HIA and skin permeability. The drug distribution results showed that most of the compounds distributed in tissue (VDss> 0.45: tissue, VDss <−0.15: plasma) with good unbound fraction scores, thus becoming available to interact with the pharmacological target. Only compound W5 and W11 were entirely unable to penetrate the blood-brain barrier (BBB) and central nervous system (CNS). In addition, 15 compounds presented a good renal elimination and were not substrates of the renal organic cation transporter 2 (OCT2). Finally, 14 compounds did not present any particular toxicity problems including AMES toxicity, maximum tolerated dose, hERG I inhibitor, hERG II inhibitor, oral rat acute toxicity (LD50), hepatotoxicity, skin sensitisation, and minnow toxicity (Fig. 7B).

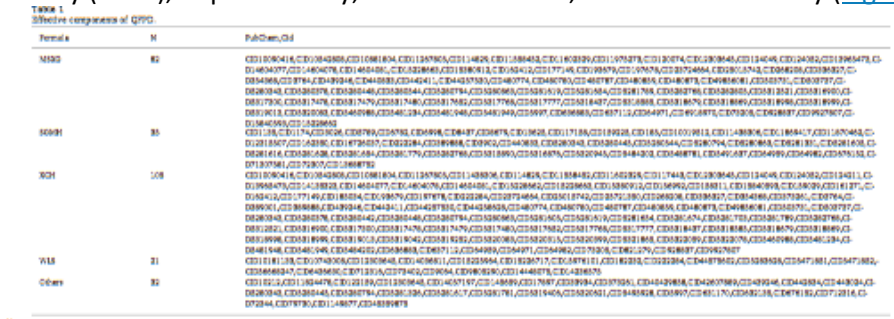

Table 2
20 potential active compounds from QFPD.

Pubchem	Molecular	Name	Structure	Pubchem	Molecular	Name	Structure
CID6918970	M3	ZINC5356864		CID10019512	S5	3-O-Methylviolanone	
CID336327	M5	Medicarpin		CID9064	W5	Cianidanol	
CID14057197	O1	–		CID182232	W11	(+)-Epicatechin	
CID42607889	O2	Alysifolinone		CID25721350	X1	ZINC13130930	
CID3902	S1	letrozole		CID14135323	X2	(2S)-dihydrobaicalein	
CID821279	X4	ZINC338038		CID439246	MXO1	naringenin	
CID440833	MS1	Leucocyanidol		CID676152	SO1	SR-01,000,767,148	
CID177149	MX16	(+)-Vestitol		CID11438306	SX1	cyclo(L-Tyr-L-Phe)	
CID114829	MX17	Liquiritigenin		CID712316	WO1	(-)-taxifolin	
CID928837	MX8	ZINC519174		CID373261	XO1	Eriodyctiol (flavanone)	

M: MXSG, S: SGMH, X: XCH, O: Others.

3.6. Molecular docking

The application of COVID-19 docking server and Discovery Studio software elucidated the interactions between the 20 lead-likeness compounds (S1, W5, MX17, MX16, W11, M5, XO1, MXO1, SO1, WO1, X4, MX8, M3, S5, SX1, O1, X2, X1, O2, MS1) and the 10 nonstructural and 2 structural proteins (Mpro, PLpro, nsp12 [RdRp with RNA], nsp12 [RdRp without RNA], nsp13 [Helicase ADP site], nsp13 [Helicase NCB site], nsp14 [ExoN], nsp14 [N7-MTase], nsp15 [endoribonuclease], nsp16 [2′-O-MTase], N protein NCB site, E protein [ion channel]). The docking scores were depicted in Table 3, Table 4. The smaller of docking score, the lower of energy would be required, which means the binding between the compounds and the targets are stronger. There are 9 compounds presenting better bonding ability than other compounds.

Table 3
Docking score between specific ingredients of QFPD and 2019-nCov proteins.

Molecule	M3	M5	S1	S5	W11	W5	X1	X2	X4	O1	O2
Main Protease	−7.7	−7.3	−6.8	−6.9	−7.2	−7.5	−7.1	−7.9	−7.3	−7.1	−7.4
Papain-like protease	−8.7	−8.5	−9.9	−7.7	−8	−8	−8.4	−8.7	−8.2	−8.6	−8.2
RdRp with RNA	−8.4	−8.5	−8.5	−8.3	−9.1	−9.1	−8.4	−8.2	−8.2	−8.3	−8.6
RdRp without RNA	−6.8	−6.8	−6.9	−6.8	−6.7	−6.9	−7.1	−7.2	−6.5	−7.3	−7.1
Helicase ADP site	−6.3	−6.3	−7.3	−6.5	−6	−6	−6.3	−6.5	−6.2	−6.1	−6.5
Helicase NCB site	−7.9	−6.9	−7.2	−7.1	−7.2	−7.2	−7.4	−7.5	−7.3	−7.4	−7.4
Nsp14(ExoN)	−6.8	−6.6	−6.3	−6.4	−6.9	−6.8	−6.7	−6.9	−6.4	−6.6	−6.9
Nsp14(N7-MTase)	−8.8	−8.1	−8.5	−7.5	−8.4	−8.4	−8.3	−8.5	−8	−8.3	−8.5
Nsp15(endoribonuclease)	−6.6	−6.3	−6.3	−5.9	−6.2	−6.2	−6.2	−6.3	−6.2	−6.4	−6.4
Nsp16(2′-O-MTase)	−7.5	−7.4	−7.5	−7.2	−8.2	−8.2	−7.7	−7.9	−7.7	−7.9	−8.4
N protein NCB site	−7.6	−7.5	−7.8	−7.6	−7.6	−7.6	−8	−8	−7.5	−7.6	−7.6
E protein(ion channel)	−8.1	−7	−7.8	−6.7	−6.4	−6.4	−7.2	−7.3	−7.2	−7.1	−6.8

M: MXSG, S: SGMH, X: XCH, O: Others.

Table 4
Evaluation of the effect of QFPD on the robustness disturbance of COVID-19 network.

Topology	MXSG	SGMH	XCH	WLS	Others	BXTM	YDBF
DTS	25.66	26.71	21.02	17.64	23.16	14.52	22.71
AC	−4.63	−5.21	−3.02	−3.49	−5.38	−2.32	−3.78
APL	13.35	13.40	9.96	6.49	10.66	4.59	11.17
CoC	−1.44	−1.64	−1.59	−1.30	−1.23	−1.15	−1.25
CIC	−6.24	−6.46	−6.45	−6.36	−5.88	−6.46	−6.52

M3 (Fig. 8A), a specific compound in formulae MXSG, showed eight interactions with E protein [ion channel] including Pi-sigma, Pi-alkyl and Alkyl, which were connected with TYR 57, ALA 32, ILE 46 and PRO 54, etc.; additionally, M3 (Fig. 8B) showed five interactions with nsp13 [Helicase NCB site] including Unfavorable Donor-Donor, Pi-alkyl and Alkyl, which were connected with ASN 559, ARG 409, LEU 42 and PRO 406. S1 (Fig. 8C), a specific compound in formulae SGMH, showed seven interactions with nsp13 [Helicase ADP site] including H-bond interactions, van der waals, Amide-Pi stacked and Pi-alkyl, which were connected with ALA 313, ASP 374, GLN 537 and SER 289, etc.; additionally, S1 (Fig. 8D) showed five interactions with PLpro including Pi-anion, Pi-Pi stacked, Pi-Pi T-shaped and Pi-alkyl, which were connected with TYR 264, ASP 164, TYR 268 and PRO 248. X2 (Fig. 8E), a specific compound in formulae XCH, showed seven interactions with Mpro including H-bond interactions, Pi-Donor hydrogen bond and Pi-alkyl, which were connected with MET 165, GLU 166, LEU 141 and CYS 145, etc. O2 (Fig. 8F), a specific compound in formulae Others, showed seven interactions with Mpro including H-bond interactions, Carbon hydrogen bond, Pi-anion, Pi-sulfur and Pi-alkyl, which were connected with MET 131, GLY 71, LEU 100 and CYS 115, etc.

MS1 (Fig. 9A), a compound in formulae MXSG and SGMH, showed eleven interactions with N protein NCB site including H-bond interactions, Pi-Donor hydrogen bond, Pi-sigma, Pi-Pi stacked and Pi-alkyl, which were connected with SER 51, THR 109, ALA 50 and PRO 42, etc.; additionally, MS1 (Fig. 9B) showed five interactions with nsp14 [ExoN] including H-bond interactions and Pi-Pi stacked, which were connected with GLU 92, PHE 190, ASP 273 and VAL 91, etc. MX16 (Fig. 9C), a compound in formulae MXSG and XCH, showed seven interactions with nsp15 [endoribonuclease] including H-bond interactions, Alkyl and Pi-alkyl, which were connected with PRO 343, VAL 275, LYS 344 and SER 293, etc. SX1 (Fig. 9D), a compound in formulae SGMH and XCH, showed two interactions with nsp14 [N7-MTase] including Pi-Pi stacked and Pi-alkyl, which were connected with PHE 426; additionally, SX1 (Fig. 9E) showed five interactions with nsp15 [endoribonuclease] including H-bond interactions, Alkyl and Pi-alkyl, which were connected with LYS 344, LYS 289, VAL 291 and PRO 343. WO1 (Fig. 9F), a compound in formulae WLS and Others, showed seven interactions with nsp16 [2′-O-MTase] including H-bond interactions, Carbon hydrogen bond, Pi-Pi T-shaped, Pi-alkyl and Pi-anion, which were connected with PHE 149, CYS 115, ASP 99 and SER 74, etc.; additionally, WO1 (Fig. 9G) showed seven interactions with nsp12 [RdRp without RNA] including H-bond interactions, Carbon hydrogen bond, Unfavorable Donor-Donor, Pi-cation and Pi-anion, which were connected with THR 556, ARG 553, ASP 623 and SER 682, etc. XO1 (Fig. 9H), a compound in formulae XCH and Others, showed ten interactions with nsp12 [RdRp with RNA] including H-bond interactions, Pi-Donor hydrogen bond, Pi-Pi T-shaped and Pi-alkyl, which were connected with CYS 813, GLY 590, LYS 593 and ASP 865, etc.

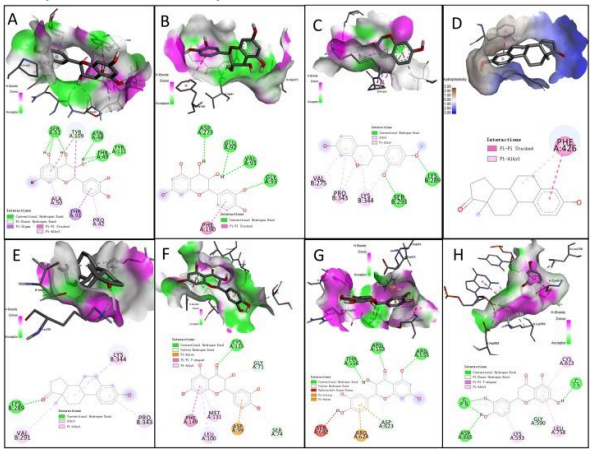

Fig. 9. Schematic (3D and 2D) representation that molecular model of common compounds of the five formulae with COVID-19 proteins. A: MS1 and N protein NCB site, B: MS1 and nsp14 [ExoN], C: MX16 and nsp15 [endoribonuclease], D: SX1 and nsp14 [N7-MTase], E: SX1 and nsp15 [endoribonuclease], F: WO1 and nsp16 [2′-O-MTase], G: WO1 and nsp12 [RdRp without RNA], H: XO1 and nsp12 [RdRp with RNA]. MS: MXSG and SGMH, MX: MXSG and XCH, SX: SGMH and XCH, WO: WLS and Others, XO: XCH and Others.

3.7. ACE2 and CD147 expression across tissues and co-expression genes

Since 2019-nCov may enter other tissues and organs through ACE2 and CD147 binding, we firstly explored the expression and distribution of ACE2 and CD147 across 53 tissues. Fig. 10A showed that the 5 top expression tissues of ACE2 were terminal ileum, testis, visceral (omentum), left ventricle and kidney cortex, which are 3 fold change higher than lung. And the 5 top expression tissues of CD147 were testis, visceral (omentum), left ventricle, aorta, atrial appendage and transformed fibroblasts. Then, to further understand whether QFPD only targets pneumonia or 2019-nCov, we obtained 200 co-expression genes of ACE2, 200 co-expression genes of CD147, 470 pneumonia-associated proteins, 119 HCoV-associated host proteins, and 476 co-expression genes of ACE2 in colonic epithelial cells. Fig. 10B displayed that QFPD had some common targets with these five sets, while specific 254 targets for QFPD, indicating other mechanisms of QFPD on COVID-19 in addition to 2019-nCov, pneumonia, ACE2 and CD147 related functions.

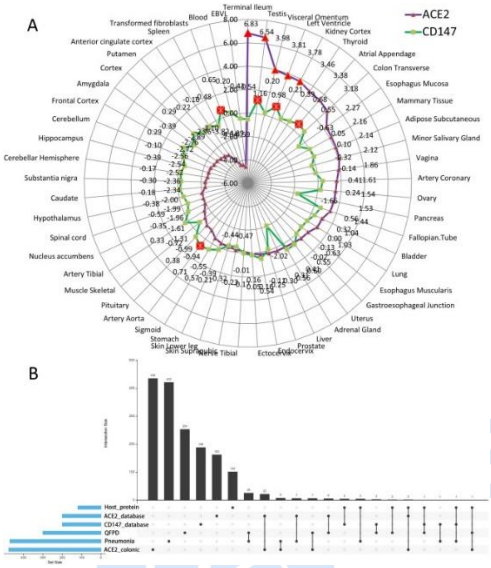

Fig. 10. ACE2 and CD147 expression across tissues and co-expression genes. A: Radar plot of ACE2 and CD147 expression across 53 tissues. The expression values were converted to base-2 logarithm. Red triangle and square mean the top 5 expression tissues. B: UpSet plot of proteins among QFPD, HCoV (Host_protein), pneumonia, ACE2 co-expression genes (ACE2_database), CD147 co-expression genes (CD147_database), and ACE2 co-expression genes in colonic epithelial cells (ACE2_colonic). The horizontal bar graph at the bottom left shows the total number of proteins for each group set. Circles and vertical lines in the x-axis mark the corresponding data sets being compared. The vertical bar graph at the top quantitates the number of proteins in the comparisons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.8. Validation of drug positioning for QFPD against COVID-19 via the robustness of disease network

Firstly, the robustness of whole networks against formula attack was assessed to evaluate QFPD attack on the COVID-19 disease network. Interestingly, Table 4 and Fig. 11 showed that MSXG, SGMH, XCH, WLS and Others attack on the COVID-19 network were characterized by greater disturbance score than negative control (BXTM), and increasing dependence on hub nodes, indicating greater fragility under formula attack. In addition, SGMH, MSXG, and Others exerted higher disturbance score than the positive control (YDBF) Table 5.

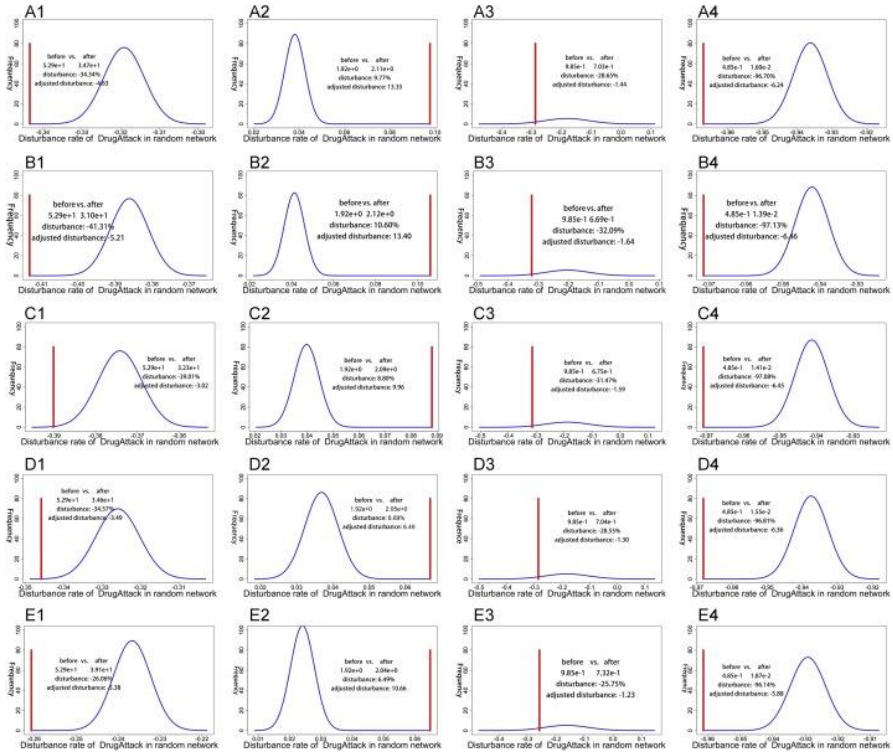

Fig. 11. Evaluation of the effect of QFPD on the robustness disturbance of COVID-19 network. Blue normal distribution: drug attack on random networks as a null distribution for the permutation test. Red vertical line: the disturbance rate of the drug to the real disease network. Fist row: MXSG, second row: SGMH, third row: XCH, fourth row: WLS, fifth row: Others. Fist column: average connectivity, second row: average length of shortest path, third row: connection centrality, fourth row: closeness centrality. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Docking score between common ingredients of QFPD and 2019-nCov proteins.

Molecule	MS1	MX16	MX17	MX8	MXO1	SO1	SX1	WO1	XO1
Main Protease	−7.6	−7	−7.8	−7.8	−7.8	−7.2	−7.1	−7.4	−7.4
Papain like protease	−8	−8.2	−8.1	−8.1	−8.2	−8.2	−9.2	−8.7	−8.3
RdRp with RNA	−8.6	−8	−7.9	−7.9	−8	−8.4	−8.2	−8.8	−9.5
RdRp without RNA	−7.1	−6.8	−6.8	−6.8	−7	−7.1	−6.9	−7.5	−7.1
Helicase ADP site	−6.2	−6.5	−6.3	−6.1	−6.2	−6.4	−6.8	−6.2	−6.8
Helicase NCB site	−7.4	−7.1	−7.2	−7.2	−7.5	−7.5	−7.4	−7.6	−7.5
Nsp14(ExoN)	−7.2	−6.6	−6.6	−6.7	−6.9	−7	−6.9	−7.1	−7.1
Nsp14(N7-MTase)	−8.6	−8.3	−8.4	−8.3	−8.6	−8.3	−9.4	−8.7	−8.7
Nsp15(endoribonuclease)	−6.2	−6.8	−6.4	−6.4	−6.3	−6.5	−6.8	−6.4	−6.6
Nsp16(2′-O-MTase)	−8.1	−7.4	−7.6	−7.8	−7.8	−8.3	−7.7	−8.4	−8.2
N protein NCB site	−8.1	−7.9	−8	−8	−7.6	−7.8	−7.5	−7.7	−7.9
E protein(ion channel)	−6.4	−6.9	−7.2	−7.2	−6.9	−6.9	−7.9	−6.8	−6.8

M: MXSG, S: SGMH, X: XCH, O: Others.

Next, to illustrate the mechanism of QFPD against COVID-19, a formula-attacked target-KEGG pathway network was constructed (Fig. 12). This network showed that MSXG, SGMH, XCH, WLS and Others interacted with 8 drug-attacked nodes (Cdc20, Ido1, Ifng, Il10, Il6, Ptger4, Spi1, Tnf), and 24 drug-attacked nodes in the COVID-19 network were related to Graft-versus-host disease, cytokine-cytokine receptor interaction, asthma, influenza A, inflammatory bowel disease, JAK-STAT signaling, etc.

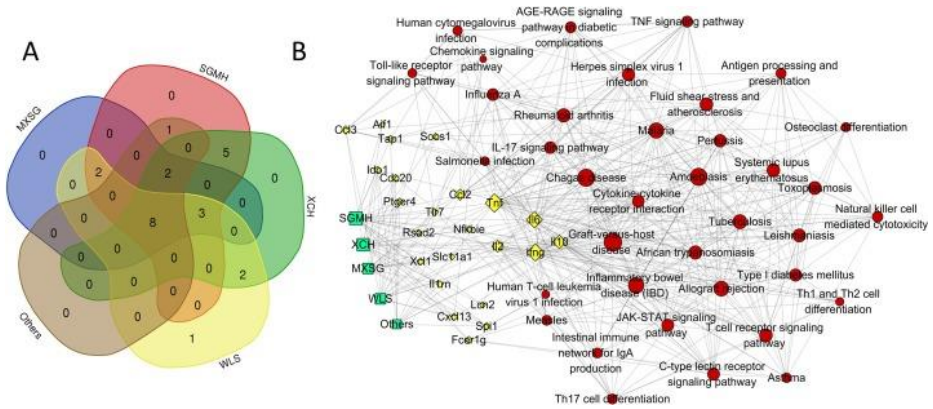

Fig. 12. Disturbance analysis of QFPD for COVID-19 network. A: Venn diagram of the five formulae' attacked targets. B: Formula-attacked target-KEGG pathway network; green square: formula, yellow diamond: attacked target, red circle: KEGG pathways. The bigger the size of the nodes is, the higher the degree is. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Novel coronavirus (2019-nCov) infection is characterized by lung and immune system damage. Severe infection can lead to acute respiratory distress syndrome (ARDS) and septicemia, and eventually lead to death [2]. In addition, a number of patients presented multi-organ damage and dysfunction [28]. However, there are no specific drugs or vaccine for the treatment of the COVID-19. This reason may partly be that a single targeted drug cannot cure a complex disease with complex biological networks [29]. Despite the lack of strong evidence-based medicine, TCM has a good potential to complement the medical service for COVID-19, including reverting radiological changes, and shortening fever duration and hospital stay. [30]. It is observed that the total effective rate of QFPD in the treatment of pneumonia patients infected by novel coronavirus is more than 90 % [31]. Therefore, we explored the mechanism of QFPD against COVID-19 by systems pharmacology, and provided a combination strategy to explore the functional units in QFPD from a holistic perspective.

To our knowledge, this is the first study to explore the mechanisms of QFPD for COVID based on intra-functional units. In this study, GO enrichment analysis showed that the common GO terms of MSXG, SGMH, XCH, WLS and Others targets were significant enriched in oxidoreductase activity, lipid metabolic process, lipid binding, small molecule metabolic process, and homeostatic process etc., suggesting QFPD may exert anti-viral activity through metabolic function. In agreement with these results, a recent research has found that lipid metabolic reprograming plays an important role in virus replication, which may be an appealing and applicable target for antiviral therapy [32]. KEGG analysis showed that in addition to lipid metabolism-related pathways, endocrine system pathways were also significantly enriched in more than four formulae, including PPAR signaling pathway and adipocytokine signaling pathway. A recent study has showed that the host can exert anti-inflammatory functions to inhibit excessive inflammatory damage through PPAR signaling pathway after H1N1 infection, thus keeping homeostasis of metabolism and development [33]. In addition, other common terms were significant enriched in more than two formulae, such as immune system process, endoplasmic reticulum, cell-cell signaling, calcium signaling pathway, vascular smooth muscle contraction,

inflammatory mediator regulation of TRP channels, cardiac muscle contraction, etc. Therefore, the multi-pathway and multi-target results of our intra functional unit of QFPD not only showed a new useful method for studying TCM, but may demonstrate the rationality of TCM compatibility. Moreover, TCMATCOV platform was used to validate these results. Interestingly, all the five FUs of QFPD showed higher disturbance score than negative control (BXTM), indicating that MSXG, SGMH, XCH, WLS and Others may protect COVID-19 independently, and target 8 specifically expressed drug-attacked nodes (Cdc20, Ido1, Ifng, Il10, Il6, Ptger4, Spi1, Tnf) which were related to the bacterial and viral responses, cytokine, immune system, signaling transduction, etc.

Currently, a number of studies have showed that 2019-nCov can cause multiple organs dysfunction, including liver [34], pancreas [35], kidney [36], throat and rectum [37], which may be the reason that a wide distribution of ACE2 across these tissues [38]. In agreement with these results, we found that ACE2 was highly expressed in terminal ileum, testis, adipose visceral omentum, heart left ventricle, kidney cortex and thyroid, etc., and QFPD has only 15.33 % common targets with 2019-nCov, pneumonia and ACE2 related genes. These results indicate that the effective treatment of QFPD for COVID-19 may be through a holistic treatment. Moreover, TTD analysis further displayed that QFPD targets were significantly enriched in many COVID-19 related disease, such as chronic inflammatory diseases, asthma, inflammatory bowel disease, chronic obstructive pulmonary disease, intrahepatic cholestasis, chronic ileitis, etc.

It is known that ADMETox prediction is an important part in evaluating if a drug can be toxic or can be absorbed during drug development process [39]. In our study, ADMETox evaluation shows that 20 compounds passed the stringent lead-like criteria (250 ≤ MW≤350 & XLOGP ≤ 3.5 & Number of rotatable bonds≤7) [40] and in silico drug-likeness test, and showed high gastrointestinal absorption. Moreover, predicted toxicity evaluation showed that the median lethal dose (LD50) of all these ingredients was above 1600 mg/kg, thus may suggesting safety and efficacy of QFPD. Combined with molecular docking results, 4 specific ingredients (M3, S1, X2 and O2) and 5 common ingredients (MS1, MX16, SX1, WO1 and XO1) of QFPD might be promising leading compounds with good molecular docking score for 2019-nCov structure and non-structure proteins, revealing that QFPD treated COVID-19 by multi-component synergy. However, these newly monomer components should provide a further research.

It has been reported that host cellular microRNAs (miRNAs) are involved in the regulation of virus infection [41]. A previous study discovered that significantly up-regulated MIR301 and down-regulated MIR183/130B were found in H1N1 patients [42]. Consistent with these results, we found that MIR183 and MIR130A/B/301 are related to four functional units of QFPD, indicating these microRNAs may exert anti−COVID-19 activity through QFPD. In addition, CDKs have played a role in the efficient replication of various viruses, including human HIV-1, papillomaviruses, human cytomegalovirus (HCMV), herpes simplex virus (HSV) type 1 and HSV-2 [43,44]. In agreement with these results, we found that CDK7 was predicted to enriched in the five formulae, suggesting that QFPD may regulate replication of COVID-19 viruses via CDK7 mediated cell cycle and RNA polymerase II transcription. Recently, a previous study showed that LXR known to regulate cholesterol homeostasis during inflammation were differentially regulated during H1N1 influenza virus

infection [45]. Based on our results that LXR was associated with MSXG, SGMH, XCH, WLS targets, we speculated that QFPD can regulate metabolic and pro-inflammatory processes to counter COVID-19 virus infection.

In summary, QFPD is effective in the treatment of COVID-19. However, some shortcomings in our study include lack of an in-depth study of predictive monomers and key targets and pathways, thus need further validation in vivo and in vitro. And the TCMATCOV platform uses SARS disease network, which is different from the COVID-19 disease network, and COVID-19-related cytokines are related to severe COVID-19 disease, so the results of platform analysis more reflect the potential efficacy of severe stage. Nevertheless, this study confirms that network pharmacology can help explore the mechanism of QFPD on the treatment of COVID-19 with time- and cost-saving. Moreover, based on our new FUNP analysis, we reveal that QFPD treat COVID-19 by a holistic treatment and multi-component synergy, and are further demonstrated by formula perturbation analysis. In addition, this study provides possible candidate monomers of QFPD and related miRNAs, kinases and TFs with potential therapeutic effect on COVID-19. This will hopefully provide evidence and new insights for further researches on the treatment of COVID-19 using QFPD.

Author contributions

Conceiving the research, Jian Chen and Yong-bing Cao; Data curation, Jian Chen, Wen-jie Sun, and Zhi-qiang Liang; Funding acquisition, Jian Chen, Zhi-qiang Liang, Bing-yong Cao and Ye-min Cao; Investigation, Ling-San Hu, Jian-ru Wang, and Bing-yong Cao; Methodology, Yong-kui Wang, Jiang-wei Yang and Ye-min Cao; Resources, Ling-San Hu and Yong-kui Wang; Visualization, Jian-ru Wang, Jiang-wei Yang; Writing – original draft, Jian Chen.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Abstract

In this study, Donghua Hospital information management system and Meikang clinical pharmacy management system were used to collect medical records of all inpatients diagnosed as coronavirus disease 2019(COVID-19) in Wuhan Third Hospital. The statistics was based on the data of the cases treated with Ganlu Xiaodu Decoction, including demographic statistics, clinical cha-racteristics before medication, outcome of after medication and efficacy of drug combination. Excel 2003 and SPSS Clementine 12.0 software were used to conduct statistics on the included cases, and Apriori algorithm and association rules were used for the association analysis on drug combination. A total of 131 cases of COVID-19 were treated with Ganlu Xiaodu Decoction combined with Chinese and Western medicine. All of the patients were cured and discharged. The drug combination mainly included Ganlu Xiaodu Decoction, abidor, Lianhua Qingwen,

moxifloxacin, Qiangli Pipa Lu, vitamin C, glycyrrhizinate diammonium, pantoprazole and Shufeng Jiedu. There is a certain regularity and effectiveness in the treatment of COVID-19 infection patients with the combination of Ganlu Xiaodu Decoction and other drugs, but the rationality and safety still need to be further verified.

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How COVID-19 (2019-nCoV) is Currently Treated in China with TCM

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Disclaimer: This article is compiled, translated and edited by John K. Chen and Lori Hsu from three references to inform the readers how COVID-19, the 2019 novel coronavirus, is currently treated in China. For readers who may have such an infection, contact and consult your primary physician, go to the hospital or the CDC immediately. For additional information, please contact the World Health Organization (WHO), the Center of Disease Control (CDC) and the Food and Drug Administration (FDA).

With Wuhan on Lockdown for More Than 10 days, the Chinese Government Announces a Major Change in Strategy: All Patients with Confirmed Infections are to Use Chinese medicine. 2/10/2020
The Medical Treatment Unit of Wuhan's Novel Coronavirus (2019-nCoV), currently named COVID-19, Prevention and Control Headquarters issued a "Notice Regarding the Agreement to Recommend the Use of Chinese Medicine in the Treatment of Pneumonia due to Infection from the Novel Coronavirus". The "Notice" emphasizes that all designated medical institutions in Wuhan will ensure that all infected patients take Chinese medicine (Chinese medicine decoction or granules) before midnight (24:00) on February 3, 2020.
A collaboration between Western and Chinese medicine is undoubtedly welcoming news, especially now as the results from the front lines in Wuhan have been very promising. See below for more.
1/29/20 The second dispatch of support from the central government to the Hubei Chinese Medicine Treatment team took over the newly created isolation ward for pneumonia patients infected by the 2019-nCoV in the Hubei Provincial Hospital of Integrated Traditional Chinese and Western Medicine. It is the only medical facility in the Hubei Province that is managed entirely under the supervision of the China's Traditional Chinese Medicine (TCM), TCM system, providing a central point responsible for determining diagnosis and treatment.
The following is reported in the patient wards under the responsibility of Guandong Provincial TCM Hospital:
Patient Rounds: The severity of patient conditions was unexpected, and the entire ward was more or less quiet as these patients were so weak, that it seemed that they did not even have the energy to moan. Most of the patients treated were urgently, critically, or severely ill. Many of them had a high fever, a thick tongue coating, slippery and wiry pulses, bowel incontinence, and little to no expression on their faces (perhaps from extreme fatigue?).
1/30/20 Herbal formulas were administered to patients according to the individual's TCM pattern diagnoses, as prescribed by Guandong's experts.
2/1/20 Patient Rounds: Some patients' fever have been reduced. There were more patients with low-grade fever. By and large,tongue coatings changed from thick and greasy to thin, and patient stools were more formed. Furthermore, these patients now had the energy to speak for up to five sentences before the onset of mild wheezing. Also, in general, coughing was significantly less compared to before. Patients' self-reports of

conditions have improved quite a bit. Patients can now sit up and practice the qigong exercise, Eight Brocade in bed and/or meditate.
As of 2/4/20: Nearly fifty patients in the ward have clearly improved. The effectiveness of Chinese herbal treatments are being actively observed and witnessed in this clinical setting.
Caption: For the first time, eight patients treated with traditional Chinese medicine or a combination of traditional Chinese and western medicine were discharged from hospital.
1/28/20, Dr. Huang Luqi, the chair of the Chinese Academy of Chinese Medical Sciences, led the medical team of Guang'anmenHospital and Beijing Xiyuan Hospital of the Chinese Academy of Chinese Medical Sciences to support Wuhan Jinyintan Hospital. Chinese medicine combined with Western medicine, treatment by syndrome differentiation, greatly improved the patients'breathing issues, fatigue, dry mouth, bitterness, chest tightness, diarrhea and other symptoms.
2/3/20, Eight confirmed patients were discharged from the first ward of the South Building of Wuhan Jinyintan Hospital. This represents the first group of patients who were discharged from a hospital using traditional Chinese medicine or a combination of traditional Chinese medicine and Western medicine. Amongst them, six were female, two were male. Of these, six were severe and two were mild cases. The age range of the patients was from twenty-six to sixty-eight. After treatment with traditional Chinese medicine and herbs, most patients had obviously improved symptoms and an overall improved mental state. At the time of discharge, each patient was sent home with a two-week dose of Chinese herbs along with instructions on appropriate exercise and proper diet in order to gain full recovery.
Please share this article and the herbal prescriptions with everyone.
In the fight against the pandemic of pneumonia from 2019-nCoV Novel Coronavirus infection, Hubei Provincial Hospital of Traditional Chinese Medicine diligently used the specialties of TCM, in cooperation with relevant departments to study and formulate pneumonia prevention and treatment programs revealing that Chinese herbs played a definitive role in positive treatment outcomes. Chinese herbal formulas harbor the unique potential to reduce fever and cough symptoms, limit disease progression, and improve overall immunity, and thus a person's ability to mount an essential immune response to the virus.
The following are the recommended formulas, acupuncture and moxibustion protocols for prevention and treatment of Coronavirus according to TCM experts. Please note, the following formulas are not to be used in place of Western medicine rather they are to be integrated into a comprehensive treatment plan utilizing both Western and Chinese medicine to ensure optimal patient outcomes. Furthermore, patients are advised not to self treat or use the formulas blindly, but rather to consult with licensed medical practitioners to ensure optimal treatment on a case by case basis.

Prevention Phase: 预防期

Formula: Pneumonia Prevention #1肺炎预防1号

- Huang Qi 黄芪 (Radix Astragali) 15g,
- Bai Zhu 炒白术 (Rhizoma Atractylodis Macrocephalae), dry fried 10g
- Fang Feng 防风 (Radix Saposhnikoviae) 10g
- Mian Ma Guan Zhong 贯众 (Rhizoma Dryopteridis Crassirhizomatis) 10g
- Jin Yin Hua 金银花 (Flos Lonicerae Japonicae) 10g
- Chen Pi 陈皮 (Pericarpium Citri Reticulatae) 6g
- Pei Lan佩兰(Herba
- Cases Eupatorii) 10g

Suitable for: Prevention of pneumonia due to viral infections, and the flu.
Acupuncture Treatment for Suspected:
The purpose is to strengthen the immune system, to help alleviate early symptoms, and to shorten the duration of the virus.
Points: Bilateral Zusanli (ST 36), Qihai (CV 6), Zhongwan (CV 12)
Method and Frequency:
Moxa Zusanli (ST 36) on both sides for 15 minutes. Moxa Qihai (CV 6) or Zhongwan (CV 12) for 10 minutes. (alternating from treatment to treatment).

Twice a day, once in the afternoon and once at night.

Influenza Phase: 流感期

Diagnosis: Wind-Cold Invading the Exterior 风寒袭表证

Clinical Manifestations: Onset of fever (mostly low-grade fever), aversion to cold and fear of cold, chills, headache, ticklish throat, soreness of muscles of limbs, no sweat or night sweats.

Examination: Lung CT negative. Tongue is pale, coating is white and thin. Floating pulse

Treatment Strategy: Expel Wind, Release the Exterior; Clear Heat, Detoxify

Herbal Formula: 葛根汤或柴葛解肌汤 (Ge Gen Tang (Kudzu Decoction) or Chai Ge Jie Ji Tang (Bupleurum and Kudzu Decoction to Release the Muscle Layer))

Flu Formula #1流感1号

- Ge Gen 葛根 (Radix Puerariae Lobatae) 15g
- Ma Huang 麻黄 (Herba Ephedrae) 10g
- Gui Zhi 桂枝 (Ramulus Cinnamomi) 6g
- Bai Shao 白芍 (Radix Paeoniae Alba) 15g
- Sheng Jiang 生姜 (Rhizoma Zingiberis Recens) 10g
- Gan Cao 生甘草 (Radix et Rhizoma Glycyrrhizae) 10g
- Da Zao 大枣 (Fructus Jujubae) 10g
- Jin Yin Hua 金银花 (Flos Lonicerae Japonicae) 20g

with headache, add Bai Zhi 白芷 (Radix Angelicae Dahuricae) 15g

with dry or ticklish throat, add She Gan 射干 (Rhizoma Belamcandae) 15g

Other possible formulas to consider: Huo Xiang Zheng Qi San

Diagnosis: Toxic Heat Attacking the Lung 热毒袭肺证

Clinical Manifestations: Fever, aversion to cold, sore and dry throat, dry cough, scanty sputum, sore and painful muscles in the limbs, weakness, headache

Examination: CT scan reveals both lungs to have scattered ground-glass opacity (GGO). Tip and sides of the tongue are red; thin white or yellow tongue coating. Floating and rapid pulse.

Treatment Strategy: Expel Wind, Release the Exterior; Clear Heat, Detoxify

Herbal Formula:银翘散加清瘟败毒散加减 (Yin Qiao San (Honeysuckle and Forsythia Powder) and Qing Wen Bai Du San(Clear Epidemics and Overcome Pathogenic Influences Powder), modified)

Flu Formula #2流感2号

- Jin Yin Hua 金银花 (Flos Lonicerae Japonicae) 10g
- Lian Qiao 连翘 (Fructus Forsythiae) 10g
- Jing Jie 荆芥 (Herba Schizonepetae) 10g
- Niu Bang Zi 牛蒡子 (Fructus Arctii) 10g
- Bo He 薄荷 (Herba Menthae) 10g
- Gan Cao 生甘草 (Radix et Rhizoma Glycyrrhizae) 10g
- Dan Zhu Ye 淡竹叶 (Herba Lophatheri) 10g
- Lu Gen 芦根 (Rhizoma Phragmitis) 15g
- Huang Lian 黄连 (Rhizoma Coptidis) 6g

Diagnosis: Damp Cold in the Lung 濕寒鬱肺

Clinical Manifestations: Aversion to cold, fever or absence of fever, dry cough, dry throat, fatigue, weakness, chest stuffiness, epigastric distention, nausea, diarrhea. Pale tongue, white greasy coating, slippery pulse.

Treatment Strategy: Expel Wind, Release the Exterior; Dispel Damp Cold

Herbal Formula:

Damp Cold Formula #1

- Cang Zhu 蒼朮 (Rhizoma Atractylodis) 15g
- Chen Pi 陳皮 (Pericarpium Citri Reticulatae) 10g
- Hou Po 厚樸 (Cortex Magnoliae Officinalis) 10g

- Huo Xiang 藿香(Herba Pogostemonis seu Agastaches) 10g
- Cao Guo 草果 (Fructus Tsaoko) 6g
- Ma Huang 生麻黄 (Herba Ephedrae) 6g
- Qiang Huo 羌活 (Rhizoma et Radix Notopterygii) 10g
- Sheng Jiang 生薑 (Rhizoma Zingiberis Recens) 10g
- Bing Lang 檳榔 (Semen Arecae) 10g

Acupuncture Treatment for Mild and Moderate Cases:
The purpose is to reduce the severity of symptoms, shorten the duration, and alleviate emotional burden.
Points: Bilateral Hegu (LI 4), Taichong (LR 3), Zusanli (ST 36), Shenque (CV 8)
Method and Frequency:
Moxa Hegu (LI 4) and Taichong (LR 3) bilaterally for 15 minutes. Moxa Zusanli (ST 36) bilaterally for 10 minutes. Moxa Shenque (CV 8) with a moxa box for 15 minutes.
Twice a day, once in the morning and once in the afternoon.

Pneumonia Phase: 肺炎期

Diagnosis: Shaoyang Syndrome with Damp 少阳夹湿证

Clinical Manifestations: Fever, which is more pronounced in the afternoon, alternating chills with fever, cough, absence of wheezing, bitter taste in the mouth, dry mouth, chest stuffiness, stifling sensation, chest and hypochondriac fullness and distention, irritability, nausea or vomiting, no appetite, weakness. Similar to the beginning stage of pneumonia.
Examination: CT scan reveals both lungs to have multiple scattered or large pieces of ground-glass opacity (GGO). Slightly red tongue, thick and greasy, white or yellow coating, slippery, rapid pulse.
Treatment Strategy: Harmonize Shaoyang Syndrome, Clear Damp-Heat

Herbal Formula: 小柴胡汤合三仁汤或甘露消毒丹 (Xiao Chai Hu Tang (Minor Bupleurum Decoction) with San Ren Tang(Three-Nut Decoction) or Gan Lu Xiao Du Dan (Sweet Dew Special Pill to Eliminate Toxins))
Pneumonia Formula #1 肺炎1号

- Chai Hu 柴胡 (Radix Bupleuri) 24g
- Huang Qin 黄芩 (Radix Scutellariae) 9g
- Sheng Jiang 生姜 (Rhizoma Zingiberis Recens) 10g
- Fa Ban Xia 法夏 (Rhizoma Pinelliae) 12g
- Ku Xing Ren 杏仁 (Semen Armeniacae Amarum) 15g
- Bai Dou Kou 白豆蔻 (Fructus Amomi Rotundus) 10g
- Yi Yi Ren 薏苡仁 (Semen Coicis) 30g
- Dan Zhu Ye 竹叶 (Herba Lophatheri) 15g
- Hua Shi 滑石 (Talcum) 15g
- Tu Fu Ling 土茯苓 (Rhizoma Smilacis Glabrae) 30g
- Gan Cao 生甘草 (Radix et Rhizoma Glycyrrhizae) 10g

Diagnosis: Damp Heat Afflicting the Lung 湿热郁肺证

Clinical Manifestations: Low-grade fever or absence of fever, dry cough, scanty sputum, dry and sore throat, fatigue, weakness, poor appetite, chest stuffiness, epigastric distention, nausea or vomiting, loose stool.
Examination: CT scan reveals both lungs to have multiple scattered or large pieces of ground-glass opacity (GGO). Pale or pink, puffy tongue with teeth marks. White or greasy white coating. Soft or slippery pulse.
Treatment Strategy: Transform Dampness, Detoxify; Disperse the Lungs and Expel Pathogens

Herbal Formula: 麻杏薏甘汤、小陷胸汤、草果知母汤 (Ma Xing Yi Gan Tang (Ephedra, Apricot Kernel, Coicis, and Licorice Decoction), Xiao Xian Xiong Tang (Minor Sinking into the Chest Decoction) and Cao Guo Zhi Mu Tang (Tsaoko and Anemarrhena Decoction)
Pneumonia Formula #2 肺炎2号

- Ma Huang 麻黄 (Herba Ephedrae) 10g
- Ku Xing Ren 杏仁 (Semen Armeniacae Amarum) 10g
- Yi Yi Ren 薏苡仁 (Semen Coicis) 30g

- Huang Lian 黄连 (Rhizoma Coptidis) 6g
- Fa Ban Xia 法夏 (Rhizoma Pinelliae) 10g
- Gua Lou Pi 瓜蒌皮 (Pericarpium Trichosanthis) 10g
- Cao Guo 草果 (Fructus Tsaoko) 10g
- Zhi Mu 知母 (Rhizoma Anemarrhenae) 10g
- Yu Xing Cao 鱼腥草 (Herba Houttuyniae) 15g
- Gan Cao 生甘草 (Radix et Rhizoma Glycyrrhizae) 10g
- Bai Dou Kou 白豆蔻 (Fructus Amomi Rotundus) 9g

Diagnosis: Toxic Stagnation Obstructing the Lung 毒瘀壅肺证

Clinical Manifestations: Cough, stifling sensation, stuffiness and distention in the chest, asthma and wheezing that worsens with exertion, accelerated respiration, thirst, irritability, reddish yellow urine.
Examination: CT scan reveals both lungs to have multiple scattered or large pieces of ground-glass opacity (GGO). Fibrotic changes of the lung are also visible. Dark purplish tongue, yellow dry tongue coating or thick and greasy yellow coating, rapid, slippery pulse.
Treatment Strategy: Detoxify, Arrest Wheezing; Transform Blood Stasis and Open Collaterals

Herbal Formula: 白虎汤加人参汤合四土汤 (Bai Hu Jia Ren Shen Tang (White Tiger plus Ginseng Decoction) with Si Tu Tang (Four Wild Decoction)
Pneumonia Formula #3 肺炎3号

- Shi Gao 石膏 (Gypsum Fibrosum) 30g
- Zhi Mu 知母 (Rhizoma Anemarrhenae) 10g
- Shan Yao 山药 (Rhizoma Dioscoreae) 15g
- Xi Yang Shen 西洋参 (Radix Panacis Quinquefolii) 5g
- Tu Fu Ling 土茯苓 (Rhizoma Smilacis Glabrae) 30g
- Tu Da Huang土大黄 (Radix Rumicis Obtusifolii) 10g
- Tu Bei Mu 土贝母 (Rhizoma Bolbostemmatis) 10g
- Tu Niu Xi 土牛膝 (Rhizoma Achyranthes Sylvestris) 10g
- Su Mu 苏木 (Lignum Sappan) 10g
- Tu Bie Chong 土鳖 (Eupolyphaga seu Steleophaga) 10g
- Ju Luo 橘络 (Vascular Citri Reticulatae) 15g
- Lai Fu Zi 莱菔子 (Semen Raphani) 20g
- Ting Li Zi 葶苈子(Semen Descurainiae seu Lepidii) 15g
- Si Gua Luo 丝瓜络 (Retinervus Luffae Fructus) 30g

Diagnosis: Closed Interior and Abandoned Exterior Syndrome 内闭外脱证

Clinical Manifestations: Mental incoherence, irritability, burning or heat sensation in the chest and abdomen, cold extremities, accelerated respiration and need for assisted breathing, scarlet purple tongue, dry yellow or yellowish brown coating, floating, forceful pulse that is empty in the deep level, or rootless.
Treatment Strategy: Open the Closed, Consolidate the Abandoned, Detoxify, Rescue Reversal

Herbal Formula: 四逆加人参汤、送服 安宫牛黄丸、紫雪散 (Si Ni Jia Ren Shen Tang (Frigid Extremities Decoction plus Ginseng), taken with An Gong Niu Huang Wan (Calm the Palace Pill with Cattle Gallstone) and Zi Xue San (Purple Snow Powder))
Pneumonia Formula #4肺炎4号

- Ren Shen 人参 (Radix et Rhizoma Ginseng) 10g
- Fu Zi 制附子(Radix Aconiti Lateralis Praeparata) 10g
- Serve with An Gong Niu Huang Wan (Calm the Palace Pill with Cattle Gallstone) and Zi Xue San (Purple Snow Powder).

Recovery Phase: 恢复期

Clinical Manifestations: Absence of fever, dry cough, chest stuffiness, shortness of breath, shortness of breath upon exertion, dry mouth, weakness.
Examination: CT reveals inflammation begins to subside as well as pulmonary interstitial changes. Pale red tongue, thick or greasy coating, thread, rapid pulse.
Treatment Strategy: Tonify Qi, Nourish Yin, Tonify Lung and Open the Collaterals

Herbal Formula: 沙参麦门冬汤(Sha Shen Mai Dong Tang (Glehnia and Ophiopogonis Decoction))

Pneumonia Formula #5肺炎5号

- Sha Shen 沙参 (Radix Glehniae seu Adenophorae) 15g
- Mai Dong 麦冬 (Radix Ophiopogonis) 15g
- Wu Wei Zi 五味子(Fructus Schisandrae Chinensis) 15g
- Ren Shen 人参 (Radix et Rhizoma Ginseng) 12g
- Lai Fu Zi 莱菔子 (Semen Raphani) 15g
- Si Gua Luo 丝瓜络 (Retinervus Luffae Fructus) 15g
- Ju Luo 橘络 (Vascular Citri Reticulatae) 15g
- Zi Su Zi 苏子(Fructus Perillae) 12g
- Zhe Bei Mu 浙贝 (Bulbus Fritillariae Thunbergii) 12g
- Ku Xing Ren 杏仁 (Semen Armeniacae Amarum) 12g
- Huang Qin 黄芩 (Radix Scutellariae) 15g
- Gan Cao 生甘草 (Radix et Rhizoma Glycyrrhizae) 10g

Acupuncture Treatment for The Recovery Phase:
The Purpose is restore Lung and Spleen functions and the body's zheng (upright) qi.
Points: Bilateral Dazhui (GV 14), Geshu (BL 17), Feishu (BL 13), Zusanli (ST 36) or Kongzui (LU 6).
Method and Frequency:
Moxa all points for 15 minutes.
Once a day.
History and experience prove that Traditional Chinese Medicine is effective against epidemic diseases.
From the Western Han Dynasty to the end of the Qing Dynasty, at least 321 large-scale plagues occurred in China. Chinese medicine has served to wage life-and-death battles against various plague consistently through time and has successfully contained the spread of epidemics in a limited area and time. There has never been a similar tragedy in China's history, such as the Spanish flu or the Black Death in Europe. These are examples of global plagues that killed tens of millions of people.
In the Chinese history, whenever a plague is rampant, Chinese medicine practitioners are always on the front lines to battle the disease. Many survived with the help of Chinese Medicine; so in this fight with the insidious and novel Coronavirus, Chinese Medicine once again should take a primary role in effective treatment and must not be absent in action!

Reference 1: https://mp.weixin.qq.com/s/qzSecLwVXQIfFBTQyHQxHQ

Reference 2: https://mp.weixin.qq.com/s/YajZ_fycSKEoTBvzhOv5Wg

Reference 3: https://mp.weixin.qq.com/s/qSUM5kYJIPJTvKkf_HfuaA?

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ABSTRACT

The novel coronavirus pneumonia broke out in 2019 and spread rapidly. In 30 different countries, there are over seventy thousand patients have been diagnosed in total. Therefore, it is urgent to develop the effective program to prevent and treat for the novel coronavirus pneumonia. In view of Traditional Chinese Medicine has accumulated a solid theoretical foundation of plague in ancient and recent decades. Meanwhile, Traditional Chinese Medicine can provide the more effective and personalized treatment via adjusting the specific medicine for each patient based on the different syndromes. In addition, TCM often has different effect on the distinct stages of diseases, contributing to the prevention, treatment and rehabilitation. Nowadays, TCM has exhibited decent effect in the in the fight against NCP. Therefore, it is convinced that Traditional Chinese Medicine is an effective treatment for 2019 novel coronavirus pneumonia.

Introduction

In December 2019, an unknown virus pneumonia broke out in Wuhan China. Later the unknown virus was identified as a novel coronavirus (2019-nCoV) and the unknown pneumonia named as novel coronavirus pneumonia (NCP) by Chinese government and scientists [1] . In early February 2020, over sixty thousand patients have been diagnosed with NCP in 30 different countries all over the world only after 1 month. And 99% of the cases have occurred in China. On account of the NCP reported worldwide for the first time, there is no specific vaccine and drug. Unfortunately, the development of novel vaccine or specific drug will take a few months, cannot keeping up with the development of NCP. Therefore, it is urgent to develop the effective treatment for NCP. Though it is no time to discovery effective drugs, the therapeutic effect of NCP is still remarkable in hospitals. Partly, this is owing to that Traditional Chinese Medicine (TCM) is applied in clinic timely as shown in Fig. 1 [2] . After National Health Commission (NHC) of China announcing the emergency situation in 20 January 2020, the National Administration of Traditional Chinese Medicine (NATCM) rapidly deployed the work, and the first batch of Chinese medicine experts arrived in Wuhan city on the day. In 29 January, National TCM Rescue Team took over Wuhan Jinyintan Hospital. Five days later, eight patients were discharged after treatment with Chinese medicine, of which six were critically ill. Similarly, the first patient diagnosed with NCP in Beijing was discharged after a combination of Chinese and Western treatment. According to the NATCM, the total effective rate of certain TCM prescription for NCP is over 90% [3] . Obviously, TCM have been playing a significant role in the combat with NCP. In fact, TCM played a unique role in the prevention and treatment of emerging infectious diseases since ancient time. For instance, TCM obtained decent clinical effect on SARS (severe acute respiratory syndrome), H7N9 (H7N9 avian influenza) and EVD (Ebola Virus Disease) at one time [4-6] . In these two thousand years, TCM has laid a solid theoretical foundation in the prevention and treatment of infectious diseases via the fight against diseases. Furthermore, doctors often adjust the specific treatment for each patient or integrate with western medicine scheme after diagnosing the syn drome through comprehensive analysis of symptoms and signs. Up to now, TCM has made a big difference in fight against NCP. The theoretical foundation of TCM for NCP Though NCP is a novel infectious disease, the similar syndrome infected by coronavirus are not unfamiliar. Firstly, the history of TCM applied for plague has lasted for over two thousand years [7] (Table. 1). And abundant theories of TCM for explosive infectious disease have formed, which have survived as treatises up to now. Meanwhile, TCM also play a significant role in the treatment of these coronavirus pneumonias broke out in the last two decades, such as SARS, MERS and H7N9 avian influenza [4-6] . Obviously, TCM has accumulated a solid theoretical foundation of plague and will be an effective treatment for NCP. The theories of TCM for infectious diseases NCP is infected by the novel coronavirus, but similar infectious diseases are not unacquainted for doctors of TCM. TCM has accumulated a wealth of experience and a lot of prescriptions, laying a solid theoretical foundation (Table 1). Two thousand years ago (BC 1046), the concept of “Yi Bing” was proposed. It pointed out that “Yi Bing ” was different from common cold. “Yi

Bing" was the disease which has in fectivity and easily leaded to epidemic. Then the dialectical relationship and treatment theory of exopathogenic disease were elaborated in AD 219. At present, plentiful Chinese herbal formulae from these theoretical foundation have been used. Meanwhile, the theories of TCM for infectious diseases had been further accumulated in Tsin Dynasty and Tang Dynasty. Especially, in as early as the 17th century, China had already owned the world's first medical book about systematic study of acute infectious disease. It described the etiology, pathogenesis, syndrome and treatment of plague. Similarly, a lot of representative theories of TCM for infectious diseases had been established. It is profound that TCM can apply the same method of treatment to patients with different kinds of disease but have the same syndrome. Obviously, TCM also can be an effective treatment for NCP based on these solid theoretical foundations. The history of treatments for coronavirus pneumonia NCP is the novel coronavirus pneumonias for TCM. But TCM have been successfully treated for other coronavirus pneumonias such as SARS, MERS and H7N9 avian influenza [4-6].

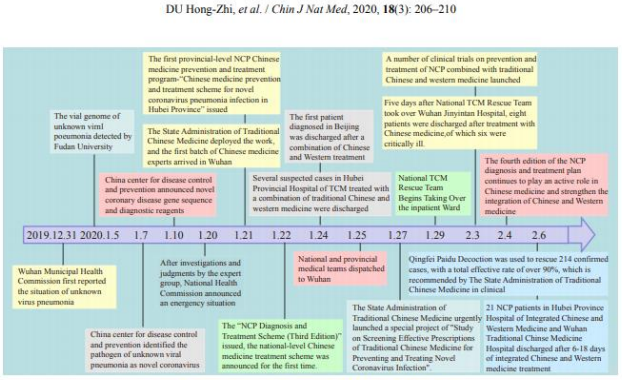

Fig. 1 The actions and treatment of TCM on NCP in China

Table 1 The representative theories of TCM for infectious diseases

No.	Data	Book Name	Related theories of TCM for infectious diseases
1	BC 1046 Zhou Dynasty	*Zhou Yi*	The concept of "Yi Bing" was proposed. It thought that "Yi Bing" was different from common cold. "Yi Bing" was the disease which has infectivity and easily leaded to epidemic.
2	AD 219 Han Dynasty	*Shang Han Lun*	The dialectical relationship and treatment theory of exopathogenic disease ("Shang Han") were elaborated. Especially, decades of specific Chinese herbal formulae were specifically treated different phenotypes of Shang Han.
3	AD 341 Tsin Dynasty	*Zhou Hou Bei Ji Fang*	*Zhou Hou Bei Ji Fang* was the first literature of emergency treatment. There were plentiful well proved clinical recipes for emergency including Wen Yi, Shang Han, pestilence and so on.
4	AD 652 Tang Dynasty	*Qian Jin Yao Fang*	In *Qian Jin Yao Fang*, there were many discussions on the theory and methods of prevention and treatment of infectious diseases. It also described the prescription drugs in detail.
5	AD 1642 Ming Dynasty	*Wen Yi Lun*	*Wen Yi Lun* was the first medical book about systematic study of acute infectious disease in the world. It described the etiology, pathogenesis, syndrome and treatment of plague. "Da Yuan Yin" was the classical prescription for plague.
6	AD 1794 Qing Dynasty	*Yi Zhen Yi De*	This book dealed with the causes of the plague, the main points of diagnosis and treatment, and the discrimination of common symptoms. The representative prescription "Qin Wen Bai Du Yin" was well recognized.

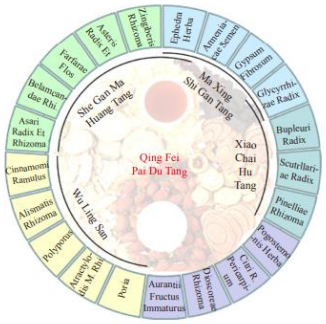

Fig. 2 The composition of the prescription—Qing Fei Pai Du Tang

In 2003, SARS broke out in China. According to statistics, there were 5327 patients diagnosed, and 7% of them died. But in Guangdong, PRC, the fatality ratio was only 3.8% while the mortality in Hong Kong, PRC was 17%, the fatality ratio in Taiwan, PRC was 11% and the fatality ratio in Canada was 14% [8] . The fundamental reason lied in the degree of TCM participation and the timing of its treatment. In Guangdong Provincal Hospital of TCM, 105 patients recovered after the treatment of integration of Chinese and western medicine among of 112 cases, not including 7 deaths from severe heart and brain and other basic diseases [9] . In 2013, H7N9 avian influenza also broke out in China. Then NHC of PRC issued the diagnosis and treatment scheme. In Beijing, the first case was also cured additionally by TCM [10] . Similarly, the Chinese Government also issued the diagnosis and treatment scheme of TCM for MERS in 2015 [11] . Thus it can be seen that TCM have long history of treatments for coronavirus pneumonia. It is believed that TCM also can be treated for NCP effectively.

The effect of TCM on NCP In the early stages of the outbreak, there is neither standard of western medicine treatment nor targeted drugs owing to the unknown of NCP. However, in these TCM hospital or Integrated

Chinese and Western Medicine Hospital, TCM has been widely used for the treatment of NCP (Fig. 1). Importantly, the effect of TCM on NCP is prominent. As shown in Fig. 2, “Qing Fei Pai Du Tang” (QFPDT) is screened out by the National Administration of Traditional Chinese Medicine (NATCM) and widely recommended nationwide [3] . The QFPDT mainly derived from 4 different classical prescriptions originated from Shang Han Lun. After treatment of QFPDT, it was reported that the overall response rates were over 90% among of 214 cases in clinic (Fig. 1). In addition, doctors of TCM still will diagnose the syndrome through comprehensive analysis of symptoms and signs, then further adjust the specific treatment for each patient. Therefore, TCM has different effect on the distinct stages of NCP. TCM prevents infection for healthy person It is well known that vaccine is the highly effective method to prevent epidemics. Unfortunately, a vaccine will take over half a year from development to approval. Although plentiful research institutes have been carrying on the research of vaccine for NCP, there is no approved vaccine for clinic at present. However, TCM has rich clinical experience in many diseases including infection, plague, emergency and so on as shown in Table. 1. Abundant Chinese medicinal formulae and Chinese patent medicine can effectively prevent healthy person from infection. As a number of studies have shown [12-14] , TCM can activate immune cells, improve phagocytosis and induce the production of cytokines. Ultimately, TCM enhance the immunocompetence of healthy person preventing infection. In 2003, TCM was successfully applied to prevent SARS in many areas of China. Therefore, a lot of hospitals and Chinese medical specialist have issued prescriptions of TCM for healthy person in defensing NCP. TCM improves symptoms for patients with mild symptoms According to the current clinical diagnosis [15] , patients at an early stage are often with fever, dry cough and fatigue, but part of the patients have suffocating, the existence of lung scattered in the exudation and other symptoms. Luckily, there exists rich clinical experience of improving these symptoms in TCM. During the treatment of NCP, not only these TCM hospitals but also those western medical hospitals almost all adopt TCM treatment plan to improve symptoms. As the the oretical foundation, TCM can diffuse the lung, outthrust the pathogen, resolve turbidity with aroma, clear heat, remove toxicity, calm panting and resolve phlegm. Meanwhile, the treatment of TCM also has a better effect on anxiety, restlessness and other emotional problems. Finally, the symptoms were improved and the pathogen was prevented from deteriorating, shortening period of treatment and promoting patient recovery. TCM controls the state for critical patients For critical patients with NCP, TCM can alleviate pulmonary effusion, inhibit the release of inflammatory factors, control oxygen saturation, reduce respiratory support and the use of antibiotics [16] . When critical patients have dyspnea and a significant decrease in oxygen saturation, respiratory support is needed and even invasive mechanical ventilation is required. On the basis of western medicine, intervention of TCM will control oxygen saturation stable, improve dyspnea and inhibit the release of inflammatory factors. Therefore, Shenmai injection, Shenfu injection and Xuebijing injection were widely used in clinical practice. Seriously, the worsening state will often result in the injury of organs and limited therapeutic effect of symptomatic and supportive treatment. At that time, some TCM therapy can clear the heart and open the orifices, tonify qi and yin, extinguish wind and increase humor. At last, TCM improves immune function to protect organ and correct electrolyte disturbance to reduce microcirculation disturbance and tissue fibrosis. In brief, TCM controls the state for critical patients via alleviating pulmonary effusion and inhibiting inflammatory overreaction. TCM facilitates the rehabilitation process for convalescent patients For NCP, the negative of nucleic acid detection is the key indicator of cure. But fatigue, cough, poor mental state and other symptoms are still present [17] . Especially, the changes of patients' lung function and clinical symptoms are not symmetrical and synchronized. In fact, the negative patients are not healthy person, since they have no infectivity but need further recover. Usually, convalescent patients with NCP also have inflammation to be absorbed only after the nucleic acid detection turning negative. In the recovery, continued TCM treatments will reinforce the healthy qi and eliminate the pathogenic factors. Obviously, TCM improves the patient's symptoms and promotes the complete repair of damaged organs and tissues. Therefore, convalescent patients often continue to take TCM after the nucleic acid detection turning negative. Conclusion The cure rate of NCP is increasing from 2% in the early days to over 20% in these days. And in partial provinces, the cure rate has exceeded 40% [18] . The application of TCM timely and widely is

one of these positive actions in the fight against this outbreak. In view of the excellent performance of TCM at present, increasing number of patients are being treated with TCM additionally. As reported [19] , about 88% patients with NCP have been receiving the treatment integrated with TCM. Firstly, TCM has accumulated a wealth of experience of prevention and treatment of emerging infectious diseases in ancient time and recent decades. Then TCM still will adjust the specific medicine for each patient after diagnosing the syndrome, providing the more effective and personalized treatment. In addition, TCM will reduce adverse reactions of western medicine (such as antiviral, antibacterial drugs and hormones.) via avoiding or decreasing the use of these drugs in clinic. Therefore, TCM has special advantage in the combat with NCP. However, there are several deficiencies in the development of TCM. As shown in Fig. 1, the national medical team of TCM dispatched to Wuhan timely, but they began to take over the inpatient ward several days later. Because there are no TCM related departments or sufficient Chinese medicine in infectious hospital and western medicine hospital. Obviously, the participation rate of TCM in these hospitals is very low. Secondly, the total number of medical staff of TCM is seriously inadequate in China. Such as this rescue, the medical staff of TCM accounted for less than 20% in the whole dispatched medical staffs from state and province. Moreover, the effect of TCM is not accepted constantly, owing to lack of modern scientific support. Nevertheless, there dozens of TCM for NCP are conducting clinical trials at present, certain specific Chinese medicines are also incorporated (Tabel. 2). As reported [20-22] , several Chinese medicines have exhibited favourable effect. In a word, the development of TCM still has long way to go. On all accounts, TCM has accumulated a solid theoretical foundation of plague. In the fight against infectious diseases broke in recent decades, TCM has played an important role in the prevention and treatment. At present, TCM also has been effectively salvaging the patient with NCP. Therefore, TCM is and will be an effective treatment for NCP.

Table 2 The representative clinical trials of TCM for NCP

No	Registration number	Scientific title	Date of Registration
1	ChiCTR2000029637	An observational study for Xin-Guan-1 formula in the treatment of 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-08
2	ChiCTR2000029628	Observational study of Xin-Guan-2 formula in the treatment of suspected 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-07
3	ChiCTR2000029605	A randomized, open-label, blank-controlled, multicenter trial for Shuang-Huang-Lian oral solution in the treatment of 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-07
4	ChiCTR2000029589	An open, prospective, multicenter clinical study for the efficacy and safety of Reduning injection in the treatment of 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-05
5	ChiCTR2000029487	Clinical Study for Gu-Biao Jie-Du-Ling in Preventing of 2019-nCoV Pneumonia (Novel Coronavirus Pneumonia, NCP) in Children	2020-02-02
6	ChiCTR2000029434	A randomized, open-label, blank controlled trial for Lian-Hua Qing-Wen Capsule/Granule in the treatment of 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-01
7	ChiCTR2000029432	A Real World Study For the Efficacy and Safety of Large Dose Tanreqing Injection in the Treatment of Patients with 2019nCoV Pneumonia (Novel Coronavirus Pneumonia, NCP)	2020-02-01

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13. Ding YW, Zeng LJ, Li RF, et al. The Chinese Prescription Lianhuaqingwen Capsule Exerts Anti-influenza Activity Through the Inhibition of Viral Propagation and Impacts Immune Function. BMC Complementary and Alternative Medicine (2017) 17:130

Abstract Background: Lianhuaqingwen Capsule (LH-C) is a traditional Chinese medicine (TCM) formula used to treat respiratory tract infectious diseases in Chinese. The aim of this study was to determine the antiviral activity of LH-C and its immunomodulatory effects on viral infection. Method: The in vitro cytotoxicity and antiviral activity of LH-C was determined by MTT and Plaque reduction assays. Time course study under single-cycle virus growth conditions were used to determine which stage of viral replication was blocked. The effect of LH-C on the nuclear export of the viral nucleoprotein was examined using an indirect immunofluorescence assay. The regulation to different signaling transduction events and cytokine/chemokine expression of LH-C was evaluated using Western blotting and real-time RT-PCR. After virus inoculation, BALB/c mice were administered with LH-C of different concentrations for 5 days. Body-weight, viral titers and lung pathology of the mice were measured, the level of inflammatory cytokines were also examined using real-time RT-PCR. Results: LH-C inhibited the proliferation of influenza viruses of various strain in vitro, with the 50% inhibitory concentration (IC50) ranging from 0.35 to 2 mg/mL. LH-C blocked the early stages (0–2 h) of virus infection, it also suppressed virusinduced NF-kB activation and alleviated virus-induced gene expression of IL-6, IL-8, TNF-a, IP-10, and MCP-1 in a dose-dependent manner. LH-C treatment efficiently impaired the nuclear export of the viral RNP. A decrease of the viral titers in the lungs of mice were observed in groups administered with LH-C. The level of inflammatory cytokines were also decreased in the early stages of infection. Conclusions: LH-C, as a TCM prescription, exerts broad-spectrum effects on a series of influenza viruses, including the newly emerged H7N9, and particularly regulates the immune response of virus infection. Thus, LH-C might be a promising option for treating influenza virus infection. Keywords: Antiviral, Lianhuaqingwen capsule, Influenza virus, Immuno-regulation

Background Influenza virus, as a common respiratory pathogen, causes seasonal epidemics and occasional severe worldwide pandemics. The most recent event including the 2009 H1N1 pandemic ("swine flu"), and

the 2013 H7N9 virus outbreak in China, which led to significant morbidity and mortality [1, 2]. Human influenza virus infections primarily affect the upper respiratory tract, resulting in clinical symptoms, including cough, fever, sore throat, rhinorrhea and congestion, occasionally followed by lower respiratory diseases like pneumonia [3, 4]. It has been reported that pneumonia causes approximately 80% mortality via influenza virus infection [5]. Upon pathogen infection of the respiratory tract, the host immune system is activated to resist and clear the infection. Airway epithelium cells and alveolar macrophages release multiple pro-inflammatory cytokines and chemokines, such as tumor necrosis factor (TNF-α), interleukin-6 (IL-6), interferon (IFN), and other chemokines, including IL-8, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein (MIP). This release results in the attraction and activation of additional inflammatory cells, including macrophages and neutrophils, into the lungs, initiating the innate immune system that is crucial for the clearance and resolution of viral particles [6, 7]. Factors implicated in severe influenza include robust cytokine production, otherwise known as the “Cytokine storm”. This effect has been considered one of the major contributors to the lethal disease caused by the 1918 pandemic strain and H5N1 viruses [8, 9]. Under physiological conditions, anti-inflammatory cytokines regulate the response of inflammation and attainment of equilibrium. However, the double-sided functions of cytokines could either be beneficial or detrimental to hosts. Under pathological conditions in which the balance is disrupted, pro-inflammatory responses may spiral out of control and excessive pro-inflammatory cytokines and inflammatory immune cells may contribute to additional tissue damage and inflammation [10, 11]. Vaccination is the most effective way to prevent influenza infection now. However, the high genetic variability of the virus renders the protection incomplete. In cases of a newly emerging strain, vaccination is only available a few months after the first appearance, leaving the population vulnerable during the crucial early phases of the pandemic [4]. Currently, two classes of antivirals are used as antiinfluenza drugs: amantadine derivatives that blocking the virus-specific M2-ion channel and two neuraminidase (NA) inhibitors: oseltamivir (Tamiflu) and zanamivir (Relenza), both of which are approved by the FDA [12, 13]. Laninamivir was approved for the treatment of influenza in Japan in 2010. These drugs interfere with the activity of viral neuraminidase. In addition, the nucleoside analogues ribavirin and favipiravir (T-705) exhibit a suppressive effect against almost all RNA based human viruses [13]. However, resistant viruses against these prophylactic agents have emerged in recent years. Amantadine resistance has been detected in human and avian H5N1 strains, and an increasing number of clinical strains have been confirmed as resistant NA inhibitors, including oseltamivir and zanamivir [12, 14]. Additionally, all of these therapies are aimed at inhibiting virus propagation and spread; thus, the inflammation resulting from infection and the disease remain untreated. Because the severe outcome of influenza virus infection is associated with the aberrant production of inflammatory cytokines, maintaining the immune system in an appropriately robust condition may be detrimental for the prevention of the severe symptoms of influenza [15, 16]. LH-C composed of 13 herbs was extended from two TCM prescriptions: Maxing Shigan Tang and Yinqiao San. Maxing Shigan Tang was originally described in a classical Chinese book Shanghan Lun of Han Dynasty for the treatments of febrile diseases, it has been prescribed in treating bronchitis, pneumonia and early stage of measles [17]. Yinqiao San from the TCM monograph Wenbing Tiaobian of Qing Dynasty was mainly used for the treatment of “Warm disease” characterized by fever, thirst and headache. LH-C has been used in treating regular seasonal influenza for decades. Recently, A randomized controlled trial for the comparison of LH-C with oseltamivir in therapeutic effects on patients with mild H1N1 infection demonstrated that LH-C has a significant effect on the alleviation of fever, cough, sore throat and fatigue, it also showed comparative therapeutic effectiveness in reduction of illness duration and viral shedding duration [18, 19]. In the present study, we attempted to elucidate the mechanisms of LH-C anti-influenza activity, we examined the effect of LH-C on different influenza virus strains, and further addressed the impact of LH-C on the cell line and BALB/c mice, with particular focus on its anti-inflammation potential. Methods Reagent preparation LH-C (Lot No. B1502001) was provided by Shijiazhuang Yiling Pharmaceutical Co., Ltd. (Shijiazhuang, China). The raw material of LH-C is black powder, comprising 13 ingredients as shown in Table 1. LH-C was dissolved in DMSO to 500 mg/mL and stored at −20 °C prior to use. Serum-free medium or saline was used as the dilution

buffer in the follow-up experiments. Cells and viruses Influenza virus A/PR/8/34 (H1N1), B/Lee/1940, A/Guangdong/GIRD02/09 (H1N1), A/Aichi/2/68 (H3N2), A/Hongkong/1/68(H3N2), A/Duck/Hongkong/Y280/97(H9N2), A/Duck/Guangdong/09 (H6N2), and A/Shanghai/ 01/2013(H7N9) were propagated in the allantoic cavity of chicken eggs.

Table 1 Composition of LH-C

Plant	Family	Weight	Used part
Forsythia suspensa (Thunb.) Vahl	Oleaceae	255 g	Fructus
Ephedra sinica Stapf	Ephedraceae	85 g	Stem
Lonicera japonica Thunb.	Caprifoliaceae	255 g	Flower bud
Isatis indigotica Fortune	Brassicaceae	255	Root
Mentha haplocalyx Briq.	Mentha	7.5 g	Menthol
Dryopteris crassirhizoma Nakai	Dryopteridaceae	255 g	Rhizoma
Rhodiola rosea L.	Crassulaceae	85 g	Rhizoma
Gypsum Fibrosum	–	255 g	–
Pogostemon cablin (Blanco) Benth.	Labiatae	85 g	Whole plant
Rheum palmatum L.	Polygonaceae	51 g	Rhizoma
Houttuynia cordata Thunb.	Saururaceae	255 g	Whole plant
Glycyrrhiza uralensis Fisch.	Leguminosae	85 g	Rhizoma
Armeniaca sibirica (L.) Lam.	Rosaceae	85 g	Seed

Table 2 Antiviral activity of LH-C against influenza viruses

Virus type and strain	LH-C	
	IC_{50} (mg/ml)[a]	SI
A/PR/8/34 (H1N1)	0.51	6.21
A/Guangzhou/GIRD02/2009 (H1N1)	0.71	4.46
A/Aichi/2/68 (H3N2)	0.2	15.85
B/Lee/1940	2	1.59
A/Duck/Guangdong/2009 (H6N2)	1	3.17
A/Duck/Guangdong/Y280/92 (H9N2)	0.42	8.80
A/Shanghai/01/2013 (H7N9)	0.85	3.68

The concentration required for TC_{50} of LH-C was 3.17 mg/ml to MDCK cells based on the MTT assay

[a]Mean of the results from three independent experiments

An oseltamivir-resistant variant of H1N1 influenza virus A/PR/8/34 (H1N1) (H274Y mut) and mouse-adapted influenza virus (A/PR/8/34, H1N1) was propagated in MDCK cells. The virus titers were determined based on a 50% tissue culture infectious dose (TCID50) assay. Madin-Darby canine kidney (MDCK) cells and A549 cells, a human alveolar type II-like epithelial cell line, were cultured in a monolayer in Minimum Essential Medium (MEM) or Dulbecco's modified Eagle's medium (DMEM) respectively, supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 µg/mL), incubated at 37 °C under 5% CO2. In vitro experiments were conducted in a biosafety level 2 containment facility. All procedures involving live H7N9 viruses were conducted at a biosafety level 3 facility. Animals Specific-pathogen-free BALB/c female mice weighing approximately 16 to 18 g were purchased from Guangdong Medical Laboratory Animal Center (Guangzhou, China). The animals were fed a standard laboratory diet and provided water ad libitum. The animal experiments were performed in accordance with the Guidelines of Guangdong Regulation for the Administration of Laboratory Animals. Cytotoxicity assay MDCK cells were plated onto 96-well plates and cultured to reach 80–90% confluence at 37 °C under 5% CO2 for 24 h. The aspirated medium contained various concentrations of LH-C (0.625–20 mg/mL, 100 µL/well), and the cells were further incubated at 37 °C for 48 h. Approximately 20 µL of Methyl Thiazolyl Tetrazolium (MTT) at concentration of 5 mg/mL was added to each well, and the cells were further incubated at 37 °C for 4 h. The medium was subsequently removed, and formazan crystals were solubilized with dimethyl sulfoxide (DMSO) (100 µL/well). The absorbance was measured at 490 nm using a microplate reader [20]. The 50% toxic concentration (TC50) was calculated using the Reed-Muench analysis [21]. Antiviral assay The anti-influenza virus activity of LH-C was examined using cytopathogenic effect (CPE) and MTT assays [22]. Briefly, MDCK cells were seeded onto 96-well plates and infected with 100 TCID50/100 µl of influenza virus at 37 °C for 2 h. The medium was aspirated, and the cells were incubated with 100 µl of serum-free MEM containing 1.5 µg/ml of trypsin, antibiotics and various concentrations of LH-C (0.03125-2 mg/mL) at 37 °C under 5% CO2 for 2–3 days. LH-C was dissolved in DMSO and diluted in culture medium to obtain various final concentrations. The concentration of DMSO in each medium was less than 1%. Approximately 20 µL/well of MTT (5 mg/ml) was subsequently added into each well, and the cells were further incubated for 4 h at 37 °C in a CO2 incubator. The crystallized formazan in the plates was dissolved in DMSO (100 µL/well). The absorbance was measured at 490 nm using a computer-controlled microplate reader (Bio-Rad, Tokyo, Japan) [20]. Plaque reduction assay MDCK cells (5 × 105 cells/well) were plated onto 12-well culture plates and incubated for 24 h. The cells were washed twice with phosphate-buffered saline (PBS) prior to incubation with viruses (including A/PR/8/34 (H1N1), A/Hongkong/1/68 (H3N2), oseltamivir-resistant viruses (H1N1) and A/Guangzhou/GIRD02/09(H1N1) diluted in serum-free MEM containing 1% penicillin and streptomycin for 2 h at 37 °C. After incubation, the cell monolayer was covered with overlay medium containing LH-C and further cultured at 34 °C under 5% CO2 for 72 h. Subsequently, the overlay medium was removed, and the cell monolayer was fixed with 10% formalin, stained with 1% crystal violet, and the plaques were counted [23]. Time course assay MDCK cells in 48-well plates were infected with virus A/PR/8/34 (H1N1) (MOI =0.1) for 2 h. After infection, the supernatant

was removed, and the cells were rinsed twice with PBS. LH-C (2 mg/mL) was added to cells at 0, 2, 4, 6, 8 and 10 h after infection. The time of addition studies were conducted under single-cycle virus growth conditions. The supernatant was harvested at 12 h post infection, and the virus titers were determined in MDCK cells [24]. Indirect immunofluorescence assay MDCK cells were seeded onto 48-well plates (200 µL/well); when the cell culture reached 50% at 37 °C under 5% CO2, the virus A/PR/8/34(H1N1) (MOI =0.1) was infected for 2 h. After incubation, the supernatant was aspirated, the cells were washed twice with PBS, and LH-C (2 mg/mL) was subsequently added to cells, followed by incubation at 37 °C under 5% CO2. At 6 and 8 h post infection, the cells were fixed with 4% PFA in PBS for 30 min at 4 °C The cells were permeabilized with 0.5% Triton X-100 in PBS for 15 min at room temperature and blocked with 5% BSA in PBS for 20 min at 37 °C, adding anti-influenza A virus NP antibody overnight at 4 °C. After further washing, the cells were incubated with FITC-labeled goat anti-mouse IgG at 37 °C for 1 h. The nuclei were stained with DAPI (5 µg/mL), and fluorescence was visualized using a Zeiss Axiovert 135 fluorescence microscope. RNA extraction, reverse transcription, and real-time quantitative PCR (qRT-PCR) The relative gene expression in A549 cells infected with A/Puerto Rico/8/34 H1N1 was analyzed using qRT-PCR. Total RNA was extracted with 1 ml of TRIzolTM reagent (Invitrogen Life Technologies) and dissolved in RNasefree water. One microgram from each RNA extract was used to generate first-strand cDNA using the PrimeScript RT-PCR Kit (Takara Bio) using both oligo (dT) and random primers. qRT-PCR was performed using an ABI7500 system (Applied Biosystems) with the following conditions: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Forward and reverse primers in combination with FAM/TAMRA probes sequences for IL-6, IL-8, IP-10, TNF-α, MCP-1 and GADPH genes were previously published and listed in Additional file 1: Table S1. Relative gene expression levels were calculated as 2-△△CT (Sym et al., [4]). Western blotting A549 cells were inoculated into a culture flask containing DMEM/F12 (1:1) culture medium (HyClone, Thermo Scientific Inc.) supplemented with 10% (v/v) fetal bovine serum (FBS). After growing to over 80% confluence, the cells were seeded onto 6-well plates at a density of 2x105 and subsequently infected with influenza A virus (PR8) (MOI = 0.1) in the absence or presence of different concentrations of LH-C. The cells were washed twice with cold PBS and subsequently lysed in commercial RIPA lysis buffer (Beyotime) containing Complete, Mini, EDTA-free protease inhibitor cocktail (Roche). Protein concentrations were determined using the BCA Protein Assay kit (Pierce) according to the manufacturer's instructions. The proteins were separated using SDS-PAGE and subsequently transferred to PVDF membranes, followed by blocking for an hour at room temperature in 5% nonfat milk in TBST. Following incubation with antibodies against phospho Ser536-NFkBp65, phospho Thr202/Tyr204-ERK, NF-kB, ERK, GAPDH (Cell Signaling) and a secondary HRPconjugated antibody, the immunocomplexes were detected using enhanced chemiluminescence (ECL, Amersham). Mouse inoculation and anti-viral treatment The mice were intranasally infected with 2 MLD50 of mouse-adapted A/PR/8/34 (H1N1) virus in a volume of 50 µL. Groups of mice were orally administered 1300 and 650 mg/kg/day of LH-C solution respectively. The control animals were treated with the solvent only. The drug was administered twice a day (at 12-h intervals) for 5 days. Sample collection, process and detection One set of 14 mice was monitored for weight loss from 3 days before to 15 days post infection of the virus. A second set of animals was sacrificed at 4, 6, and 8 days after infection and the lung samples were harvested. Lung tissues from euthanized mice were extracted and homogenized in MEM containing antibiotics (0.1% penicillin-streptomycin). The obtained specimens were centrifuged at 12,000 rpm for 5 min at 4 °C, aliquoted and stored at −80 °C for further analysis. The lung homogenates were determined according to the virus titer using end-point titration in MDCK cells and real-time RT-PCR for mRNA expression as previously described. Histopathological analysis The lungs were inflated with 10% formaldehyde solution. The tissues were processed for paraffin embedding and cut into 4-µm-thick sections. The tissue samples were subjected to standard hematoxylin and eosin staining. Statistical analysis The data are expressed as the means ± S.E.M. Statistical differences between two groups were determined using Student's t test. For multiple groups, one-way ANOVA analysis was used to compare the means. Statistical analyses were conducted using SAS 9.1. $P < 0.05$ was considered statistically significant. For survival studies, a log-rank (Mantel-Cox) test using GraphPad Prism (GraphPad 5.0 Software) was conducted.

Results In vitro anti-influenza activity of LH-C against different influenza viruses In a first set of experiments we investigated whether LH-C exerts antiviral effect against influenza viruses infection in cultured cells. Therefore the cells were infected with virus and incubated for 72 h with LH-C at various concentrations. For infection, we used different virus strains, including the highly pathogenic avian influenza (HPAI) H7N9 influenza virus A/Shanghai/01/2013 (H7N9), swine-origin influenza A virus A/Duck/Guangdong/2009 (H6N2) and/Duck/Guangdong/Y280/92 (H9N2) and the influenza B virus prototype isolate B/Lee/1940. As shown in Table 2, LH-C exhibited inhibitory activities against several influenza viruses from different human isolates and avian influenza viruses, with IC50 ranging from 0.35 to 2 mg/mL, and a selective index (SI) ranging from 1.56 to 15.6. LH-C showed the best inhibitory effect in A/Aichi/2/68(H3N2) and A/Duck/Guangdong/Y280/92 (H9N2) (SI = 15.85 and 8.80, respectively). Interestingly, LH-C displayed no effect towards B/Lee/1940. The anti-influenza virus activities of LH-C against A/GIRD02/09 (H1N1), A/Aichi/2/68 (H3N2) and A/PR/8/34 (H1N1) (H274Y mut) were examined by plaque reduction assay (Fig. 1). LH-C treatments blocked the early stages of viral replication process To determine the step in the virus replicate cycle that is affected by LH-C, supernatants from cells that were treated with LH-C at different time points pre- and post-infection were analyzed for their content of progeny virus (Fig. 2a) and viral gene expression (Fig. 2b). Interestingly, most prominent reductions of virus titers were obtained in the early stages of viral replication (0–2 h). In the late stages of viral replication (4–8 h), LH-C displayed an insignificant effect. It suggested that the antiviral mechanism of LH-C involves the inhibition of early-stage influenza virus replication. LH-C inhibits nuclear export of virus nucleocapsid protein (RNP) in the infected A549 cells Earlier findings clearly indicated that the inhibition of NF-kB pathway resulted in efficient retention of influenza virus RNP complexes in the nucleus of infected cells without affecting accumulation of viral proteins [25]. Therefore, we investigated whether LH-C could block nuclear export of viral RNP in the infected A549 cells. As shown in Fig. 3, Immunofluorescence staining of the viral NP, which is one of the constituents of the RNP complexes reveals that the export of viral RNP were efficiently impaired in the presents of LH-C.

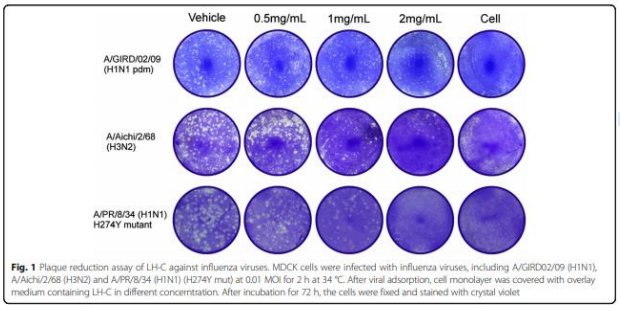

Fig. 1 Plaque reduction assay of LH-C against influenza viruses. MDCK cells were infected with influenza viruses, including A/GIRD02/09 (H1N1), A/Aichi/2/68 (H3N2) and A/PR/8/34 (H1N1) (H274Y mut) at 0.01 MOI for 2 h at 34 °C. After viral adsorption, cell monolayer was covered with overlay medium containing LH-C in different concentration. After incubation for 72 h, the cells were fixed and stained with crystal violet

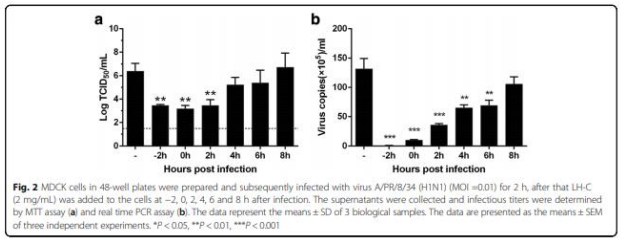

Fig. 2 MDCK cells in 48-well plates were prepared and subsequently infected with virus A/PR/8/34 (H1N1) (MOI =0.01) for 2 h, after that LH-C (2 mg/mL) was added to the cells at −2, 0, 2, 4, 6 and 8 h after infection. The supernatants were collected and infectious titers were determined by MTT assay (**a**) and real time PCR assay (**b**). The data represent the means ± SD of 3 biological samples. The data are presented as the means ± SEM of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

LH-C inhibits influenza A virus-induced NF-κB activation in A549 cells A variety of cellular signal pathways can be triggered in response to influenza A virus infection [26, 27]. The activation these signal pathways can be manipulated by viruses for efficient replication or the induction of host defense mechanisms against invading viruses [28]. Thus, we examined the effect of LH-C on different signaling transduction mechanisms after influenza A virus infection. Viral infection significantly increased the activation of NF-κB and Raf/MEK/ERK signaling in human alveolar epithelial (A549) cells (Fig. 4, Lane 1, 3). LH-C treatment suppressed influenza A virus-induced NF-kB activation but not the Raf/MEK/ERK cascade in virusinfected cells over a 24-h period (Fig. 4, Lane 3). LH-C inhibits influenza A virus-induced cytokine/chemokine

expression in A549 cells NF-κB pathway plays as a major regulator of cytokine and chemokine expression after influenza virus infection [28]. The overexpression of cytokines and chemokines induced through influenza virus infection depends on the NF-κB signaling pathway. A previous study showed that influenza A/PR/8 virus infection with specific NFκB inhibitor treatment decreased the production of inflammatory cytokines, including IL-8 and IL-6 [29]. To examine the NF-kB inhibition effect of LH-C on virusinduced inflammatory responses, we used qRT-PCR to measure cytokine/chemokine expression in A549 cells infected with PR8 (MOI = 0.1) at 24 h p.i. As shown in Fig. 5, virus infection induced a robust increase in the gene expression of IL-6, IL-8, TNF-α, IP-10, MCP-1, whereas LH-C treatment exhibited a prominent inhibitory effect in a dose-dependent manner.

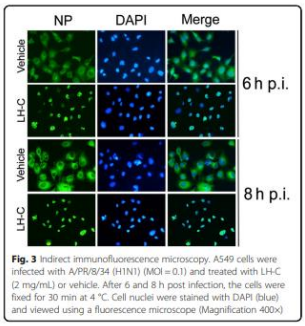

Fig. 3 Indirect immunofluorescence microscopy. A549 cells were infected with A/PR/8/34 (H1N1) (MOI = 0.1) and treated with LH-C (2 mg/mL) or vehicle. After 6 and 8 h post infection, the cells were fixed for 30 min at 4 °C. Cell nuclei were stained with DAPI (blue) and viewed using a fluorescence microscope (Magnification 400×)

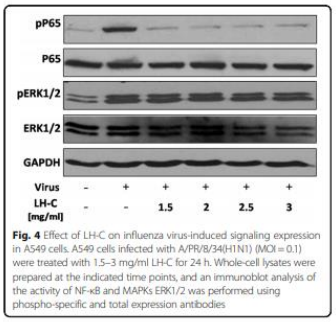

Fig. 4 Effect of LH-C on influenza virus-induced signaling expression in A549 cells. A549 cells infected with A/PR/8/34(H1N1) (MOI = 0.1) were treated with 1.5–3 mg/ml LH-C for 24 h. Whole-cell lysates were prepared at the indicated time points, and an immunoblot analysis of the activity of NF-κB and MAPKs ERK1/2 was performed using phospho-specific and total expression antibodies

Administration of LH-C exhibits antiviral functions in vivo To confirm the viral inhibitor properties of LH-C, groups of mice are inoculated with A/PR/8 (H1N1) virus in 2 MLD50 and subsequently administered LH-C and placebo, respectively. At 2, 4, 6 and 8 days post infection; the lungs were then collected from infected mice (3 mice per day) for the detection of the virus titer. As is shown in Fig. 6, A decrease (>2 log) of the viral titers in the lungs of mice by TCID50 was observed in groups administered LH-C (1300 mg/kg/day) compared with placebo at 6 and 8 days post challenge. Low-dosage LH-C treatment groups also presented reduced lung viral titers, although these values were not significantly different from those of the control group. These results suggested that LH-C has potential antiviral activity against influenza A viruses in mice. Administration of LH-C reduced the inflammatory response in the lungs of mice after influenza A virus infection To determine the properties of LH-C in modulating cytokine production during influenza virus infection, we examined the level of cytokines in the lungs of infected mice at 4, 6 and 8 days post infection. As shown in Fig. 7, the expression of pro-inflammatory cytokines (TNF-α and IL-6) and chemokines (KC and MCP-1) was strongly reduced in the presence of LH-C from 4 to 6 days post infection. The levels of IL-1β and IP-10 were also suppressed in a dose-dependent manner. Administration of LH-C reduced lung pathology induced by influenza infection Lung histology was examined to determine whether LHC treatment alleviated lung pathology resulting from influenza infection. As shown in Fig. 8, using hematoxylin and eosin (HE) staining, the lung tissues of infected animals showed considerable inflammation, with cell exudates in the lung parenchyma and small airways. Following LH-C treatment, the significant eradication of perivascular inflammation and fewer cell exudates were observed. Considering previous results showing viral loads following LH-C administration, these results suggested that LH-C treatment improved the lung pathology of influenza-infected mice. Discussion LH-C has been widely used for more than 10 years in China. Previous studies have confirmed the curative effects of LH-C on several diseases, such as acute bronchitis, asthma, and COPD. A randomized controlled trial for the comparison of LH-C with oseltamivir in therapeutic effects on mild H1N1 infection demonstrated that LH-C has a comparative effect in viral clearance and performs even better in symptom relief [18]. However, the mechanism of LH-C action remains unclear. In this study, demonstrated that LH-C could inhibit different strains of influenza viruses, including HPAI A (H7N9) virus, the newly emerged A (H1N1) pdm09 virus and oseltamivir-resistant viruses (A/PR/8 H274Y). Treatment with LH-C following infection had an inhibitory effect on plaque reduction of the human or avian influenza viruses tested. Additionally, LH-C inhibited viral replication when added between 0 and 6 h, and particularly 0–2 h, after infection. Similar to all other viral pathogens, influenza virus utilizes the host cellular machinery to support replication. NF-kB pathway plays an

important role in the maintenance of host defense responses [30], independent studies have demonstrated that the pathway is critical for the efficient replication of influenza virus. The results of our experiments that LH-C could suppress A/PR/8/34 virusinduced phosphorylation of p65 in cells. Previous reports have demonstrated that viruses support NF-kB-dependent expression of proapoptotic factor, FasL and TRAIL, which activates caspases that subsequently regulate the nuclear export of the viral RNP complexes. Here, we demonstrated that LH-C could block the nuclear export of the viral RNP regardless of the virus-induced activation of Raf/MEK/ERK pathway, indicating that the antiviral effect of LH-C was predominantly via its NF-kB inhibiting activity to suppress viral RNP export and subsequent viral propagation. The NF-kB signaling pathway is not only involved in viral replication but is also the main regulator of cytokine and chemokine production in general and particularly during severe influenza infections [31–33]. We also demonstrated that the levels of cytokine/ chemokine mRNA (including IL-6, 8, MCP-1 MIG, and IP-10) in infected cells were reduced in the presence of LH-C, indicating the regulatory activity of LH-C in an NF-kB-dependent manner. Blocking the NF-kB pathway as a potent strategy in influenza treatment has recently been considered, as this strategy will not only block virus propagation but also inhibit the development of related inflammation [25–29]. Previous studies have reported that NF-kB inhibitors show a considerable protective effect in mice against HPAI A virus infection [30], indicating the availability of NF-kB inhibitors for the treatment of HPAI virus. Inflammatory cytokines and chemokines are produced during influenza virus infection. However, the multiple functions of cytokines could either be beneficial or detrimental to virus-infected hosts. To assess whether the antiviral and anti-inflammation properties of LH-C observed in cell culture would also be relevant in vivo compared with the placebo group, a notable pattern of regulation with cytokine was observed in LH-C-treated mice, particularly in NF-kB-dependent cytokines. We observed that the production of pro-inflammation TNF-α and IL-6 and the immunoregulatory IFN-β, MCP-1 and KC were significantly decreased later in infection (at 6 or 8 dpi) compared with non-treated mice, indicating the accelerated recovery from the immunity situation resulting from infection. The adoptive concentrations (650 and 1300 mg/d) used in the in vivo study were based on the practical concentrations used in humans, though these concentrations were higher than conventional medicines, the results still showed no toxic side effects in mice, which is still in the range of the safety dose. In conclusion, LH-C might be a promising option as a new antiviral agent to fight IAV infections.

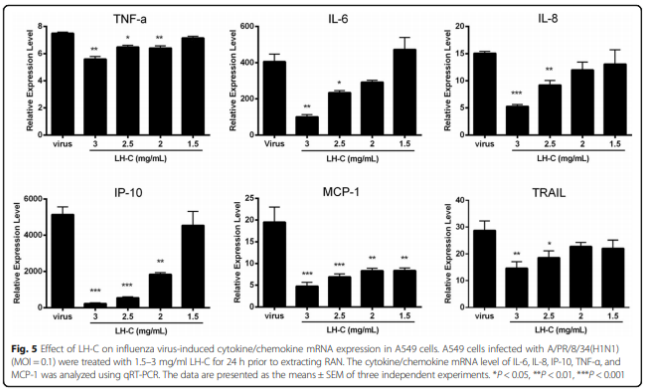

Fig. 5 Effect of LH-C on influenza virus-induced cytokine/chemokine mRNA expression in A549 cells. A549 cells infected with A/PR/8/34(H1N1) (MOI = 0.1) were treated with 1.5–3 mg/ml LH-C for 24 h prior to extracting RAN. The cytokine/chemokine mRNA level of IL-6, IL-8, IP-10, TNF-α, and MCP-1 was analyzed using qRT-PCR. The data are presented as the means ± SEM of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

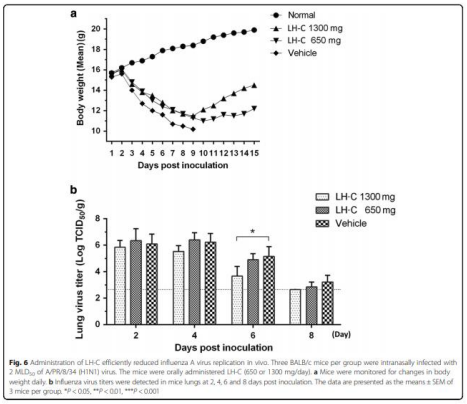

Fig. 6 Administration of LH-C efficiently reduced influenza A virus replication in vivo. Three BALB/c mice per group were intranasally infected with 2 MLD_{50} of A/PR/8/34 (H1N1) virus. The mice were orally administered LH-C (650 or 1300 mg/day). **a** Mice were monitored for changes in body weight daily. **b** Influenza virus titers were detected in mice lungs at 2, 4, 6 and 8 days post inoculation. The data are presented as the means ± SEM of 3 mice per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

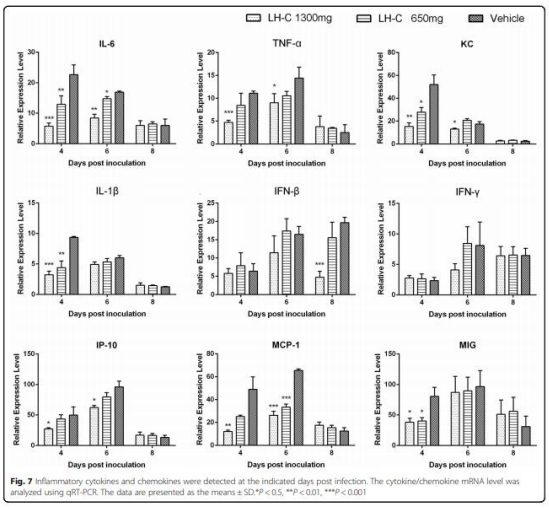

Fig. 7 Inflammatory cytokines and chemokines were detected at the indicated days post infection. The cytokine/chemokine mRNA level was analyzed using qRT-PCR. The data are presented as the means ± SD.*$P < 0.5$, **$P < 0.01$, ***$P < 0.001$

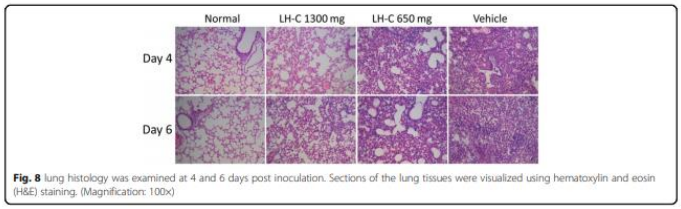

Fig. 8 lung histology was examined at 4 and 6 days post inoculation. Sections of the lung tissues were visualized using hematoxylin and eosin (H&E) staining. (Magnification: 100×)

Conclusions

LH-C, as a TCM prescription, has shown a broad spectrum of effects on a series of influenza viruses, including the newly emerged H7N9. LH-C exerts its anti-influenza activity by interfering with both viral and host reactions. Specifically, LH-C regulates the immune response of virus infection. Thus, LH-C might be a promising option in treating Influenza disease.

14. Fan T, Chen Y, Bai Y, Ma F, Wang H, Yang Y, Chen J, Lin Y .Zhejiang Da Xue Xue Bao Yi Xue Ban. 2020 May 25;49(2):260-269.

Abstract

Objective: To analysis the medication characteristics of the prescriptions issued via open channel by the National and Provincial Health Committee and the State Administration of Traditional Chinese Medicine in treating coronavirus disease 2019 (COVID-19).

Methods: We collected the data of traditional Chinese medicine related to treatment plans published by the National and Provincial Health Committee and the State Administration of Traditional Chinese Medicine from the start of COVID-19 outbreak to February 19, 2020. The frequency analysis, cluster analysis and association analysis were performed.

Results: The study collected 4 national and 34 regional prevention and treatment plans, 578 items, 84 traditional Chinese formulations, 60 Chinese patent medicines, and 230 Chinese herbs. The high frequently used herbs were *Liquorice, Scutellariabaicalensis, Semen armeniacaeamarae*, and *Gypsum*. The commonly used traditional formulations included *Maxing Shigan* decoction, *Yin Qiao* powder, and *Xuanbai Chengqi* decoction. The Chinese patent drugs included *Angong Niuhuang* pill, *Xuebijing* injection,

and *Lianhua Qingwen* capsule. The most common paired medications were *Ephedra* and *Semen armeniacaeamarae, Fructusforsythiae* and *Liquorice.* Two core combinations and one novel formula were discovered in the study.

Conclusions: *Yin Qiao* powder and *Huopo Xialing* decoction are the basic formulations for *Weifen* syndrome of COVID-19. In addition, *Maxing Shigan* decoction, *Liang Ge* powder, *Qingwen Baidu* decoction and *Da Yuan* decoction are the basic formulations for *Qifen* syndrome of COVID-19. The main medication characteristics are clearing heat, entilating lung, removing toxicity and removing turbidity. It shows that removing toxicity and eliminating evil are the prescription thought in treating epidemic disease of traditional Chinese medicine.

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Abstract

The elderly are at high risk of contracting respiratory infectious diseases, including COVID-19 infection. The recent pandemic has the potential to cause significant physical and mental damage in older adults. Similarly to other mind-body exercises in Traditional Chinese medicine, Qigong features regulation of breath rhythm and pattern, body movement and posture, and meditation. Given these traits, Qigong has the potential to play a role in the prevention, treatment, and rehabilitation of respiratory infections, such as COVID-19. Potential mechanisms of action include stress reduction, emotion regulation, strengthening of respiratory muscles, reduction of inflammation, and enhanced immune function. Three forms of Qigong; abdominal breathing, Ba Duan Jin and Liu Zi Jue, all of which are gentle, smooth, and simple for the elderly to practice, are recommended in this context.

INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19), that was first reported by local health facilities in Wuhan, China, in December 2019, has rapidly spread throughout the world. Older people and patients with existing medical conditions are prone to have severe complications associated with Covid-19 infection.[1] Clinical practitioners and researchers are working to find effective treatments for COVID-19, and complementary and alternative medicine may be a feasible and valuable option. In China, Traditional Chinese medicine (TCM) and Qigong have played important roles in the battle aga#inst this disease. The Chinese herbs Qingfei Paidu decoction have been recommended by the National Health Commission of China as a treatment for COVID-19 infected patients with mild to moderate symptoms.[2] For COVID-19 infected patients with severe respiratory symptoms, the herbs were used in combination with Western medicine. In the Wuhan Fangcang Hospitals, field hospitals for the isolation of mild cases instead of home quarantine, patients practiced Ba Duan Jin Qigong under the guidance of TCM doctors for treatment and exercise.[3] This review discusses the application of Qigong in older people for the prevention, treatment, and rehabilitation of respiratory infectious diseases, including COVID-19.

Methods

Studies for inclusion were identified by querying Pubmed, the China national knowledge infrastructure, the China Science and Technology Journal Database, and Wanfang data. The main mechanisms for the occurrence and development of COVID-19 are immunosuppression and cytokine storm.[4] COVID-19 patients with severe symptoms may develop respiratory impairment and need rehabilitation, including respiratory muscle training, whole-body movement and psychological rehabilitation.[5] We searched in the above databases using terms to address the potential mechanism of Qigong in the prevention, treatment and rehabilitation of COVID-19, including “immune function,” “inflammation,” “cytokine,” “respiratory muscle,” “stress,” “mood” and “emotion,” combined with terms which address different types of Qigong: “Qigong,” “Qi Gong,” “Tai-Chi,” “Tai Chi,” “Taichi,” “Taiji,” “Yi Jin Jing,” “Yijinjing,” “Wu Qin Xi,” “Wuqinxi,” “Liu Zi Jue,” “Liuzijue,” “Ba Duan Jin,” “Baduanjin,” as well as “abdominal breathing” and “abdominal respiration.” Given that clinical studies on the intervention of Qigong for COVID-19 are limited, we used the terms “respiratory infection” and “respiratory rehabilitation,” combined with terms mentioned above to search for clinical evidence about the application of Qigong in the treatment and rehabilitation of respiratory infection. The abstract and the full text of each article were reviewed and included if it identified as clinical research or as a clinical systematic review published in English or Chinese.

Understanding Qi and Qigong

"Qigong" is composed of two Chinese characters “Qi” and “Gong.” "Qi" refers to the energy that motivates human life activities, and "Gong" refers to the regulation of Qi through practice.

The concept of Qi in TCM is very broad, and it is involved in nearly all physiological and pathological processes.[6] According to its different functions, Qi can be divided into different types, for example defensive Wei Qi, and the organ Qi that regulates the function of each organ. The channels through which Qi moves in the body are called meridians, which are distributed on the surface of the limbs and trunk and extend to the inside organs.

Qigong is a mind-body training skill that can regulate body, breath and mind under the guidance of theory of TCM to guide Qi operation in the meridian, to regulate physical function, and to prevent and treat diseases.[7] Qigong regulates the body through an adjustment of body movement and posture. Qigong's body regulation is aimed at relaxation, so the movements are typically gentle and smooth. Regulation of breath involves changes in respiratory movement, rhythm, and pattern. Breath in Qigong needs to be slow, long, and deep. Sometimes changes in breath pattern are also required, such as abdominal breathing, and breathing with phonation, both of which are typical patterns of Qigong respiration. Abdominal breathing refers to a breathing pattern with obvious abdomen movement, and breathing with phonation is a combination of breath and the production of speech sounds. Regulation of mind includes focusing attention and visualization. Most operations of mind regulation are similar to meditation, therefore Qigong is also considered a meditative movement.[8]

Qigong originated in the primeval time of China as a means of self-care. According to the first historical record in China “Shang Shu,” 4,000 years ago, ancient Chinese people found that stretching and dancing could release pain. This is the rudiment of Qigong. Almost all religions and philosophical schools, such as Taoism, Buddhism, TCM, and martial arts, have elements of Qigong practice methods, with different appellations. In the 1950s, experts and scholars reached a consensus and coined this methodology "Qigong", and the first Qigong institute was established in China in 1954.

Many studies on Qigong have been carried out through modern research methods, including the observation of physiological and psychological changes during or after Qigong practice, along with clinical trials of treating various diseases with Qigong. Qigong is particularly appropriate for older people due to its gentle and smooth movements, and there are wide applications of Qigong in geriatric medicine,[9] including in the treatment of musculoskeletal disorders, pain relief, and muscle strengthening. As a mind-body skill, Qigong has been found to impact internal and psychosomatic diseases, such as asthma, hypertension, peptic ulcers and diabetes. Qigong is also used as a meditative movement for treating geriatric mental conditions including mood disorders and cognitive impairment.[10,11]

Classification of Qigong

According to different operations, Qigong techniques can be divided into two groups: dynamic or active Qigong, and passive or meditative Qigong. Dynamic Qigong refers to those techniques that primarily focus on body movements and involve more movements of the whole body or limbs. Tai-chi, Yi Jin Jing (Muscle Change Classic), Wu Qin Xi (Five-animal Exercise), Liu Zi Jue (Six-Healing Sounds), and Ba Duan Jin (The Eight Brocades) are examples of dynamic Qigong that have gained worldwide popularity. Contrastingly, passive Qigong techniques have almost no body movement, but require maintaining a certain posture and carrying out exercises mainly involving the breath and mind.

Dynamic Qigong is more successful than passive Qigong with regards to physical regulation, therefore it can be more effective in treating musculoskeletal and psychosomatic disease. Practitioners who have difficulty focusing their attention can concentrate on movements and actions in dynamic Qigong, which is an easier skill to master. Passive Qigong pays more attention to mind regulation. Attention training is an important and common technique of mind regulation that asks practitioners to focus attention on an object or on the present, which is similar to mindfulness meditation. According to theory of TCM, through extensive practice of focusing attention, practitioners can enter a state of tranquility. Passive Qigong has few requirements for physical strength, as it can be practiced in any posture without movement. In addition, for those with impaired body movement ability, passive Qigong is a better choice than dynamic Qigong. A study on mindfulness, conducted by Lacaille et al.,[12] indicated that prolonged mindfulness practice was associated with an increase in mindful responding, which was in turn associated with increased positive affect and with less perceived stress and negative affect. Thus, those who engage in extensive practice of passive Qigong may be likely to experience better psychological outcomes.

How Qigong Can Treat Respiratory Infective Disease Utilizing TCM Theories

Respiratory infectious diseases belong to the category of external pathogens diseases in TCM. Its pathogenesis is that external pathogens invade the human body and produce tension in the balance between “good and evil.” The “evil” refers to exogenous pathogens, which can be considered similar to the pathogen of infection. “Good” refers to the defensive function of the human body. When exogenous pathogens invade the human body, defensive Wei Qi fights against them. It can be considered that Wei Qi represents immune function from the perspective of modern medicine. The relationship between Wei Qi and exogenous pathogens determines whether the disease will develop and the prognosis of the disease. If Wei Qi is strong enough to defend against the exogenous pathogen, the disease would not occur, or would be easier to heal, and the prognosis would be good.

Because of a decline in organ function and an increase in chronic medical conditions, older people are considered to be in a state of weakness or insufficient energy, conceptualized as Qi and blood deficiency in TCM. Wei Qi is thought of as being scarce in the elderly. Therefore, according to theories of TCM, when encountering infectious diseases such as COVID-19, the elderly are more likely to be affected, and infections are more likely develop into severe diseases with poor prognoses. Given that Qigong regulates the function of Qi in the human body, in which Wei Qi is included, it may prevent respiratory infection or promote recovery from respiratory infection in the elderly.

Potential Mechanisms of Qigong in Respiratory Infectious Diseases

1. Management of Stress and Emotion

Outbreaks and illness are a source of stress, and stress reactions or emotional problems can occur in hospital inpatients, people in isolation, and those in the general population. Benson et al.[13] observed physiological changes during meditation, and found that meditation can counteract stress response. Benson coined the physiological change elicited by meditation a “relaxation response.” As a meditative movement, Qigong has been studied as a tool for stress management. Ryu et al.[14] observed changes in stress hormones during Qigong practice, and found that beta-endorphins increased in the middle of training while levels of adreno-cortico-tropic-hormone declined mid and postpractice suggesting decreased stress levels. It has been suggested that Qigong regulates emotion through enhancing nonreactivity to aversive thoughts and impulses by focusing attention, regulating the hypothalamus-pituitary-adrenal axis reactivity and the balance of the autonomic nervous system, and through changing the function of the brain, limbic system, and expression of genes linked to inflammatory responses and stress-related pathways.[15] In a meta-analysis on treating chronic obstructive pulmonary disease (COPD) with Qigong, Wu et al.[16] reported that Qigong alleviated depression and anxiety among patients with COPD. Meanwhile, during the Severe Acute

Respiratory Syndrome outbreak, practicing Qigong was found to strengthen individuals' sense of control, and taking part in a practicing group improved their senses of social support.[17,18]

2.

Strengthening the Respiratory Muscles

Qigong can enhance physical strength through the training of specific muscle groups. Liu et al.[19] found through measuring grip strength, jumping height, and toe contact test in older people that Qigong can increase muscle strength. With regards to respiratory muscles, studies in stroke patients found that the myoelectricity and activity of the diaphragm increased after a 3-month abdominal respiration training when compared with thoracic breathing.[20] In COPD patients, Wu et al.[21] observed improvement of respiratory muscle strength after 3-month Liu Zi Jue practice.

3.

Reducing Inflammation

Qigong can reduce both inflammatory factors and inflammatory response. Irwin et al.[22] examined the cytokines in older adults who had participated in a 6-month Tai-Chi program, and found reductions in levels of IL-6 in subjects in the intervention group who previously showed high levels of this inflammatory marker. In another study, Irwin et al.[23] found that in older adults with insomnia, Tai-Chi reduced proinflammatory gene expression and marginally reduced C-reactive protein by the end of the 4-month practice, and reduced monocyte production of proinflammatory cytokines at the end of the program and at the follow-up after 7 and 16 months, when compared to the control group. Additionally, a 12-week program of Tai-Chi has been found to increase levels of the anti-inflammatory cytokine IL-10 in middle-aged adults.[24] Chen et al.[25] found that in COPD patients, 60-day Liu Zi Jue practice lowered the level of IL-4,IL-13 and IL-17, and increased the level of IL-10 when compared to a regular treatment control group.

4.

Enhancing the Immune Function

Qigong's enhancement of immune function has manifested in both nonspecific immune response and specific immune response. Regarding nonspecific immune response, Qigong can increase the amount or activity of immune cells in the body. Yeh et al.[24] found that in middle-aged healthy people, after a 12-week program of Tai-Chi practice, there was a significant increase in the ratio of T helper to suppressor cells (CD4/CD8), CD4CD25 regulatory T cells, and the production of the regulatory T cell mediators transforming growth factor β. Qiu et al.[26] studied a sample of elderly people using Ba Duan Jin and found CD4, CD4/CD8 and NK cell percentage increased after a 24-week program when compared to a control group who received health education without exercise. Yu et al.[27] observed the effect of Wu Qin Xi on NK-cell activity in elderly adults tested by lactate dehydrogenase release assay, and found that the activities of NK cells increased after practicing for half a year when compared with a blank control group. Study results indicated that Wu

Qin Xi is a moderate intensity exercise for older people. According to Nieman,[28] moderate exercise can lower the risk of respiratory tract infection, and heavy exercise can increase the risk of infection.

The effect of Qigong on specific immune response can be observed in the increase of immune cells and immunoglobulin. Chiang et al.[29] selected 20 sedentary males as subjects, and found that Tai-Chi increased the number of circulating myeloid dendritic cells when compared with a blank control group, but that plasmacytoid dendritic cells remained the same in both groups. The degree of growth in myeloid dendritic cell significantly increased with the years of practice. Vera et al.[30] examined the acute effect of Qigong on adults who participated in a 1-month-Taoist Qigong program. The researchers took blood samples the day before the experiment commenced and 1 hour after the last session of the training program ended, and found higher values in the number and the percentage of B lymphocytes, as compared with a control group that did not engage in the practice. Niu[31] investigated the effect of Tai-Chi on middle-aged participants and observed that blood IgA, IgG and IgM levels increased significantly in the Tai-Chi exercise group with increasing exercise time.

The promotion of Qigong in the specific immune response has also been reflected in the level of antibodies after vaccination. In Irwin's study,[32] participants took part in Tai-Chi or health education for 25 weeks, and after 16 weeks of intervention, subjects were vaccinated with Varivax. They found the Tai-Chi group showed higher levels of cell-mediated immunity to VZV than the health education group. In Yang's study,[33] older adults practiced Tai-Chi and Qigong for 5 months and then received the influenza vaccine during the first week of the intervention. Improvement of the magnitude and duration of the antibody response to influenza vaccine were observed when compared to the control group.

Clinical Evidence on Qigong in Respiratory Infectious Diseases

1.

Application of Qigong to the Prevention of Respiratory Infectious Diseases

Some studies have demonstrated the effectiveness of Qigong in preventing respiratory infectious diseases. Hu et al.[34] selected elderly men as experimental subjects and randomly divided participants into either a Qigong intervention group or a control group who performed jogging. Compared with the control group, the experimental group experienced significantly fewer respiratory tract infections after Qigong exercise for two years, and the difference between the two groups increased with exercise time. Wright et al.[35] found that in swimmers who practiced Qigong at least once per week, cold and flu symptoms showed a significant nonlinear association with frequency of Qigong practice, with a strong, inverse relationship between practice frequency and symptom scores.

2.

Use of Qigong in Treating Respiratory Infectious Diseases

There are few studies on the intervention of Qigong in the acute phase of respiratory infection, but according to limited research results Qigong can be found to shorten the course of infection. In Ties' study,[36] 90 female healthy students were separated into three groups; a control group, a three times a week movement group, and a five times a week movement group, after a six-month Tai-Chi training. In the two Tai-Chi groups, the levels of IgA and IgG became higher, as compared with the control group. There was no difference in the frequency of respiratory tract infection in the three groups, however the duration of each onset became shorter in the Tai-Chi group.

3.

Application of Qigong to Rehabilitation in Respiratory Infectious Diseases

Severe respiratory infections can cause reduced respiratory function and require rehabilitation. Although research on the application of Qigong in the rehabilitation of respiratory infections is limited, researchers have shown that Qigong can promote the rehabilitation of other respiratory diseases which cause impaired respiratory function. Tong's meta-analysis[37] on 10 studies examined 993 participants who were in the stable stage of COPD infection. Results indicated that Qigong can improve lung function in COPD patients, including forced expiratory volume in 1s (FEV1), forced vital capacity rate of 1s (FEV1/FVC), and forced expiratory volume in 1s/predicted (FEV1/pre). Additionally, results showed that it can improve exercise capacity, Functional Task Evaluation, COPD Assessment Test for exercise, and increase the score of Short Form-36 Health Quality Survey, which indicates improvement in quality of life. The researchers analyzed the function of Ba Duan Jin, Yi Jin Jing and Liu Zi Jue respectively, and found the first two resulted in significant improvement, and that Liu Zi Jue did not have significant effect. However, other studies have shown Liu Zi Jue to be effective in the rehabilitation of COPD patients. A study by Li et al.[38] indicated that COPD patients' lung function (FEV1/pred, FEV1/FVC) improved significantly with Liu Zi Jue practice, as did the 6-minute walking test, 30-second sit-to-stand test, and St George's Respiratory Questionnaire score. Chen[39] observed that in chronic bronchitis patients who practiced Ba Duan Jin, hospital stays decreased, and lung function (FEV1/FVC) and arterial blood gas analysis (PaO2, PaCO2) improved when compared with the control group who received regular treatment.

How to Learn and Practice Qigong

Some simple Qigong can be learned independently through watching Qigong videos. Before learning Qigong, consulting with doctors is necessary for safety reasons. Learners can begin with physical movements of the forms. After practitioners acquire the sequences of both isometric and isotonic segmental movements in upper and lower extremities, they can try to combine breathing techniques and focus their attention on movement, breath and Qi.

Tips for Practice

Practice environment: Qigong can be practiced both indoors and outdoors, though indoor practice is more appropriate during an epidemic of infectious disease. The practice environment should be clean and quiet

for breathing exercises and concentration. Practice space needs to be chosen according to the type of Qigong being practiced. For patients with fall risks, an instructor who is experienced in working with elderly people may be necessary.

Practice time: Qigong practicing needs to be persistent. Fixed time can help in forming a habit. Being either too hungry or too full is not suitable for practice.

Intensity: Attention needs to be paid to personal condition at each practice, on which intensity should depend. As mentioned above, moderate exercise is the best option for respiratory infection patients. However, what can be considered moderate exercise differs between individuals, thus the increase in heart rate can be a indicator of intensity. Increasing heart rate by 60%-80% is recommended.

Recommending Forms of Qigong

Considering the physiological characteristics of the elderly, the pathological features of respiratory diseases, and the psychosocial factors in the face of the COVID-19 epidemic, we recommend Ba Duan Jin, Liu Zi Jue, and abdominal breathing. According to the research results mentioned above, these three kinds of Qigong are often used in the prevention and treatment of respiratory infections, for the movement is smooth with low intensity, and easy to learn. In addition, the range of these three Qigong movements is small, and the space requirements are not significant. Thus, they are suitable for home practice during the current epidemic.

1. Abdominal Breathing

Abdominal breathing can be found in a variety of mind-body exercises, including yoga, meditation, and Qigong. The technique is very straight forward: consciously move your stomach when inhaling, tightening your stomach muscles, and let them fall inward as you exhale, while focusing attention on breathing. Do not hold your breath.

Abdominal breathing can enhance respiratory function, and create a relaxation response. Abdominal breathing has been found to stimulate the vagus nerve,[40] which can regulate breathing and help with relaxation. Focusing attention on breath is one of the simplest ways to achieve emotional regulation and to decrease anxiety-related dysfunctional thoughts about the pandemic. Abdominal breathing requires the least amount of exercise of the three Qigong exercises recommended, and can be practiced standing, sitting, or lying on the back so that it can meet the needs of practitioners with poor physical conditions or more serious illness. Since abdominal breathing can be practiced anytime, anywhere, and in most physical conditions, it is a highly recommended method for coping with COVID-19 related stress.

2. Ba Duan Jin

Ba Duan Jin exercise consists of eight separate, delicate, and smooth exercise movements, to achieve self-psychosomatic regulation and enhance function. Since Ba Duan Jin exercises emphasize body and Qi, slow body movements along with musculoskeletal stretching should be combined with physical relaxation, deep breathing, and mental concentration.[41] Movements of the whole body can enhance physical well-being. According to TCM theory, stretching the upper limbs, where the lung meridians are, can facilitate Qi moving in the respiratory organs and thereby promote recovery from respiratory symptoms. Deep, rhythmic breathing along with slow bodily movements accompanied by mental focusing frequently leads to a state of meditation, which can produce a relaxation response and stress reduction. We recommend using Ba Duan Jin for the prevention of COVID-19 and treatment of respiratory symptoms if infected, as well as for the management of stress derived from the pandemic.

3.

Liu Zi Jue

Liu Zi Jue combines abdominal breathing and pursed lip breathing with uttering six different sounds, along with corresponding mild-body movements and a calm state of mind. This exercise is easy to learn and can be performed in any position including standing (preferred), sitting, or lying down, since the exercise mainly involves mild upper-body movements.[42]

The type of respiratory pattern of pursed lip breathing performed by expiration to produce six different sounds (xu, he, hu, si, chui, and xi) is similar to the pursed-lips breathing in rehabilitation training for COPD patients.[43] It can modify rapid shallow breathing patterns and retard the expiratory flow rate. Additionally, the different sounds can produce vibrations with different frequencies, which is commonly used in neurorehabilitation[44] and tension relaxation.[45] Research indicates that Liu Zi Jue might help tissue and organs in respiratory recovery through these vibrations. Liu Zi Jue is a good choice for people seeking to recuperate from respiratory dysfunction and sequela of COVID-19 infection.

Conclusions

The available biological and psychological evidence suggest Qigong may be potentially useful for the prevention, treatment, and rehabilitation of respiratory infections, including COVID-19. The elderly, in particular, could benefit from Qigong during the ongoing pandemic, for it is easy to practice. Future studies are needed to confirm the effectiveness of Qigong in this context and to provide more evidence on this topic.

Author contributions

Albert Yeung: Design of the work; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Fan Feng: Literature searching; Preparation of the manuscript.

Sylvie Tuchman: Preparation of the manuscript.

John W. Denninger: Preparation of the manuscript.

Gregory L. Fricchione: Preparation of the manuscript.

DISCLOSURE

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Abstract

Objective: To investigate traditional Chinese medicine (TCM) common syndrome characteristic of coronavirus disease 2019 (COVID-19), thus providing evidence for clinical differentiation.

Methods: The COVID-19 TCM treatment plan and syndrome related literature published before February 24, 2020 was searched and a database was established. TCM common syndrome characteristics of COVID-19

were analyzed by the method of latent structure and system clustering combined with frequency and constituent ratio, which were conducted by Lantern 5.0 and SPSS 20.0.

Results: Forty-two literatures about treatment plans, and 212 syndromes records were enrolled. Latent structure model was established based on 53 symptoms with frequency over 10, and 14 latent variables and 7 syndromes were concluded by comprehensive clustering, including syndrome of pathogenic heat invading lung, internal block and outward desertion, syndrome of dampness heat accumulating lung, syndrome of Qi and Yin deficiency, syndrome of epidemic virus closing lung, syndrome of cold dampness closing lung and syndrome of Qi deficiency of lung and spleen. Factor analysis was conducted for 53 symptoms, which were reported more than 10 times, and 14 common factors were obtained. Symptoms with load coefficient over 0.3 were clustered and 6 syndromes were obtained, including syndrome of epidemic virus closing lung, syndrome of pathogenic heat invading lung, syndrome of Qi and Yin deficiency, internal block and outward desertion, syndrome of cold dampness closing lung, and syndrome of dampness heat accumulating lung. The literatures included 25 syndromes, and the syndromes with constituent ratio over 5% were internal block and outward desertion (14.62%), syndrome of epidemic virus closing lung (13.68%), syndrome of dampness heat accumulating lung (12.74%), syndrome of Qi deficiency of lung (10.85%), spleen and syndrome of cold dampness closing lung (8.50%), syndrome of Qi and Yin deficiency (8.50%), syndrome of pathogenic heat invading lung (8.02%) and syndrome of dampness repressing defensive Qi of lung (5.66%). Eighty-seven symptoms whose cumulative frequency was 2 838 were referred, including greasy fur (5.25%), fever (4.83%), red tongue (4.37%), rapid pulse (3.74%) and fatigue (3.46%). According to the results above, the common syndromes and their symptoms of COVID-19 were: (1) syndrome of pathogenic heat invading lung: fever, cough, throat-drying, headache, all of the body distressed and constipation, etc.; (2) syndrome of cold dampness closing lung: aversion to cold, all of the body distressed, nausea and vomiting, abdominal distention and loose stool, etc.; (3) syndrome of dampness heat accumulating lung: cough, sticky phlegm, anorexia, thirst without desire to drink and constipation, etc.; (4) syndrome of epidemic virus closing lung: fever, cough, yellow phlegm, wheezing, suffocation and purple lips, etc.; (5) internal block and outward desertion: coma, feel fidgety, suffocation, sweating and feel cold and purple lips, etc.; (6) syndrome of Qi and Yin deficiency: dry cough, sweating, fatigue, thirsty, feverish feeling in palms and soles and loose stool, etc.; (7) syndrome of Qi deficiency of lung and spleen: cough, wheezing, sweating, fatigue, nausea and vomiting, and loose stool, et al.

Conclusions: The common syndromes of COVID-19 were syndrome of pathogenic heat invading lung, syndrome of cold dampness closing lung, syndrome of dampness heat accumulating lung, syndrome of epidemic virus closing lung, internal block and outward desertion, syndrome of Qi and Yin deficiency and syndrome of Qi deficiency of lung and spleen. This study could provide reference for clinical differentiation.

17. ***Gao K, Song YP, Chen H, Zhao LT, Ma L. Therapeutic efficacy of Qingfei Paidu decoction combined with antiviral drugs in the treatment of corona virus disease 2019: A protocol for systematic review and meta analysis. Medicine (Baltimore). 2020 May 29;99(22):e20489. doi: 10.1097/MD.0000000000020489.***

Abstract

Background: The corona virus disease 2019 (COVID-19) has caused a global pandemic, there are no specific drugs and vaccines for epidemic control at present. More and more clinical practice shows that traditional Chinese medicine has played an important role in the outbreak. Among them, Qingfei Paidu decoction (QPD) combined with antiviral drugs can enhance the therapeutic efficacy for COVID-19. However, there is still a lack of comprehensive and systematic evidence, which urgently requires us to verify its therapeutic efficacy. Hence, we provide a protocol for systematic review and meta-analysis.

Methods: We will search the studies in MEDLINE/PubMed, China National Knowledge Infrastructure, Wanfang database, VIP database, the Cochrane Library, Chinese Biomedical Database and Chinese Science Citation Database. Searches are limited to clinical studies published in Chinese and English. Next, the quality

of each study is assessed according to the criteria of the Cochrane Handbook for Systematic Reviews of Interventions. Then, the outcome data are recorded and pooled by Review Manager 5.3 and STATA 16.0 software.

Results: The systematic review and meta-analysis aims to review and pool current clinical outcomes of QPD combined with antiviral drugs for the treatment of COVID-19.

Conclusion: This study will provide a high-quality evidence of QPD for the treatment on COVID-19 patients.

1 Introduction

Since December 2019, the sudden epidemic of corona virus disease 2019 (COVID-19) has seriously threatened the healthy life of the people. As of April 25, 2020, there have been approximately 2,840,000 confirmed cases of COVID-19 worldwide, with 200,000 deaths. Among them, a total of 84,330 cases of COVID-19 (including overseas imported cases) were diagnosed in China, with 78,384 cured cases and 4642 deaths. From these data, it is not difficult to find that China's prevention and control of COVID-19 epidemic is beginning to bear fruit, which is not only due to measures such as restricting the flow of people and vigorously publicizing, but also to the important factor of medical assistance. In particular, the widespread use of Traditional Chinese Medicine (TCM) has played a huge role in the prevention and control of this epidemic.[1,2]

During the epidemic, the National Health Commission of the People's Republic of China have formulated and issued “the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version)” based on clinical manifestations and pathology of the disease, as well as accumulated experience in diagnosis and treatment.[3] Among them, Qingfei Paidu decoction (QPD) was included the treatment protocol as a recommended prescription. Its scope of application includes light, moderate, and severe patients, and it can be used reasonably in combination with the actual situation of patients in the treatment of critically ill patients. The prescription consists of Mahuang (Ephedrae Herba), Zhigancao (Glycyrrhizae Radix et Rhizoma Praeparata cum Melle), Xingren (Armeniacae Semen Amarum), Shengshigao (Gypsum Fibrosum), Guizhi (Cinnamomi Ramulus), Zexie (Alismatis Rhizoma), Zhuling (Polyporus), Baizhu (Atractylodis Macrocephalae Rhizoma), Fuling (Poria), Chaihu (Bupleuri Radix), Huangqin (Scutellariae Radix), Jiangbanxia (Pinelliae Rhizoma Praeparatum cum Zingibere et Alumine), Shengjiang (Zingiberis Rhizoma Recens), Ziwan (Asteris Radix et Rhizoma), Donghua (Farfarae Flos), Shegan (Belamcandae Rhizoma), Xixin (Asari Radix et Rhizoma), Shanyao (Dioscoreae Rhizoma), Zhishi (Aurantii Fructus Immaturus), Chenpi (Citri Reticulatae Pericarpium), and Huoxiang (Pogostemonis Herba). QPD has been proved to have a significant therapeutic effect on COVID-19, the effective cure rate of QPD for COVID-19 is more than 90%.[4] However, there is still a lack of comprehensive and systematic evidence. Therefore, we will present a meta-analysis protocol of the therapeutic efficacy of QPD combined with antiviral drugs vs antiviral drugs alone on COVID-19.

2 Materials and methods

The study protocol has been registered on International prospective register of systematic reviews (PROSPERO ID: CRD42020182409). The protocol followed Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) guidelines.[5]

2.1 Data resources and search strategies

We will search the studies in MEDLINE/PubMed, China National Knowledge Infrastructure, Wanfang database, VIP database, the Cochrane Library, Chinese Biomedical Database, and Chinese Science Citation Database. Searches are limited to clinical studies published in Chinese and English. The target databases are searched for capturing all potentially relevant clinical literature by 2 reviewers independently and disagreements are settled by discussion with a third reviewer.

The following search strategies will be used to identify publications: ((Qingfei Paidu Decoction [Title/Abstract]) AND ((((((((((Antiviral Agents [Title/Abstract]) OR (Agents, Antiviral [Title/Abstract])) OR (Antivirals [Title/Abstract])) OR (Antiviral Drugs [Title/Abstract])) OR (Drugs, Antiviral [Title/Abstract])) OR (α-Interferon [Title/Abstract])) OR (Lopinavir [Title/Abstract])) OR (Ritonavir [Title/Abstract])) OR (Ribavirin [Title/Abstract])) OR (Chloroquine phosphate [Title/Abstract])) OR (Arbidol [Title/Abstract]))) AND ((((((((((COVID-19 [Title/Abstract]) OR (2019 novel coronavirus disease [Title/Abstract])) OR (COVID19 [Title/Abstract])) OR (COVID-19 pandemic [Title/Abstract])) OR (SARS-CoV-2 infection [Title/Abstract])) OR (COVID-19 virus disease [Title/Abstract])) OR (2019 novel coronavirus infection [Title/Abstract])) OR (2019-nCoV infection [Title/Abstract])) OR (coronavirus disease 2019 [Title/Abstract])) OR (coronavirus disease-19 [Title/Abstract])) OR (2019-nCoV disease [Title/Abstract])) OR (COVID-19 virus infection [Title/Abstract])).

2.2 Inclusion and exclusion criteria

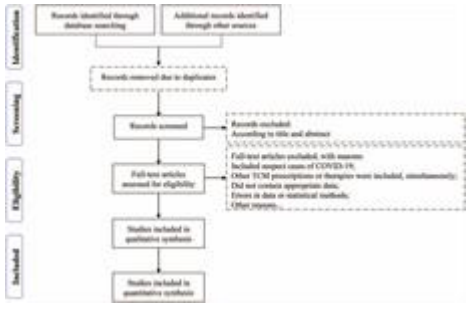
The inclusion criteria were designed as following:

- (1) The clinical trials involved were randomized controlled trials.
- (2) Patients diagnosed with COVID-19 by the following criteria: “the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version)” was formulated and issued by the National Health Commission of the People's Republic of China.
- (3) The patients in the experimental group received antiviral-based therapy with QPD, whereas patients in the control group were treated with antiviral-based therapy only. Here, antiviral therapy was defined as the administration of antiviral chemical drugs, such as α-interferon, Lopinavir, Ritonavir, Ribavirin, Chloroquine phosphate, Arbidol, etc. In addition, 2 groups of patients can be given circulatory support, effective oxygen therapy, and other support therapies.
- (4) The measurement indicators of clinical studies should include one of the following indicators at least: clinical efficacy, relief time of main symptoms (such as fever, cough, and lung CT improved), hematology index (such as the levels of lymphocyte, C-reactive protein, and albumin), and adverse reactions.

We also set exclusion criteria as following:

- (1) The patients were only suspected cases of COVID-19 (not confirmed cases).
- (2) Articles of the following types should be excluded: comments, non-clinical experiments, self-control studies, case reports, random method error studies, and reviews.
- (3) If there were repetitively published clinical literatures, only the latest publications with large sample sizes and more comprehensive studies were included.
- (4) If the treatment of COVID-19 patients involved other TCM prescriptions, TCM patent prescriptions, or acupuncture and moxibustion, it should not be included.

The PRISMA flow chart showed the full screening process (Fig. 1).



2.3 Quality assessment of included studies

Two investigators will evaluate independently the methodological quality and bias risk in each trial using "Assessment of Study Quality" in Cochrane Handbook for Systematic Reviews of Interventions,[6] which includes random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. If there is a disagreement during the evaluation process, it will be resolved through discussion with a third investigator.

2.4 Data collection and analysis

In the process, 2 investigators will extract detailed information and available data from the qualified studies, such as sample size, interventions, duration of intervention, and outcome measures. If there are disagreements during the evaluation process, it will be resolved through discussion with a third investigator. All analyses will be performed with Review Manager 5.3 and STATA 16.0 software.

We will select appropriate statistical methods for meta-analysis according to the data types of each indicator. The dichotomous variables are expressed as a risk ratio (RR) with 95% confidence intervals (95% CI), while the continuous variables are expressed as a mean difference (MD) with 95% CI. Heterogeneity tests are performed by Cochrane's homogeneity test, while I^2 tests are used to quantify the degree of heterogeneity. When $I^2 \leq 25\%$, the data is considered to be homogeneous. When $25\% < I^2 \leq 50\%$, the data has lower heterogeneity, and a fixed effect model is used; when $I^2 > 50\%$, the data has obviously heterogeneity, and a random effects model is used.[7] We also seek possible sources of heterogeneity, and attempt to clarify the causes of heterogeneity through subgroup analysis. The publication bias is explored graphically by funnel plots, and detected by Egger test and Harbord test.

3 Discussion

Due to the highly contagious and limited medical resources, the epidemic has caused high mortality worldwide, and it has been declared a public health emergency of international concern by the World Health Organization.[8,9] Although the existing antiviral drugs have a certain effect, they still cannot be used as special drugs anti-COVID-19,[10] prompting the need for novel treatment options.

However, TCM could effectively alleviate the development of the epidemic due to its own unique advantages. TCM has the characteristics of overall regulation and the treatment is based on syndrome differentiation. For thousands of years, TCM has formed a unique set of theories, diagnostic, and therapeutic systems as the significant means of treating diseases clinically in China. TCM prescription is a material carrier based on the basic theory of TCM to treat diseases. To achieve the treatment effects of pestilence, it is important to balance yin and yang, promote the body resistance, and eliminate pathogenic factors through a multi-component-target-pathway approach.[11] Also, the network pharmacology results showed that the active ingredients from QPD could contribute to recovery of different disease progresses during COVID-19.[12] Through the implementation of this study protocol, we can comprehensively evaluate the efficacy and safety of QPD in patients with COVID-19, thereby providing reasonable complementary therapy for the clinical treatment of COVID-19.

Author contributions

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Writing – review & editing: Yan-Ping Song.

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ABSTRACT

Objective: To seek potential Chinese herbal medicine (CHM) for the treatment of coronavirus : To seek potential Chinese herbal medicine (CHM) for the treatment of coronavirus disease 2019 (COVID-19) through the molecular docking of the medicine with SARS-CoV-2 3CL hydrolytic disease 2019 (COVID-19) through the molecular docking of the medicine with SARS-CoV-2 3CL hydrolytic enzyme and the angiotensin converting enzyme enzyme and the angiotensin converting enzyme Ⅱ (ACE2) as receptors, using computer virtual screening (ACE2) as receptors, using computer virtual screening technique, so as to provide a basis for combination forecasting. technique, so as to provide a basis for combination forecasting. Methods: The molecular docking of CHM with : The molecular docking of CHM with the SARS-Cov-2 3CL hydrolase and the ACE2 converting enzyme, which were taken as the targets, was the SARS-Cov-2 3CL hydrolase and the ACE2 converting enzyme, which were taken as the targets, was achieved by the Autodock Vina software. The CHM

monomers acting on 3CLpro and ACE2 receptors were achieved by the Autodock Vina software. The CHM monomers acting on 3CLpro and ACE2 receptors were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, the retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, the active ingredients were selected, and the key CHMs and compounds were speculated. Based on the perspective active ingredients were selected, and the key CHMs and compounds were speculated. Based on the perspective of network pharmacology, the chemical-target network was constructed, and the functional enrichment analysis of network pharmacology, the chemical-target network was constructed, and the functional enrichment analysis of gene ontology and the pathway enrichment analysis of Kyoto encyclopedia of genes and genomes were of gene ontology and the pathway enrichment analysis of Kyoto encyclopedia of genes and genomes were carried out by DAVID to speculate about the mechanism of action of the core drug pairs. carried out by DAVID to speculate about the mechanism of action of the core drug pairs. Results Results: There are : There are 6 small molecule compounds that have the optimal binding energy with the two target proteins. Among 238 6 small molecule compounds that have the optimal binding energy with the two target proteins. Among 238 potential anti-COVID-19 herbs screened in total, 16 kinds of CHM containing the most active ingredients, and potential anti-COVID-19 herbs screened in total, 16 kinds of CHM containing the most active ingredients, and 5 candidate anti-COVID-19 herbs that had been used in high frequency, as well as a core drug pair, namely, 5 candidate anti-COVID-19 herbs that had been used in high frequency, as well as a core drug pair, namely, Forsythiae Fructus Forsythiae Fructus-Lonicerae Japonicae Flos Lonicerae Japonicae Flos were selected. were selected. Conclusion Conclusion: The core drug pair of : The core drug pair of Forsythiae Forsythiae Fructus-Lonicerae Japonicae Flos Lonicerae Japonicae Flos containing multiple components and targets is easy to combine with 3CLpro containing multiple components and targets is easy to combine with 3CLpro and ACE2, and exerts an anti-COVID-19 pneumonia effect through multi-component and multi-target, and plays and ACE2, and exerts an anti-COVID-19 pneumonia effect through multi-component and multi-target, and plays the role of anti-COVID-19 pneumonia in multi-pathway. KEYWORDS KEYWORDS molecular docking, COVID-19, SARS-CoV-2 3CL hydrolytic enzyme, angiotensin converting molecular docking, COVID-19, SARS-CoV-2 3CL hydrolytic enzyme, angiotensin converting enzyme Ⅱ, Chinese medicine

COVID-19 caused by the new coronavirus is characterized by rapid and extensive spread, strong infectivity and general susceptibility of the population. Currently, there is no specific drug for it.(1) On February 11, 2020, the International Commission on the Classification of Viruses announced the official designation of the new coronavirus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, formerly known as 2019-ncov). On the same day, the World Health Organization (WHO) named the disease caused by this virus as Corona Virus Disease 2019 (COVID-19). Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome. The new coronavirus isolated in Wuhan is found to be the 7th corona virus that can infect humans.(2) The s-protein expressed by the cov-2 virus binds to the angiotensin-converting enzyme Ⅱ (ACE2) in the human body, thereby infecting cells, invading the body and causing diseases.(3) Recently, the team of Rao ZH and Yang HT from Shanghai University of Science and Technology has obtained a high-resolution crystal structure of 2019-nCoV coronavirus 3CL hydrolase (Mpro) which is currently considered to be an effective target of the COVID-19 virus.(4) These studies have brought hope for seeking effective clinical drugs to prevent and control COVID-19, which may help us to develop more effective ways to fight against COVID-19. COVID-19 belongs to the category of "epidemic" disease of Chinese medicine (CM). Since CM has played an active role through integration of Chinese and Western medicine in the treatment of COVID-19, its clinical efficacy has been widely praised. The study of Wang, et al(5) showed that the cure rate of patients with new corona virus pneumonia might be positively correlated with the participation rate of CM treatment. In the "Diagnosis and Treatment Program of Novel Coronavirus Pneumonia (3rd trial edition)",(1) the specific methods of CM prevention and control were identified, and were constantly supplemented and improved in the 4th to 7th editions,(1) playing an important role in the prevention and control of COVID-19.(1) Due to the holistic "multi-target, multipathway and multi-effect" therapeutic characteristics, single herbs and CHM

compounds are advantageous and potential in the treatment of some diseases. Molecular docking is a method of drug design based on the characteristics of the receptor and the way the receptor interacts with the drug molecule. As an emerging research method combining the physical and chemical principles and with the scientific calculation algorithm, it provides a feasible strategy for exploring the basis and mechanism of the pharmacodynamic substances of CHM, and promotes the modern research process of CHM.(6) This study took the SARS-CoV-2 3CL hydrolytic enzyme and the ACE2 as receptors, and molecular docking of the two was performed to select potential antiviral active ingredients, so as to provide reference for seeking effective and quick-acting anti-COVID-19 chemical components. METHODS Database and Software Data used in this study were downloaded from the database system: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmsp.php), Protein Data Bank (PDB, https://www.rcsb.org/), the Swiss Target Prediction (http://www.swisstargetprediction.ch/), the STRING Online Database (https://string-db.org/), and the Biological Information Annotation Database DAVID, (https://david.ncifcrf.gov/summary.jsp, Version 6.8). The software used included AutoDockTools1.5.6 Software, AutoDockVina Software (http://vina.scripps.edu/), biological information analysis tools Cytoscape v3.8.1, data analysis tools R 3.6.2, protein molecules and visualization software PyMOL. Molecular Docking A total of 12,541 compounds in MOL2 format were downloaded from the TCMSP database, and 3D structures of them were downloaded from PDB database, to establish a virtual screening small molecule database for molecular docking technology. The 3D structure of ACE2 (PDB ID: 1R42) protein (in PDB format) was downloaded from PDB database (https://www.rcsb.org/). SARS-CoV-2 is determined to be a high-resolution crystal structure (PDB ID: 6LU7) of SARS-CoV-2 3CL hydrolase (Mpro) by Rao ZH and Yang HT's research group from Shanghai University of Science and Technology. Visualization and analysis of protein-ligand complexes was performed using PyMOL version 0.99 Software (De Lano Scientific LLC, CA, USA) and high-throughput molecular docking was carried out by Autodock Vina and R. According to the optimal binding energy (affinity) of each compound with the 3CL hydrolase and ACE2 converting enzyme, a small molecule database of anti-COVID-19 CM was established. Based on ADME Assessment "ADME" refers to the absorption, distribution, metabolism and excretion process of exogenous chemicals in the body, and screening ADME properties is an important means to further develop candidate drugs.(7) In this study, the active ingredients that met the drug screening criteria [oral availability (OB) 30% and drug-likeness (DL) 0.18] in the small-molecule database were selected, so as to fi nd compounds with good pharmacokinetic properties and bioavailability, and to improve the screening effi ciency. Screening of Anti-COVID-19 CHM Based on the above screening results, we speculated the anti-COVID-19 novel corona virus pneumonia CHM. We selected CHM varieties frequently used in prevention and treatment of COVID-19 issued by the country and various institutions, and predicted the antiviral core drug pair according to the distribution of active ingredients in these CHM varieties. Prediction of Molecular Mechanism of Core Drug Pair Based on Network Pharmacology The main active components of the herbs were screened by TCMSP extraction, and the potential targets were predicted by the Swiss Target Prediction server. The STRING database was used to analyze the relationship between drug targets, and the visual analysis was conducted with the help of the Cytoscape 3.5.1 software. Then, the potential targets of the selected active components were submitted to the bioinformatics database DAVID 6.8 for functional annotation of Gene Ontology (GO) genes and enrichment analysis of the Kyoto encyclopedia of genes and genomes (KEGG) pathway in order to further understand the functions of the targets and their role in the signaling pathway, thereby exploring and predicting the potential molecular mechanism of the core drug pair. RESULTS Anti-COVID-19 Molecule Database In order to minimize the probability of false positive results, the optimal binding energy of small molecule compounds in CHM was compared with that of the currently recommended clinical chemical drugs in this study, and the binding energy in the screening criteria was changed to –5.0 kCal/mol (–20 kJ/mol). The partial results are shown in Table 1. The binding energy of the molecular docking between puerarin and the two target proteins was lower than that of the molecular docking between clinical recommended chemical drugs, and the same two proteins, which indicated that the binding activity of puerarin and target proteins was stronge, which came from Radix Bupleuri, Cyathulae Radix, Puerariae Flos, Hemerocallis Radix and Radix Puerariae. Bicuculline is derived from Corydalis Bungeanae

Herba, Forsythiae Fructus, Corydalis Decumbens (Thunb.) Pers, and Corydalis Rhizoma, luteolin is originated from Lonicerae Japonicae Flos, Forsythiae Fructus, Ephedra Herba and other 89 herbs. Anti-COVID-19 Core Drug Pairs The binding activity of 6 active ingredients with the two target proteins were better than that of the recommended chemical drugs with these two targets, and a total of 238 potential anti-COVID-19 CHM were chosen, and 42.02% of them possess the character of Fei (Lung) meridian tropism. Among the potential CHM, heat-clearing herbs accounted for the largest proportion (29.41%), followed by tonic herbs (9.66%, Figure 1). Based on the CM prevention and treatment plans issued by the municipal, provincial and national health administration departments, 5 kinds of frequently used CHMs containing the most active ingredients from 16 herbs (Figure 2). Among these selected CHMs, Forsythiae Fructus and Lonicerae Japonicae Flos are the most widely distributed active ingredients, and thus selected as the predicted core drug pair in this study.

Table 1. Binding Energy of Representative Components and Clinically Recommended Chemical Drugs with SARS-CoV-2 3CL and ACE2

Compound	Formula	MW	3CLpro (kJ/mol)	ACE2 (kJ/mol)
Puerarin	$C_{21}H_{20}O_{10}$	432.38	−33.47	−38.07
Bicuculline	$C_{20}H_{17}NO_6$	367.40	−26.78	−41.42
Luteolin	$C_{15}H_{10}O_6$	286.24	−26.78	−36.82
Quercetin	$C_{15}H_{10}O_7$	302.24	−26.36	−36.40
Isorhamnetin	$C_{16}H_{12}O_7$	316.27	−25.95	−35.15
Irisolidone	$C_{17}H_{14}O_6$	314.29	−25.53	−38.49
Lopinavir	$C_{37}H_{48}N_4O_5$	628.80	−22.59	−37.24
Ritonavir	$C_{37}H_{48}N_6O_5S_2$	720.94	−24.69	−36.40
Remdesivir	$C_{27}H_{35}N_6O_8P$	602.58	−25.94	−36.40
Arbidol	$C_{22}H_{25}BrN_2O_3S$	531.89	−28.03	−30.54
Chloroquine	$C_{18}H_{26}ClN_3$	319.87	−24.30	−27.20
Ribavirin	$C_{37}H_{48}N_6O_5S_2$	720.96	−25.52	−32.22
Nitazoxanide	$C_{12}H_9N_3O_5S$	307.28	−23.85	−34.73

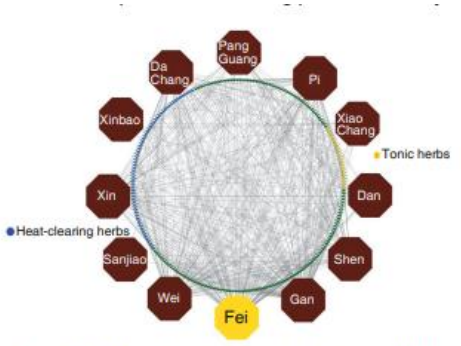
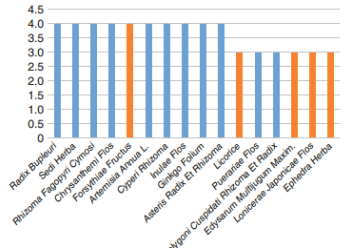


Figure 1. Main Categories of Selected CHMs

GO Enrichment and KEGG Pathway Annotation Analysis of Potential Targets of Forsythiae Fructus-Lonicerae Japonicae Flos Forsythiae Fructus -Lonicerae Japonicae Flos contains 46 active ingredients with good pharmacokinetic properties and bioavailability, with a total of 412 potential targets, 121 of which are targeted for viral pneumonia (Figure 3).



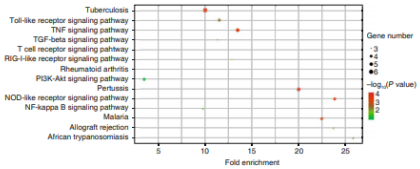


Figure 4. Enrichment Analysis of Potential Target KEGG Pathway of *Forsythiae Fructus-Lonicerae Japonicae Flos*

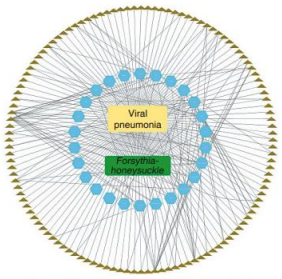


Figure 3. Molecule-Target-Pathway Network of *Forsythiae Fructus-Lonicerae Japonicae Flos*

Potential targets were imported into the DAVID database for GO term and KEGG pathway enrichment analyses. GO term enrichment results were divided into biological process (BP), cell compound (CC), and molecular function (MF), and showed 35 BP, 4 CC and 18 MF enriched for these targets had a P value less than 0.05. In addition, 56 KEGG pathways were recognized as P value less than 0.05. In addition, 56 KEGG pathways were recognized as P <0.05 among which 14 were coincide with the super pathway of viral pneumonia (Figure 4). Pertussis, tuberculosis, T cell receptor (TCR) and the tumor necrosis factor (TNF) signaling pathways had the smallest P values and the most enriched targets.

DISCUSSION

The COVID-19 belongs to the category of pestilent disease in CM. As mentioned in Detailed Analysis of Warm Diseases (Wen Bing Tiao Bian), a book on epidemic febrile disease written by WU Ju-tong, "the pestilence is severe and prevalent." That exactly describes the strong infectivity of the new corona virus pneumonia as a kind of "Wenyi (pestilence) disease". YE Tian-shi in his Treatise on Warm-Heat Disease (Wen Re Lun) stated that "Lung (Fei) is the fi rst to be attacked when a person suffers from warm-heat evils." In this study, a total of 238 potential anti-COVID-19 CHMs were selected based on the analysis of 2 target proteins SARS-CoV-2 3CL hydrolytic enzyme and ACE2. As a pestilence disease, COVID-19 fi rst causes lung diseases. Among the 238 anti-COVID-19 CHMs selected in our study, 42.02% of them possess the character of meridian tropism in Fei. Besides, among the 238 CHMs, heat-clearing herbs (29.41%) accounted for the largest proportion, followed by tonic herbs (9.66%). It is suggested that CM may treat COVID-19 by clearing heat (resisting virus and inflammation) and tonifying deficiency (immunomodulation). The pathological study of COVID-19 also confirmed that the pathological changes were mainly caused by damage to the lung and immune system. Due to varied foundation diseases, other viscera suffered differently, mostly secondary damage.(1) It is also described in WU Ju-tong's "Wen Bing Tiao Bian" that " Warm diseases include Feng Wen, Wen Re, Wen Nue, Wen Du, Shu Wen, Shi Wen, Qiu Zao, Dong Wen and Wen Yi." A study by Yu, et al(8) showed that in patients in China, the main affected organ of COVID-19 infections was the Fei, and the etiology and mechanism of COVID-19 in CM was mainly due to "dampness". Ran, et al(9) found that the disease pattern of 30.14% COVID-19 patients in Chongqing are pathogenic-heat obstructing in Fei. Among the 238 potential CHMs, heat-clearing herbs accounted for the largest proportion (29.41%), and the drug pair of Forsythiae Fructus-Lonicerae Japonicae Flos is the most widely distributed active ingredient, and is selected as the predicted core drug pair in this study. The pair of Forsythiae FructusLonicerae Japonicae Flos is a treatment method suitable for the early stage of COVID-19 when the disease pattern is heat. We suggest that the COVID-19 patients should be treated by integrated CM and Western medicine as soon as possible, and the pair of Forsythiae Fructus-Lonicerae Japonicae Flos should be adopted at the early stage of COVID-19 when the disease pattern is heat. In this way the transmission of Sanjiao and Wei-Qi-YingXue in CM can be prevented, and the possibility of the disease aggravation may be reduced. The drug pair of Forsythiae Fructus-Lonicerae Japonicae Flos has 121 targets for viral pneumonia. And 14 pathways are coincided with viral pneumonia. One study showed that the pair of Forsythiae Fructus-Lonicerae Japonicae Flos had antipyretic, anti-inflammatory, anti-free radical damage and immune-enhancing effects in fever rats, and the combined use of the two herbs was better than a single use.(10) The antiviral, antibacterial, antipyretic and anti-inflammatory effects of this drug pair were also revealed by a study by Ding, et al.(11) TCR is a receptor on the surface of T cells and plays a key role in the function of T cells and the formation of immune synapses. It provides the connection between T cells and antigen presenting cells. TCR can specifically recognize peptides, and transform extracellular recognition into signals that can be transmitted to the interior of the cell. It can promote many signal cascade reactions by inducing tyrosine kinase activation and activating downstream mitogen-activated protein kinases, protein kinase C, calcium and other signal pathways, and thereby fi nally achieve T cell activation.(12) One study showed that the extract of Flos Lonicerae had an immunosuppressive effect, and was capable of signifi cantly inhibiting the phagocytosis of mononuclear macrophages in mice and suppressing various infl ammatory reactions in the early, middle and late stages of infl ammation.(13) In this, we found that the pathways enriched with more than 4 targets were pertussis, tuberculosis, TCR and the TNF signaling pathways in the order of P value from small to large in the drug pair of Forsythiae Fructus -Lonicerae Japonicae Flos . Therefore, this drug pair may play anti-inflammatory and immunomodulatory roles in COVID-19 through these signaling pathways, demonstrating the multitarget, multi-channel and multi-effect features of CHM. The results of this study provided the basis for further studies on the molecular mechanism of the drug pair of Forsythiae Fructus-Lonicerae Japonicae Flos in the treatment of COVID-19. In this study, the molecular compounds of CHM were mined from the TCMSP database based on virtual screening via molecular docking technology and network pharmacology, with SARS-CoV-2 3CL hydrolytic enzyme and ACE2 as receptors, and a reasonable virus inhibition scheme of CHMs was put forward. The results fully reflected the complexity of the mechanism of

action of multi-component and multi-target CHM, as well as the versatility and extensiveness of the pharmacological action of CHM. The method proposed in this study can provide reference for the development of effective combinations of CHMs for treating COVID-19. In view of the limitations of the virtual screening results, further in vitro and in vivo experiments are needed to verify the results of this study, so as to provide the experimental basis for the development of natural antiviral drugs. Confl ict of interest The authors declare that they have no confl ict of interests. Author Contributions All authors participated in the review of the manuscript. Gao LQ, Xu J and Chen SD analyzed data and wrote the manuscript.

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Abstract

Introduction

The World Health Organization declared the coronavirus disease (COVID-19) as a pandemic on 11 March 2020, after the number of confirmed cases outside China increased 13-fold. As the epicentre of the initial outbreak, China has been updating the National COVID-19 Diagnostic and Treatment Guideline with up-to-date information about the disease. To facilitate the implementation of integrative Chinese–Western

Medicine in COVID-19 management, Traditional Chinese medicine (TCM) has been recommended in recent editions of the national guideline.

Methods

The national guideline summarised the opinions and frontline experience of medical experts across the country to provide by far the best management for COVID-19. We extracted the case definition and clinical classifications of COVID-19 in China along with relevant TCM treatments cited in the seventh edition of the guideline, with an intent to disseminate practical information to TCM clinicians and researchers around the world.

Results

We present the most recent case definition, clinical classifications, and relevant TCM treatments of COVID-19 in accordance with the recommendations in the Chinese guideline. TCM treatments are stratified into two groups based on patients' disease status. Four types of Chinese patent medicines are recommended for suspected COVID-19 cases. Several herbal formulae are recommended for confirmed COVID-19 cases according to their clinical classification and TCM pattern diagnoses. Two herbal formulae are also recommended for rehabilitation of recovering cases.

Conclusion

To control the waves of COVID-19 outbreak, countries must ensure the adherence of their citizens to local public health measures. Medical professionals should diagnose and treat patients according to up-to-date guidelines. Future evaluation of the outcomes of implementing TCM recommendations will strengthen the evidence base for COVID-19 management for the sake of public health and the internationalisation of TCM.

1. Background

The coronavirus disease (COVID-19) unfolded in Wuhan, China, in December 2019 [1]. Without effective control measures, the disease has spread across the globe with more than one hundred countries reporting confirmed cases [2]. Having realised that the number of new confirmed cases outside China has increased 13-fold, the World Health Organization finally decided to characterise COVID-19 as a pandemic on 11 March 2020 and requested member states to scale up their emergency response mechanisms [2].

Being the epicentre of the initial outbreak, China developed and has been constantly updating its National COVID-19 Diagnostic and Treatment Guideline with up-to-date information about the aetiology, epidemiology, pathology, clinical features, diagnosis, and treatments of the disease. With strong support from the Chinese government [3], Traditional Chinese Medicine (TCM), as a core component of the national healthcare system, has also been recommended in recent editions of the national guideline for the treatment of COVID-19. A month after the implementation of the guideline, Chinese officials reported that the preliminary outcome of the integrative Chinese–Western Medicine treatment approach appeared to be promising [4]. To disseminate practical information to TCM clinicians and researchers around the world, we extracted and present the case definition and clinical classifications of COVID-19 in China along with relevant TCM treatments citied in the seventh edition of the National COVID-19 Diagnostic and Treatment Guideline released on 3 March 2020 [5].

TCM treatments recommended in the Chinese national guideline are stratified into two groups based on patients' disease status (*suspected COVID-19 case* or *confirmed COVID-19 case*). Four types of Chinese patent medicines are recommended for suspected COVID-19 cases according to their clinical features, while

different herbal formulae are recommended for confirmed COVID-19 cases according to their clinical classification (*mild*, *moderate*, *severe*, or *critical*) and TCM pattern diagnosis. Two herbal formulae are also recommended for rehabilitation of recovering cases.

In the Chinese guideline, healthcare professionals are encouraged to offer integrative Chinese–Western Medicine treatments for COVID-19 patients, regardless of their disease status and clinical classification, as soon as possible to achieve the best clinical outcome [5]. Those who would like to implement an integrative treatment approach may consult the conventional medicine section of the Chinese national guideline, which describe how the two types of the interventions may be used in a coordinated manner [6].

2. Methods

We extracted the case definition and clinical classifications of COVID-19 in China along with relevant TCM treatments cited in the seventh edition of the guideline. The current translation is for educational and non-profit purposes. We have satisfied the conditions of fair use of open-source materials [7]. Since the guideline is an administrative document issued by The National Health Commission of the People's Republic of China, it is not necessary to obtain approval from Chinese officials according to the country's Copyright Law [8].

3. Results

3.1. Case definition in China

3.1.1. Suspected COVID-19 case

To be classified as a suspected case in China, the patient should fulfil one of the following epidemiological risks criteria and two of the following clinical features:

• *Epidemiological risks criteria* – (1) travelled to or lived in Wuhan or other Chinese cities with confirmed cases in the last 14 days before symptom onset; (2) contacted with a confirmed case (tested positive for viral nucleic acid) in the last 14 days before symptom onset; (3) contacted with a person with fever or respiratory symptoms who travelled to or lived in Wuhan or other Chinese cities with confirmed cases in the last 14 days before symptom onset; or (4) cluster onset.

• *Clinical features* – (1) fever and/or respiratory symptoms; (2) radiological characteristics of COVID-19; or (3) normal or reduced total white blood cell count, or normal or reduced lymphocyte count in early-onset.

3.1.2. Confirmed COVID-19 case

To be classified as a confirmed case in China, the suspected case should fulfil one of the following pathological or serological criteria:

(1) Test positive for SARS-CoV-2 nucleic acid in real-time rRT-PCR;

(2) Viral genome sequencing reveals a high similarity to SARS-CoV-2; or

(3) Test positive for serum SARS-CoV-2-specific IgM and IgG, serum SARS-CoV-2-specific IgG seroconversion, or a fourfold or greater rise in SARS-CoV-2-specific IgG titre between acute- and convalescent-phase sera.

3.2. Clinical classifications in China

3.2.1. Mild case

• Mild clinical features without radiological characteristics of pneumonia.

3.2.2. Moderate case

• Fever and respiratory symptoms with radiological characteristics of pneumonia.

3.2.3. Severe case

• Fulfils one of the following: (1) tachypnoea with respiratory rate ≥ 30 breaths per minute; (2) resting peripheral capillary oxygen saturation ≤ 93%; or (3) arterial oxygen partial pressure (PaO_2) / fractional inspired oxygen (FiO_2) ≤ 300 mmHg.

3.3. Critical case

• Fulfil one of the following: (1) respiratory failure and requires invasive mechanical ventilation; (2) shock; or (3) multiple organ failure and requires admission into intensive care unit.

3.4. Traditional Chinese Medicine treatments

3.4.1. Suspected COVID-19 case

Clinical features 1 – Muscle fatigue accompanied by gastrointestinal discomfort

^**Recommended Chinese patent medicine* –

• Huoxiang Zhengqi capsules

⁺Ingredients: Pogostemonis Herba, Glycyrrhizae Radix et Rhizoma Praeparata cum Melle, Atractylodis Macrocephalae Rhizoma, Pinelliae Rhizoma, Citri Reticulatae Pericarpium, Magnoliae Officinalis Cortex, Platycodonis Radix, Perillae Folium, Arecae Pericarpium, Poria, Angelicae Dahuricae Radix, Zingiberis Rhizoma Recens, and Jujubae Fructus

Clinical features 2 – Muscle fatigue accompanied by fever

^**Recommended Chinese patent medicines* –

• Jinhua Qinggan granules

⁺Ingredients: Lonicerae Japonicae Flos, Gypsum Fibrosum, Ephedrae Herba Praeparata cum Melle, Armeniacae Semen Amarum, Scutellariae Radix, Forsythiae Fructus, Fritillariae Thunbergii Bulbus, Anemarrhenae Rhizoma, Arctii Fructus, Artemisiae Annuae Herba, Menthae Haplocalycis Herba, and Glycyrrhizae Radix et Rhizoma

• Lianhua Qingwen capsules

⁺Ingredients: Forsythiae Fructus, Lonicerae Japonicae Flos, Ephedrae Herba Praeparata cum Melle, Armeniacae Semen Amarum, Gypsum Fibrosum, Isatidis Radix, Dryopteridis Crassirhizomatis Rhizoma, Houttuyniae Herba, Pogostemonis Herba, Rhei Radix et Rhizoma, Rhodiolae Crenulatae Radix et Rhizoma, Menthae Haplocalycis Herba, and Glycyrrhizae Radix et Rhizoma.

• Shufeng Jiedu capsules

[+]Ingredients: Polygoni Cuspidati Rhizoma et Radix, Forsythiae Fructus, Isatidis Radix, Bupleuri Radix, Patriniae Herba, Verbenae Herba, Phragmitis Rhizoma, and Glycyrrhizae Radix et Rhizoma.

[^]*The recommended Chinese patent medicine should only be used under the instruction of a qualified TCM clinician*.

**The recommended Chinese patent medicines are registered in China. If they are not available outside China, they may be offered to patients in the form of herbal decoction.*

[+]No endangered animal species are included.

3.4.2. Confirmed COVID-19 case

• Qingfei Paidu Decoction

Application– Based on the clinical observations made by TCM clinicians across different regions, this is a basic Chinese herbal medicine formula and applies to mild cases, moderate cases, and severe cases. It may also apply to critical cases, depending on the condition of individual patients. Where appropriate, medical professionals may choose to prescribe other formulae introduced in the subsequent sections of this article, based on the TCM diagnosis of patients.

[+@]*Basic formula*– Ephedrae Herba 9 g, Glycyrrhizae Radix et Rhizoma Praeparata cum Melle 6 g, Armeniacae Semen Amarum 9 g, Gypsum Fibrosum 15–30 g (decoct first), Cinnamomi Ramulus 9 g, Alismatis Rhizoma 9 g, Polyporus 9 g, Atractylodis Macrocephalae Rhizoma 9 g, Poria 15 g, Bupleuri Radix16 g, Scutellariae Radix6 g, Pinelliae Rhizoma Praeparatum cum Zingibere et Alumine 9 g, Zingiberis Rhizoma Recens 9 g, Asteris Radix et Rhizoma 9 g, Farfarae Flos 9 g, Belamcandae Rhizoma 9 g, Asari Radix et Rhizoma 6 g, Dioscoreae Rhizoma 12 g, Aurantii Fructus Immaturus 6 g, Citri Reticulatae Pericarpium 6 g, and Pogostemonis Herba 9 g.

Method of usage– Decoct the above medicinals with water. One decoction per day in two doses. Consume one warm dose every morning and evening, 40 min after meals. Three decoctions per treatment course. When possible, consume a half bowl of rice soup after each dose. Patients with a dry tongue and fluid-humour depletion may consume one bowl of rice soup. Start another course when the patient has his or her symptoms improved but is not yet cured. The formula of the second course may be amended as appropriate when the patient has other conditions or comorbidities. Stop the medication when the patient presents with no symptoms.

• Treatment strategies for mild cases based on differential Chinese medicine diagnosis

(1) Cold-dampness obstructing the lung

o

Clinical features– Fever, muscle fatigue, muscle pain, coughing, expectoration of sputum, chest discomfort, shortness of breath, loss of appetite, nausea, vomiting, and ungratifying defecation. Pale tongue with teeth-marked, or pale red tongue. White, thick and curdy tongue fur, or white and slimy tongue fur. Soggy or slippery pulse.

[+@]*Recommended formula*– Ephedrae Herba 6 g, Gypsum Fibrosum 15 g, Armeniacae Semen Amarum 9 g, Notopterygii Rhizoma et Radix15 g, Descurainiae Semen & Lepidii Semen 15 g, Dryopteridis Crassirhizomatis Rhizoma 9 g, [#]Pheretima (Earthworm; *not endangered*) 15 g, Cynanchi Paniculati Radix et Rhizoma 15 g, Pogostemonis Herba 15 g, Eupatorii Herba 9 g, Atractylodis Rhizoma 15 g, Poria 45 g, Atractylodis Macrocephalae Rhizoma 30 g, Crataegi Fructus Tostum 9 g, Hordei Fructus Germinatus Tostum 9 g, Massa Medicata Fermentata Tostum 9 g, Magnoliae Officinalis Cortex15 g, Arecae Semen Tostum 9 g, Tsaoko Fructus Tostum 9 g, and Zingiberis Rhizoma Recens 15 g.

Method of usage– Decoct the above medicinals with 600 ml of water. One decoction per day in three doses. Consume one dose every morning, noon, and evening, before meals.

(2) Dampness-heat in the lung

Clinical features– Mild fever or no fever, slight aversion to cold, muscle fatigue, heaviness in the head and body, muscle pain, dry coughing with small amounts of sputum, sore throat, dry mouth without a desire to drink, chest discomfort, absence of sweating or difficulty in sweating, loss of appetite, nausea, vomiting, and watery stool or ungratifying defecation. Pale red tongue. White, thick and slimy tongue fur, or yellow and thin tongue fur. Slippery and rapid pulse, or soggy pulse.

[+@]*Recommended formula*– Arecae Semen 10 g, Tsaoko Fructus 10 g, Magnoliae Officinalis Cortex10 g, Anemarrhenae Rhizoma 10 g, Scutellariae Radix10 g, Bupleuri Radix10 g, Paeoniae Radix Rubra 10 g, Forsythiae Fructus 15 g, Artemisiae Annuae Herba 10 g (decoct later), Atractylodis Rhizoma 10 g, Isatidis Folium 10 g, and Glycyrrhizae Radix et Rhizoma 5 g.

Method of usage– Decoct the above medicinals with 400 ml of water. One decoction per day in two doses. Consume one decoction every morning and evening.

• Treatment strategies for moderate cases based on differential Chinese medicine diagnosis

(3) Dampness toxin obstructing the lung

Clinical features– Fever, coughing with small amounts of sputum or coughing with yellow sputum, chest discomfort, shortness of breath, abdominal distension, and constipation. Dark red and enlarged tongue. Yellow and slimy tongue fur, or yellow and dry tongue fur. Slippery and rapid pulse, or string-like and soggy pulse.

[+@]*Recommended formula*– Ephedrae Herba 6 g, Armeniacae Semen Amarum 15 g, Gypsum Fibrosum 30 g, Coicis Semen 30 g, Atractylodis Rhizoma 10 g, Pogostemonis Herba 15 g, Artemisiae Annuae Herba 12 g,

Polygoni Cuspidati Rhizoma et Radix20 g, Verbenae Herba 30 g, Phragmitis Rhizoma 30 g, Descurainiae Semen & Lepidii Semen 15 g, Citri Grandis Exocarpium 15 g, and Glycyrrhizae Radix et Rhizoma 10 g.

Method of usage– Decoct the above medicinals with 400 ml of water. One decoction per day in two doses. Consume one decoction every morning and evening.

(4) Cold-dampness obstructing the lung

Clinical features– Mild or no fever, feeling of feverishness, dry coughing with small amounts of sputum, fatigue, chest discomfort, stomach discomfort, nausea, and watery stool. Pale or pale red tongue. White tongue fur, or white and slimy tongue fur. Soggy pulse.

+@Recommended formula– Atractylodis Rhizoma 15 g, Citri Reticulatae Pericarpium 10 g, Magnoliae Officinalis Cortex10 g, Pogostemonis Herba 10 g, Tsaoko Fructus 6 g, Ephedrae Herba 6 g, Notopterygii Rhizoma et Radix10 g, Zingiberis Rhizoma Recens 10 g, and Arecae Semen 10 g.

Method of usage– Decoct the above medicinals with 400 ml of water. One decoction per day in two doses. Consume one decoction every morning and evening.

•Treatment strategies for severe cases based on differential Chinese medicine diagnosis

(5) Epidemic toxin obstructing the lung

Clinical features– Fever, flushed face, coughing with small amounts of sticky yellow sputum or with blood, panting, shortness of breath, fatigue, dry mouth with bitter taste and sticky feeling in the mouth, loss of appetite, nausea, ungratifying defecation, reddish urine with reduced amount. Red tongue. Yellow and slimy tongue fur. Slippery and rapid pulse.

+@Recommended formula (Huashi Baidu Decoction)– Ephedrae Herba 6 g, Armeniacae Semen Amarum 9 g, Gypsum Fibrosum 15 g, Glycyrrhizae Radix et Rhizoma 3 g, Pogostemonis Herba 10 g (decoct later), Magnoliae Officinalis Cortex10 g, Atractylodis Rhizoma 15 g, Tsaoko Fructus 10 g, Pinelliae Rhizoma Praeparatum 9 g, Poria 15 g, Rhei Radix et Rhizoma 5 g (decoct later), Astragali Radix10 g, Descurainiae Semen & Lepidii Semen 10 g, and Paeoniae Radix Rubra 10 g.

Method of usage– Decoct the above medicinals with 100–200 ml of water. One to two decoction(s) with two to four doses per day. Oral administration or feeding via nasogastric tube.

(6)Blazing of both qi and nutrient

Clinical features– High fever, agitation, thirsty, panting, shortness of breath, delirium, loss of consciousness, blurred vision, purpura, hematemesis, nasal bleeding, and convulsion. Crimson tongue. Less or no tongue fur. Sunken and fine pulse, or floating, big and rapid pulse.

o

[+@]*Recommended formula*– Gypsum Fibrosum 30–60 g (decoct first), Anemarrhenae Rhizoma 30 g, Rehmanniae Radix 30–60 g, [#]Bubali Cornu (buffalo horn; *not endangered*) 30 g (decoct first), Paeoniae Radix Rubra 30 g, Scrophulariae Radix30 g, Forsythiae Fructus 15 g, Moutan Cortex15 g, Coptidis Rhizoma 6 g, Lophatheri Herba 12 g, Descurainiae Semen & Lepidii Semen 15 g, and Glycyrrhizae Radix et Rhizoma 6 g. *Method of usage*– Decoct the above medicinals with 100–200 ml of water. One to two decoction(s) with two to four doses per day. Oral administration or feeding via nasogastric tube.

Treatment strategy for critical cases

Internal block and external collapse

Clinical features– Difficulty in breathing, panting after slight movement (may require invasive mechanical ventilation), convulsion, agitation, sweating, and cold extremities. Dark purple tongue. Thick and slimy tongue fur, or dry tongue fur. Floating and big pulse without root.

[+@]*Recommended formula*– Ginseng Radix et Rhizoma 15 g, Aconiti Lateralis Radix Praeparata 10 g (decoct first), and Corni Fructus 15 g, along with Suhexiang pills or Angong Niuhuang pills. May prescribe Rhei Radix et Rhizoma 5–10 g to patients with invasive mechanical ventilation having abdominal distension or constipation. May prescribe Rhei Radix et Rhizoma 5–10 g and Natrii Sulfas to 5–10 g with sedatives and muscle relaxants when ventilator-patient dyssynchrony occurs.

Method of usage– Depends on the condition of the patient, as well as a consensus between TCM and conventional medicine clinicians.

Treatment strategies for patients in the recovery period based on differential Chinese medicine diagnosis

Lung-spleen qi deficiency

Clinical features– Shortness of breath, fatigue, loss of appetite, nausea, vomiting, stomach fullness, difficulty in defecation, and watery stool. Pale and enlarged tongue. White and slimy tongue fur.

[+@]*Recommended formula*– Pinelliae Rhizoma Praeparatum 9 g, Citri Reticulatae Pericarpium 10 g, Codonopsis Radix15 g, Astragali Radix Praeparata cum Melle 30 g, Atractylodis Macrocephalae Rhizoma Tostum 10 g, Poria 15 g, Pogostemonis Herba 10 g, Amomi Fructus 6 g (decoct after), and Glycyrrhizae Radix et Rhizoma 6 g.

Method of usage– Decoct the above medicinals with 400 ml of water. One decoction per day in two doses. Consume one dose every morning and evening.

Dual deficiency of qi and yin

Clinical features– Muscle fatigue, shortness of breath, dry mouth, thirsty, palpitation, profuse sweating, loss of appetite, mild or no fever, and dry coughing with small amounts of sputum. Dry tongue. Fine or vacuous pulse.

[+@]*Recommended formula*– Adenophorae Radix10 g, Glehniae Radix10 g, Ophiopogonis Radix15 g, Panacis Quinquefolii Radix6 g, Schisandrae Chinensis Fructus 6 g, Gypsum Fibrosum 15 g, Lophatheri Herba 10 g, Mori Folium 10 g, Phragmitis Rhizoma 15 g, Salviae Miltiorrhizae Radix et Rhizoma 15 g, and Glycyrrhizae Radix et Rhizoma 6 g.

Method of usage– Decoct the above medicinals with 400 ml of water. One decoction per day in two doses. Consume one dose every morning and evening.

[@]*The names of herbal medicines are stated in accordance with the Pharmacopoeia of the People's Republic of China 2015* [9].

[+]*No endangered animal species are included.*

[#]*These are animal products and may not be available outside China.*

4. Discussion

4.1. Rigorous evaluation of Traditional Chinese Medicine as an epidemic response

The integrative Chinese–Western Medicine treatment approach has been widely implemented in China. For instance, up to 67 % of confirmed cases in Zhejiang province received TCM interventions, in conjunction to conventional treatment [10]. The Chinese official reported that this has yielded promising outcomes, with 23 confirmed cases in Wuhan discharged from hospital after receiving integrative medicine treatment [11]. Considering the urgency of treating an increasing number of patients in some countries and jurisdictions, TCM clinicians suggested that the practical application of Chinese herbal medicine should be given priority [12]. It seems to be impossible to conduct a rigorous evaluation of its efficacy and effectiveness in the midst of an epidemic.

Individuals sceptical of TCM posed a serious doubt to this suggestion, criticising that the Chinese government is battling against the COVID-19 with “politicised pseudoscience” [13]. However, the Ebola experience demonstrated the feasibility of rigorously evaluating therapeutic measures during an epidemic [14], and the evaluation of Chinese herbal medicine for COVID-19 is by no means an exception. Indeed, in the case of COVID-19 where experimental infection could not be used to facilitate the conduct of randomised trials, the current outbreak provides the only opportunity for evaluating the efficacy of Chinese herbal medicine. It is now the prime time to start randomised trials as the peak of the epidemic has pasted in China [15], and the healthcare system has more capacity to plan for evaluation. In fact, on 3 February 2020, the Ministry of Science and Technology has launched a clinical research programme on integrative medicine treatment for COVID-19, in which COVID-19 treatment centres in Hubei, Beijing, Tianjin, Hebei and Guangdong will participate [16].

The launching of this programme represents an important step towards researching integrative treatment. However, divergent views on what constitute the most promising investigational TCM intervention seem to challenge the coordinated effort. Despite the prompt development of a national guideline, only four provinces, namely Shanxi, Anhui, Fujian and Qinghai, have adopted it, and Beijing, Tianjin and Xinjiang implemented its revised version [17]. Shanghai, Henan and Chongqing decided to develop their own Chinese

herbal medicine treatment scheme [17]. The National Administration of Traditional Chinese Medicine initiated another clinical research programme in Shanxi, Shaanxi, Hebei and Heilongjiang, investigating a single Chinese herbal formula intended for different stages of the disease [18].

To seize the opportunity of conducting quality research, establishing a national coalition of stakeholders across these initiatives is urgently needed. A multi-disciplinary team would be the key to success; it should consist of members who have expertise in COVID-19, TCM, clinical research methodology, as well as ethics and regulations, and those who are patient representatives. Additional research investment in terms of resources and personnel will enable the immediate systematic collection of data on outcomes which could later be shared across centres in a coordinated manner. Such data are expected to provide insight as to how TCM interventions impact prognosis. Besides, they will help identify which promising Chinese herbal medications should be prioritised for further assessment in randomised trials. Since Chinese herbal medicine has already been widely prescribed across the nation, an efficacy-driven approach could be adopted, focusing on phase II or III randomised trials.

In the midst of the epidemic, a trial design should be practical yet reliable, aiming to quickly generate interpretable efficacy and short-term safety results. A rigorous randomised trial conducted in a transparent manner would help in clearly determining the worthiness of the current national policy on the use of Chinese herbal medicine.

5. Conclusion

The World Health Organization has characterised COVID-19 as a pandemic. Whenever possible, healthcare professionals may diagnose and treat patients with reference to the most recent guidelines on COVID-19. More importantly, the global community must endeavour to ensure the adherence of public health measures, such as the recommendations from Public Health England who have strongly encouraged their citizens to stay at home unless they have legitimate reasons, to stay two metres away from each other, and to wash hands frequently for 20 s using soap and water [19]. We hope that future evaluation of the outcomes of implementing TCM recommendations will strengthen the evidence base for COVID-19 management not only for the sake of public health but also for the promotion of TCM status in the world.

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CRediT authorship contribution statement

Leonard T.F. Ho: Writing - original draft, Writing - review & editing, Methodology. **Karina K.H. Chan:** Writing - original draft. **Vincent C.H. Chung:** Methodology, Supervision, Writing - original draft. **Ting Hung Leung:** Conceptualization.

Declaration of Competing Interest

Dr Vincent CH Chung is a member of the editorial board for the European Journal of Integrative Medicine. The remaining authors declare that there are no conflicts of interest regarding the publication of this paper.

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Dr Vincent CH Chung is a Visiting Associate Professor of the Bachelor of Science in Biomedical Sciences & Bachelor of Medicine (Chinese Medicine) Programme, School of Biological Science, Nanyang Technological University, Singapore at the time of writing this manuscript.

Appendix A. Supplementary data

Download all supplementary files included with this article

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[ABSTRACT] The novel coronavirus pneumonia broke out in 2019 and spread rapidly. In 30 different countries, there are over seventy thousand patients have been diagnosed in total. Therefore, it is urgent to develop the effective program to prevent and treat for the novel coronavirus pneumonia. In view of Traditional Chinese Medicine has accumulated a solid theoretical foundation of plague in ancient and recent decades. Meanwhile, Traditional Chinese Medicine can provide the more effective and personalized treatment via adjusting the specific medicine for each patient based on the different syndromes. In addition, TCM often has different effect on the distinct stages of diseases, contributing to the prevention, treatment and rehabilitation. Nowadays, TCM has exhibited decent effect in the in the fight against NCP. Therefore, it is convinced that Traditional Chinese Medicine is an effective treatment for 2019 novel coronavirus pneumonia.

Introduction

In December 2019, an unknown virus pneumonia broke out in Wuhan China. Later the unknown virus was identified as a novel coronavirus (2019-nCoV) and the unknown pneumonia named as novel coronavirus pneumonia (NCP) by Chinese government and scientists [1] . In early February 2020, over sixty thousand patients have been diagnosed with NCP in 30 different countries all over the world only after 1 month. And 99% of the cases have occurred in China. On account of the NCP reported worldwide for the first time, there is no specific vaccine and drug. Unfortunately, the development of novel vaccine or specific drug will take a few months, cannot keeping up with the development of NCP. Therefore, it is urgent to develop the effective treatment for NCP. Though it is no time to discovery effective drugs, the therapeutic effect of NCP is still remarkable in hospitals. Partly, this is owing to that Traditional Chinese Medicine *(TCM) is applied in clinic timely as shown in Fig. 1 [2]. After National Health Commission (NHC) of China announcing the emergency situation in 20 January 2020, the National Admin- istration of Traditional Chinese Medicine (NATCM) rapidly deployed the work, and the first batch of Chinese medicine experts arrived in Wuhan city on the day. In 29 January, Na- tional TCM Rescue Team took over Wuhan Jinyintan Hospit- al. Five days later, eight patients were discharged after treat- ment with Chinese medicine, of which six were critically ill. Similarly, the first patient diagnosed with NCP in Beijing was discharged after a combination of Chinese and Western treat- ment. According to the NATCM, the total effective rate of certain TCM prescription for NCP is over 90% [3]. Obviously,TCM have been playing a significant role in the combat with NCP.*

In fact, TCM played a unique role in the prevention and treatment of emerging infectious diseases since ancient time. For instance, TCM obtained decent clinical effect on SARS (severe acute respiratory syndrome), H7N9 (H7N9 avian in- fluenza) and EVD (Ebola Virus Disease) at one time [4-6]. In these two thousand years, TCM has laid a solid theoretical foundation in the prevention and treatment of infectious dis- eases via the fight against diseases. Furthermore, doctors of- ten adjust the specific treatment for each patient or integrate with western medicine scheme after diagnosing the syn drome through comprehensive analysis of symptoms and signs. Up to now, TCM has made a big difference in fight against NCP.

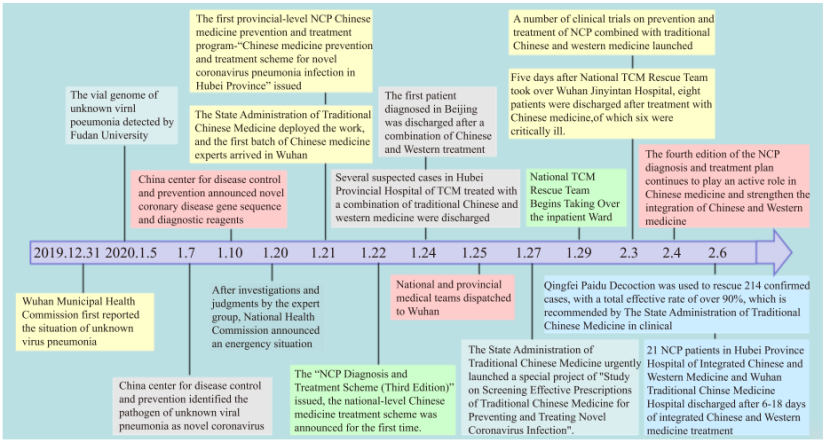

Fig. 1 The actions and treatment of TCM on NCP in China

The theoretical foundation of TCM for NCP Though NCP is a novel infectious disease, the similar syndrome infected by coronavirus are not unfamiliar. Firstly, the history of TCM applied for plague has lasted for over two thousand years [7](Table. 1). And abundant theories of TCM for explosive infectious disease have formed, which have sur- vived as treatises up to now. Meanwhile, TCM also play a significant role in the treatment of these coronavirus pneumonias broke out in the last two decades, such as SARS, MERS and H7N9 avian influenza [4-6]. Obviously, TCM has accumulated a solid theoretical foundation of plague and will be an effective treatment for NCP.

Table 1 The representative theories of TCM for infectious diseases

No.	Data	Book Name	Related theories of TCM for infectious diseases
1	BC 1046 Zhou Dynasty	*Zhou Yi*	The concept of "Yi Bing" was proposed. It thought that "Yi Bing" was different from common cold. "Yi Bing" was the disease which has infectivity and easily leaded to epidemic.
2	AD 219 Han Dynasty	*Shang Han Lun*	The dialectical relationship and treatment theory of exopathogenic disease ("Shang Han") were elaborated. Especially, decades of specific Chinese herbal formulae were specifically treated different phenotypes of Shang Han.
3	AD 341 Tsin Dynasty	*Zhou Hou Bei Ji Fang*	*Zhou Hou Bei Ji Fang* was the first literature of emergency treatment. There were plentiful well proved clinical recipes for emergency including Wen Yi, Shang Han, pestilence and so on.
4	AD 652 Tang Dynasty	*Qian Jin Yao Fang*	In *Qian Jin Yao Fang*, there were many discussions on the theory and methods of prevention and treatment of infectious diseases. It also described the prescription drugs in detail.
5	AD 1642 Ming Dynasty	*Wen Yi Lun*	*Wen Yi Lun* was the first medical book about systematic study of acute infectious disease in the world. It described the etiology, pathogenesis, syndrome and treatment of plague. "Da Yuan Yin" was the classical prescription for plague.
6	AD 1794 Qing Dynasty	*Yi Zhen Yi De*	This book dealed with the causes of the plague, the main points of diagnosis and treatment, and the discrimination of common symptoms. The representative prescription "Qin Wen Bai Du Yin" was well recognized.

The theories of TCM for infectious diseases NCP is infected by the novel coronavirus, but similar infectious diseases are not unacquainted for doctors of TCM. TCM has accumulated a wealth of experience and a lot of prescriptions, laying a solid theoretical foundation (Table 1). Two thousand years ago (BC 1046), the concept of "Yi Bing" was proposed. It pointed out that "Yi Bing " was different from common cold. "Yi Bing" was the disease which has in western medicine among of 112 cases, not including 7 deaths from severe heart and brain and other basic diseases [9]. In 2013, H7N9 avian influenza also broke out in China. Then NHC of PRC issued the diagnosis and treatment scheme. In Beijing, the first case was also cured additionally by TCM [10].

Similarly, the Chinese Government also issued the diagnosis and treatment scheme of TCM for MERS in 2015 [11]. Thus it can be seen that TCM have long history of treatments for coronavirus pneumonia. It is believed that TCM also can be treated for NCP effectively.

The effect of TCM on NCP In the early stages of the outbreak, there is neither standard of western medicine treatment nor targeted drugs owing to the unknown of NCP. However, in these TCM hospital or Integrated

Chinese and Western Medicine Hospital, TCM has been widely used for the treatment of NCP (Fig. 1). Importantly, the effect of TCM on NCP is prominent. As shown in Fig. 2, “Qing Fei Pai Du Tang” (QFPDT) is screened out by the National Administration of Traditional Chinese Medicine (NATCM) and widely recommended nationwide [3].

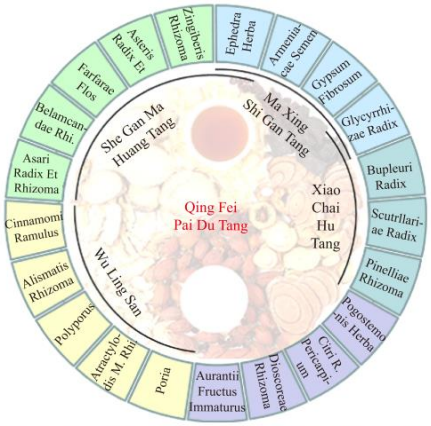

Fig. 2 The composition of the prescription—Qing Fei Pai Du Tang

Fig. 2 The composition of the prescription—Qing Fei Pai Du Tang In total, the prescription contains 21 kinds of Chinese medi- cine, which mainly derived from 4 different classical pre- scriptions originated from Shang Han Lun: Ma Xing Shi Gan Tang, Xiao Chai Hu Tang, She Gan Ma Huang Tang and Wu Ling San.

The QFPDT mainly derived from 4 different classical prescrip- tions originated from Shang Han Lun. After treatment of QFPDT, it was reported that the overall response rates were over 90% among of 214 cases in clinic (Fig. 1). In addition, doctors of TCM still will diagnose the syndrome through comprehensive analysis of symptoms and signs, then further adjust the specific treatment for each patient. Therefore, TCM has different effect on the distinct stages of NCP. TCM prevents infection for healthy person It is well known that vaccine is the highly effective method to prevent epidemics. Unfortunately, a vaccine will take over half a year from development to approval. Al- though plentiful research institutes have been carrying on the research of vaccine for NCP, there is no approved vaccine for clinic at present. However, TCM has rich clinical experience in many diseases including infection, plague, emergency and so on as shown in Table. 1. Abundant Chinese medicinal for- mulae and Chinese patent medicine can effectively prevent healthy person from infection. As a number of studies have shown [12-14], TCM can activate immune cells, improve phagocytosis and induce the production of cytokines. Ultimately, TCM enhance the immunocompetence of healthy person pre- venting infection. In 2003, TCM was successfully applied to prevent SARS in many areas of China. Therefore, a lot of hospitals and Chinese medical specialist have issued prescrip- tions of TCM for healthy person in defensing NCP. TCM improves symptoms for patients with mild symptoms According to the current clinical diagnosis [15], patients at an early stage are often with fever, dry cough and fatigue, but part of the patients have suffocating, the existence of lung scattered in the exudation and other symptoms. Luckily, there exists rich clinical experience of improving these symptoms in TCM. During the treatment of NCP, not only these TCM hospitals but also those western medical hospitals almost all adopt TCM treatment plan to improve symptoms. As the the quired. On the basis of western medicine, intervention of TCM will control oxygen saturation stable, improve dyspnea and inhibit the release of inflammatory factors. Therefore, Shenmai injection, Shenfu injection and Xuebijing injection were widely used in clinical practice. Seriously, the worsen- ing state will often result in the injury of organs and limited therapeutic effect of symptomatic and supportive treatment. At that time, some TCM therapy can clear the heart and open the orifices, tonify qi and yin, extinguish wind and increase humor. At last, TCM improves immune function to protect organ and correct electrolyte disturbance to reduce microcir- culation disturbance and tissue fibrosis. In brief, TCM con- trols the state for critical patients via alleviating pulmonary effusion and inhibiting inflammatory overreaction. TCM facilitates the rehabilitation process for convalescent patients For NCP, the negative of nucleic acid detection is the key

indicator of cure. But fatigue, cough, poor mental state and other symptoms are still present [17]. Especially, the changes of patients' lung function and clinical symptoms are not symmetrical and synchronized. In fact, the negative pa- tients are not healthy person, since they have no infectivity but need further recover. Usually, convalescent patients with NCP also have inflammation to be absorbed only after the nucleic acid detection turning negative. In the recovery, con- tinued TCM treatments will reinforce the healthy qi and eliminate the pathogenic factors. Obviously, TCM improves the patient's symptoms and promotes the complete repair of dam- aged organs and tissues. Therefore, convalescent patients of- ten continue to take TCM after the nucleic acid detection turning negative.

Table 2 The representative clinical trials of TCM for NCP

No	Registration number	Scientific title	Date of Registration
1	ChiCTR2000029637	An observational study for Xin-Guan-1 formula in the treatment of 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-08
2	ChiCTR2000029628	Observational study of Xin-Guan-2 formula in the treatment of suspected 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-07
3	ChiCTR2000029605	A randomized, open-label, blank-controlled, multicenter trial for Shuang-Huang-Lian oral solution in the treatment of 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-07
4	ChiCTR2000029589	An open, prospective, multicenter clinical study for the efficacy and safety of Reduning injection in the treatment of 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-05
5	ChiCTR2000029487	Clinical Study for Gu-Biao Jie-Du-Ling in Preventing of 2019-nCoV Pneumonia (Novel Coronavirus Pneumonia, NCP) in Children	2020-02-02
6	ChiCTR2000029434	A randomized, open-label, blank controlled trial for Lian-Hua Qing-Wen Capsule/Granule in the treatment of 2019-nCoV pneumonia (novel coronavirus pneumonia, NCP)	2020-02-01
7	ChiCTR2000029432	A Real World Study For the Efficacy and Safety of Large Dose Tanreqing Injection in the Treatment of Patients with 2019nCoV Pneumonia (Novel Coronavirus Pneumonia, NCP)	2020-02-01

Conclusion

The cure rate of NCP is increasing from 2% in the early days to over 20% in these days. And in partial provinces, the cure rate has exceeded 40% [18]. The application of TCM timely and widely is one of these positive actions in the fight against this outbreak. In view of the excellent performance of TCM at present, increasing number of patients are being treated with TCM additionally. As reported [19], about 88% patients with NCP have been receiving the treatment integ- rated with TCM. Firstly, TCM has accumulated a wealth of experience of prevention and treatment of emerging infec- tious diseases in ancient time and recent decades. Then TCM still will adjust the specific medicine for each patient after diagnosing the syndrome, providing the more effective and personalized treatment. In addition, TCM will reduce ad- verse reactions of western medicine (such as antiviral, anti- bacterial drugs and hormones.) via avoiding or decreasing the use of these drugs in clinic. Therefore, TCM has special ad- vantage in the combat with NCP. However, there are several deficiencies in the development of TCM. As shown in Fig. 1, the national medical team of TCM dispatched to Wuhan timely, but they began to take over the inpatient ward several days later. Because there are no TCM related departments or sufficient Chinese medicine in infectious hospital and western medicine hospital. Obvi- ously, the participation rate of TCM in these hospitals is very low. Secondly, the total number of medical staff of TCM is seriously inadequate in China. Such as this rescue, the medic- al staff of TCM accounted for less than 20% in the whole dispatched medical staffs from state and province. More- over, the effect of TCM is not accepted constantly, owing to lack of modern scientific support. Nevertheless, there dozens of TCM for NCP are conducting clinical trials at present, certain specific Chinese medicines are also incorpor- ated (Tabel. 2). As reported [20-22], several Chinese medicines have exhibited favourable effect. In a word, the development of TCM still has long way to go. On all accounts, TCM has accumulated a solid theoretical foundation of plague. In the fight against infectious dis- eases broke in recent decades, TCM has played an important role in the prevention and treatment. At present, TCM also has been effectively salvaging the patient with NCP. There- fore, TCM is and will be an effective treatment for NCP.

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Abstract

Background: Coronavirus disease 2019 (Covid-19) has resulted in a global outbreak. Few existing targeted medications are available. Lianhuaqingwen (LH) capsule, a repurposed marketed Chinese herb product, has been proven effective for influenza.

Purpose: To determine the safety and efficacy of LH capsule in patients with Covid-19.

Methods: We did a prospective multicenter open-label randomized controlled trial on LH capsule in confirmed cases with Covid-19. Patients were randomized to receive usual treatment alone or in combination with LH capsules (4 capsules, thrice daily) for 14 days. The primary endpoint was the rate of symptom (fever, fatigue, coughing) recovery.

Results: We included 284 patients (142 each in treatment and control group) in the full-analysis set. The recovery rate was significantly higher in treatment group as compared with control group (91.5% vs. 82.4%, $p = 0.022$). The median time to symptom recovery was markedly shorter in treatment group (median: 7 vs. 10 days, $p < 0.001$). Time to recovery of fever (2 vs. 3 days), fatigue (3 vs. 6 days) and coughing (7 vs. 10 days) was also significantly shorter in treatment group (all $p < 0.001$). The rate of improvement in chest computed tomographic manifestations (83.8% vs. 64.1%, $p < 0.001$) and clinical cure (78.9% vs. 66.2%, $p = 0.017$) was also higher in treatment group. However, both groups did not differ in the rate of conversion to severe cases or viral assay findings (both $p > 0.05$). No serious adverse events were reported.

Conclusion: In light of the safety and effectiveness profiles, LH capsules could be considered to ameliorate clinical symptoms of Covid-19.

Introduction

Since the initial epidemics in Wuhan city, China, coronavirus disease 2019 (Covid-19) has rapidly spread globally, with 84,237 and 2,314,621 laboratory-confirmed cases in China and throughout the world as of April 21st, 2020, respectively. The number of cases might have been underestimated, possibly because asymptomatic viral carriers and patients with mild diseases have insidious or atypical symptoms and signs (WHO Collaborating Centre For Infectious Disease Modelling, 2020). The rapid outbreak worldwide could be reflected by the basic reproductive number that reached to 2.68 (Wu et al., 2020). Covid-19 is caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which potently elicits pulmonary inflammation and infiltration, as well as systemic inflammatory cytokine storms. Timely treatment is cardinal to the management of Covid-19, the adverse impacts of which has been estimated to be significantly greater than those of severe acute respiratory syndrome (Yang et al., 2020).

Currently, supportive therapies are the cornerstone for the management of Covid-19. Development of a novel class of medication would not be practical within a short span during the public emergency event such as Covid-19. In light of the long history of evolution and the proven efficacy in patients with influenza (Duan et al., 2011), the traditional Chinese medicine has recently been repurposed for the clinical management of Covid-19 (Xia et al., 2020). Several candidates with possible antiviral effects have been explored (Wang et al., 2020). In the latest publication, Lianhuaqingwen (LH) capsule (Shijiazhuang Yiling Pharmaceutical Co. Ltd., Shijiazhuang, China) was a manufactured product of the traditional Chinese medicine formula marketed in China that could significantly inhibit SARS-CoV-2 replication, alter the viral morphology and confer anti-inflammatory activity *in vitro* (Li et al., 2020). Moreover, LH capsules could significantly ameliorate the cardinal symptoms (i.e., fever, cough, fatigue) and shorten the course of Covid-19 (Cheng and Li, 2020; Lu et al., 2020). According to the *Diagnosis and Treatment Protocol for Coronavirus Pneumonia (Trial version 7)* (*Diagnosis and Treatment Protocol for Coronavirus Pneumonia*. 2020), LH capsule

has been endorsed by the National Health Commission for the treatment of Covid-19. However, no existing study has been conducted with a sufficient sample size and prospective randomized designs in multicenter settings.

We hypothesized that LH capsules could effectively ameliorate symptoms (including fever, cough and fatigue) and shorten the duration of viral shedding. On the basis of usual treatment, we sought to explore the safety and efficacy of LH capsules in patients with Covid-19 by conducting a multicenter randomized controlled trial in mainland China.

Methods

Study oversight

In this prospective, open-label, randomized controlled trial, we recruited patients with Covid-19 from 23 hospitals in nine provinces throughout mainland China. The study protocol has been approved by the ethics committee of each participating site. The protocol was designed based on the *Good Clinical Practice* guidelines and *The Declaration of Helsinki*, and has been registered with China Clinical Trial Registry website (www.chictr.org/cn/, No.: Chi CTR-TRC-2000029434). All patients signed written informed consent.

Patients

We recruited 284 patients with Covid-19 between February 2nd and February 15th, 2020 **(Figure 1**). Eligibility criteria consisted of the following: 1) Laboratory-confirmed cases with Covid-19 according to the *Protocol for Diagnosis and Treatment of Novel Coronarvirus Pneumonia (4th edition)* which was issued by the National Health Commission (General Office Of The National Health And Health Commission, 2020) (**Panel 1**) Being symptomatic (either having fever, coughing, or fatigue) plus radiologic abnormalities consistent with pneumonia; 3) Patients aged 18 years or greater of either sex.

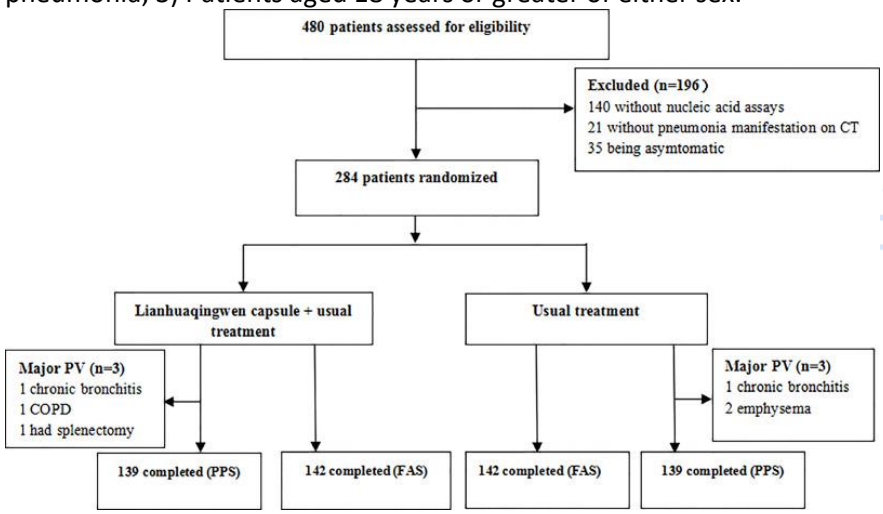

Fig. 1. **Study flow chart**. PV: protocol violation; FAS: full analysis set; PPS: per protocol set.

Key exclusion criteria included: 1) respiratory tract bacterial infections due to primary or secondary immunodeficiency, congenital respiratory malformation, congenital heart disease, gastroesophageal reflux, and lung malformation; 2) asthma or other chronic airway diseases needing maintenance therapy, acute respiratory tract bacterial infection (i.e., bronchiectasis, tonsillitis, bronchitis, rhinosinusitis, otitis media), severe pulmonary interstitial diseases; 3) severe pneumonia needing mechanical ventilation; 4) severe systemic diseases (i.e., malignancy, autoimmune diseases, liver or renal diseases) or surgeries (splenectomy, organ transplantation) that in the judgement of the investigators could affect the assessment of efficacy; 5) women during pregnancy or lactation; 6) participation in clinical trials within 3 months; 7) known allergies to the investigational medications; 8) other conditions judged by the investigators. See the study protocol in *Online Supplement* for the withdrawal criteria.

Study medications

The major ingredients of LH consisted of *Forsythia suspensa, Lonicera japonica, Ephedra sinica, Isatis indigotica, Pogostemon cablin, Rheum palmatum, Glycyrrhiza uralensis, Dryopteris crassirhizoma, Rhodiola crenulata, Houttuynia cordata, Prunus sibirica*, gypsum and 1-menthol (see Online Supplement text for details). LH capsules were manufactured based on *The Pharmacopedia of People's Republic of China*. LH

capsules were provided by the manufacturer, supplied to the participating sites, and dispensed by the designated research nurses after randomization. The routine treatment of the two groups was determined based on the *Diagnosis and Treatment Protocol for Coronavirus Pneumonia (Trial version 7)* (Diagnosis and Treatment Protocol for Coronavirus Pneumonia. 2020), at the discretion of the attending clinicians (General Office Of The National Health And Health Commission, 2020). Routine treatment generally consisted of the supportive treatment such as oxygen therapy, antiviral medications and symptomatic therapies.

Randomization

An open-label study was conducted because of the urgency of major public health events. Randomization numbers were generated using SAS statistical software package (SAS Inc., Cary, USA). A computer-generated 1:1 block randomization scheme was used to assign patients to either treatment group or control group. Each consecutively coded patient was randomly enrolled by the sub-site investigators until the total number of cases allocated to the site was reached. Competitive recruitment was adopted for enrollment.

Procedures

Eligible patients were randomized to receive usual treatment alone based on *The Protocol for Diagnosis and Treatment of Novel Coronarvirus Pneumonia (4th edition)* (control group) or the combination of LH capsules (4 capsules thrice daily for 14 days). Adherence to the study medications, clinical outcomes, the use of concomitant medications and adverse events were recorded. Vital signs, laboratory testing, chest computed tomography and nucleic acid assays of SARS-CoV-2 were evaluated at baseline after randomization and on day 14.

In our study, symptom recovery denoted a complete remission of at least one major symptom (coughing, fever, or fatigue). An experienced radiologist who was blinded to the study allocation reviewed the chest computed tomography (CT) images from all patients. An improvement in chest CT images was defined as a decreased area of infiltration, a decreased area of any radiologic abnormality, or decreased density of the ground-glass opacity or nodules. We defined clinical cure as having met all of the following criteria: recovery of body temperature for more than 3 days, symptom recovery, marked improvement in chest CT images, and two consecutive negative SARS-CoV-2 RNA testing (at least one day apart).

Study endpoints

The primary endpoint was the rate of symptom (fever, fatigue, and coughing) recovery. Fever denoted the subaxillary temperature being 37.3 degrees or greater. The magnitude of fatigue and coughing was self-reported by the patients. Recovery of symptoms was defined as the complete resolution of fever, fatigue and coughing.

Secondary endpoints consisted of the time to symptom recovery, the rate of and the time to the recovery of individual symptoms, the proportion of patients with improvement on chest computed tomography, the proportion of patients with clinical cure, the timing and rate of conversion of SARS-CoV-2 RNA assay.

Safety monitoring

No major reports of the adverse events of LH capsules have been documented after marketing (Cai et al., 2012). In this study, we recorded the timing, severity, duration, measures and consequence of adverse events, and determined the association with the use of study medications.

Statistical analysis

Assuming the length of hospital stay for 14 days, and the rate of recovery of clinical symptoms (fever, fatigue, or coughing) in the control group and treatment group of 82% and 94%, we estimated a total of 240 cases (120 cases in each group) after taking into account the drop-out rate of 10% or lower.

All statistical analyses were conducted with SAS®9.4 software (SAS Institute, Cary, North Carolina). All patients were included in the full-analysis set (FAS) after randomization, while patients with major protocol deviation (PV) were removed from per protocol set (PPS). All statistical testing was two-sided, with $p < 0.05$ being considered statistically significant. Count (percentage) was adopted for summarizing the categorical

variables, and compared with Chi-square tests. Continuous variables were presented with mean ± standard deviation, and compared with independent t-test or Wilcoxon rank-sum test. The time to events was presented as the median duration and 95% confidence interval (95%CI), and analyzed with Kaplan-Meier analysis. The hazards ratio (HR) of the events (i.e. symptom recovery) was also demonstrated.

Results

Patient characteristics

Of the 480 patients who were assessed for eligibility, 196 were excluded due to the lack of symptoms (not having fever, fatigue or coughing), SARS-CoV-2 assay findings, or radiologic abnormality on chest CT. Therefore, 284 patients were included in the FAS (142 each in treatment and control group). Three patients in each group had a major PV and were therefore excluded from the PPS. The treatment group had a good compliance with the study medication, with the average duration being 14.0 days (95%CI: 12.0 ~ 15.0). The study flow chart is shown in **Fig. 1**.

At baseline, most patients were aged above 45 years and males accounted for approximately half of the patients. More than 40% of patients had a recent of contact with people from Wuhan city. Both groups were comparable in terms of the demographic characteristics, vital signs, symptoms and concomitant treatment. 85.2% of patients each in the treatment and control group received antiviral medications such as oseltamivir ($p > 0.05$, **Table 1**).

Table 1
Baseline demographic and clinical characteristics.

Variables	Treatment group N = 142	Control group N = 142	*p* value
Age (yr, $\bar{x} \pm s$)	50.4 ± 15.2	51.8 ± 14.8	0.420
Age ≥ 45 yr	89 (62.7%)	102 (71.8%)	0.100
Males (No., %)	79 (55.6%)	71 (50.0%)	0.342
Temperature (℃, $\bar{x} \pm s$)	37.1 ± 0.7	37.09 ± 0.668	0.932
Systolic blood pressure (mmHg, $\bar{x} \pm s$)	128.8 ± 14.4	131.1 ± 15.83	0.203
Diastolic blood pressure (mmHg, $\bar{x} \pm s$)	80.0 ± 10.9	81.2 ± 10.29	0.367
Heart rate (bpm, $\bar{x} \pm s$)	87.5 ± 12.8	85.5 ± 13.58	0.196
Respiratory rate (bpm, $\bar{x} \pm s$)	20.2 ± 2.1	20.7 ± 3.0	0.104
Recent contact with people from Wuhan (No., %)	64 (45.1%)	62 (43.7%)	0.811
Clustered cases (No., %)	27 (19.0%)	28 (19.7%)	0.881
Duration from symptom onset to hospitalization (days, $\bar{x} \pm s$)	9.5 ± 5.1	9.9 ± 5.9	0.591
Symptoms			
Fever (No., %)	71 (50.0%)	77 (54.2%)	0.476
Fatigue (No., %)	75 (52.8%)	69 (48.6%)	0.476
Coughing (No., %)	123 (86.6%)	127 (89.4%)	0.465
Concomitant medications			
Antiviral (No., %)	121 (85.2%)	121 (85.2%)	> 0.999
Antibiotics (No., %)	95 (66.9%)	95 (66.9%)	> 0.999
Immune modulators (No., %)	77 (54.2%)	81 (57.0%)	0.633
Systemic corticosteroids (No., %)	32 (22.5%)	34 (23.9%)	0.779

Primary endpoints

The rate of symptom recovery at day 14 was significantly higher in treatment group as compared with control group (FAS: 91.5% *vs*. 82.4%, mean difference: 9.2%, 95%CI: 1.3% ~ 17.1%; PPS: 91.4% *vs*. 82.0%, mean difference: 9.4%, 95%CI: 1.3% ~ 17.4%, both $p = 0.022$) (**Fig. 2-A**).

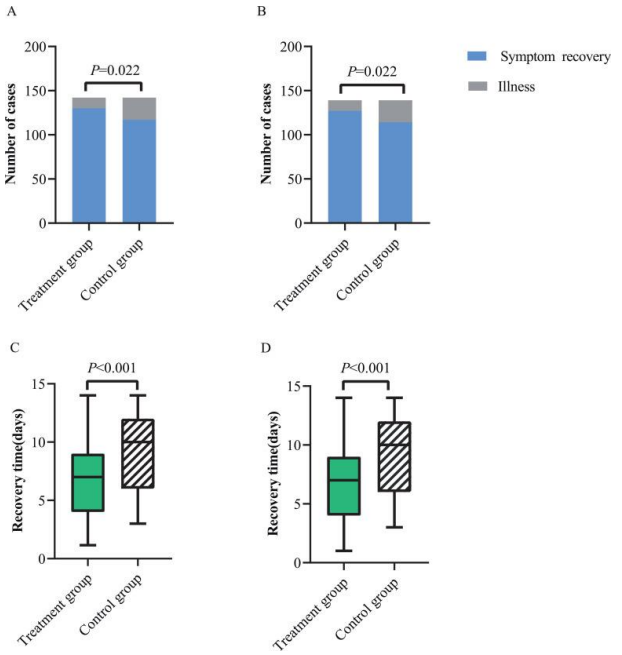

Fig. 2. **The rate of and the time to symptom recovery**.

Fig. 2-A. Comparison of the rate of symptom recovery in treatment and control group in the full analysis set,

Fig. 2-B. Comparison of the rate of symptom recovery in treatment and control group in the per protocol set,

Fig. 2-C. Comparison of the time to symptom recovery in treatment and control group in the full analysis set,

Fig. 2-D. Comparison of the time to symptom recovery in treatment and control group in the per protocol set,

Shown in the figure are the median value and 95% confidence intervals (95%CI).

Secondary endpoints

A significantly shorter median time to symptom recovery was observed in treatment group as compared with control group (FAS: 7 days *vs*. 10 days, HR: 1.72, 95%CI: 1.33 ~ 2.22; PPS: 7 days *vs*. 10 days, HR: 1.70, 95%CI: 1.32 ~ 2.21; both $p < 0.01$) (**Fig. 2-B, Fig. 3**). Moreover, the treatment group yielded a significantly shorter time to the recovery of fever (FAS: 2 days *vs*. 3 days, HR: 1.39, 95%CI: 1.00 ~ 1.94, $p = 0.017$; PPS: 2 days *vs*. 3 days, HR=1.46, 95%CI: 1.04 ~ 2.05, $p = 0.007$), fatigue (FAS: 3 days *vs*. 6 days, HR: 1.78, 95%CI: 1.26~2.54; PPS: 3 days *vs*. 6 days, HR: 1.72, 95%CI: 1.21 ~ 2.45; both $p < 0.001$) and coughing (FAS: 7 days *vs*. 10 days, HR: 1.71, 95%CI: 1.30~2.23; PPS: 7 days *vs*. 10 days, HR: 1.66, 95%CI: 1.27~2.18; both $p < 0.001$) (**Fig. 4**).

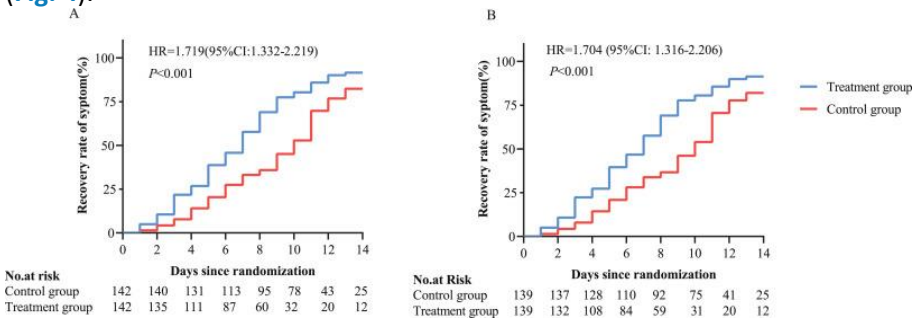

Fig. 3. **Dynamic changes in the recovery rate**.

Fig. 3-A. Dynamic changes in the recovery rate in the full analysis set,

Fig. 3-B. Dynamic changes in the recovery rate in the per protocol set,

Shown is the Kaplan Meier curve for the rate of recovery of the symptoms (including fever, coughing and fatigue). The percentage of patients who achieved the symptom recovery at individual time points was demonstrated for the control group and treatment group.

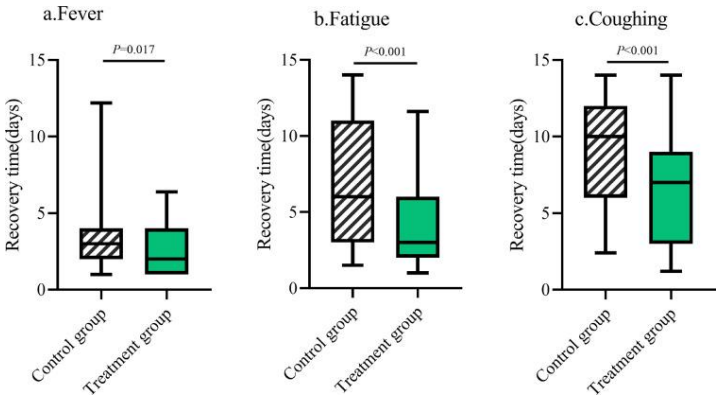

Fig. 4. Time to individual symptom recovery in the full analysis set.

The overall rate of clinical cure was significantly higher in the treatment group as compared with control group in FAS (78.9% *vs*. 66.2%, mean difference: 12.7%, 95%CI: 2.3%22.7%, p <0.05), and PPS (79.1% *vs*. 66.9%, mean difference: 12.2%. 95%CI: 1.8%22.3%, both p = 0.022) (**Fig. 5**). The rate of recovery of chest CT manifestations was also markedly higher in treatment group as compared with control group (FAS: 83.8% *vs*. 64.1%, mean difference: 19.7%, 95%CI: 9.6%~ 29.4%: PPS: 84.2% *vs*. 64.7%, mean difference: 19.4%, 95%CI: 9.2%~ 29.1%; both p < 0.001) (**Fig. 5**). **Fig. 6** demonstrates the CT manifestations in a patient of the treatment group and the other in the control group.

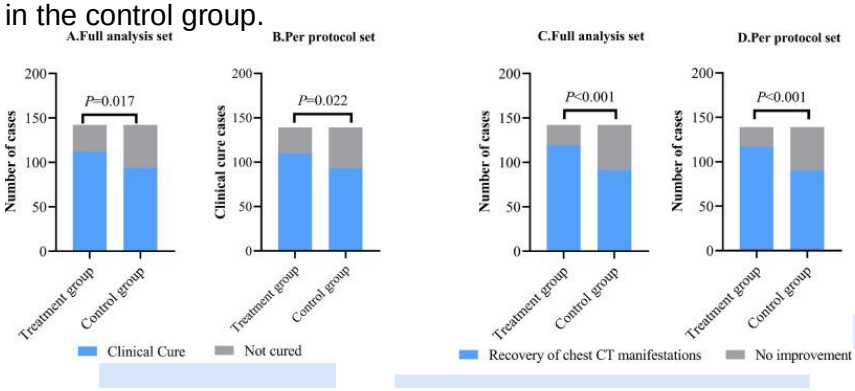

Fig. 5. **The rate of clinical cure and the improvement of chest computed tomographic imaging**.

We defined the clinical cure as having met all of the following criteria: recovery of body temperature for more than 3 days, symptom recovery, marked improvement in chest CT images, and two consecutive negative SARS-CoV-2 RNA testing (at least one day apart).

An improvement in chest CT images was defined as a decreased area of infiltration, a decreased area of any radiologic abnormality, or decreased density of the ground-glass opacity or nodules.

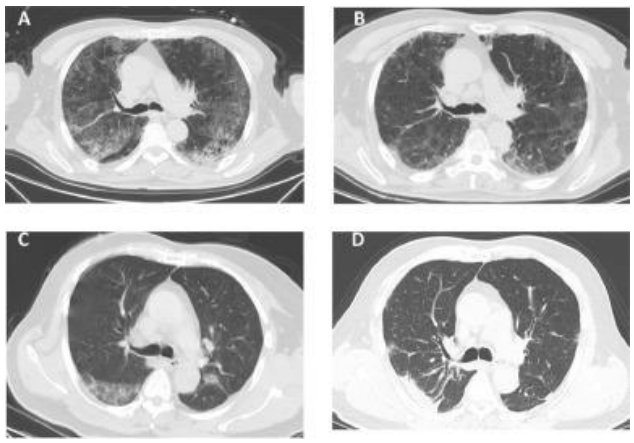

Fig. 6. **Chest computed tomographic manifestations at enrolment and after treatment**.

Shown are the chest computed tomographic manifestations in a patient of the treatment group and the other in the control group. Panel A: A 71-year-old male who presented with bilateral pulmonary infiltrates at the initiation of treatment with LH capsules; Panel B: Marked absorption of bilateral pulmonary infiltrates at day 12 of the treatment with LH capsules; Panel C: A 66-year-old male who presented with bilateral pulmonary infiltrates (the right lower lobe being more prominent) at the initiation of usual treatment; Panel D: Consolidation of the right lower lobe and absorption of the infiltrations in the left lower lobe at day 10 of the usual treatment.

LH: Lianhuaqingwen

Treatment with LH capsules was not associated with a higher conversion rate of SARS-CoV-2 viral assay findings (FAS: 76.8% *vs*. 71.1%, mean difference: 5.6%, 95%CI: -4.6%~ 15.7%, $p = 0.279$; PPS: 77.0% *vs*. 71.2%, mean difference: 5.8%, 95%CI: -4.5%~ 15.9%, $p = 0.273$). The rate of conversion to severe cases in the treatment group was similar as compared with the control group (FAS: 2.1% *vs*. 4.2%, mean difference: -2.1%, 95%CI: -7.0%~ 2.4%, $p = 0.498$; PPS: 0% *vs*. 2.2%, mean difference: -1.4%, 95%CI: -76.2%~ 3.1%, $p = 0.247$). Furthermore, there was no significant difference in the median viral assay conversion time between the treatment group and control group (FAS: 11.0 *vs*. 12.0 days, HR: 1.21, 95%CI: 0.92 ~ 1.59; PPS: 11.0 *vs*. 12.0 days, HR = 1.21, 95%CI: 0.92 ~ 1.59; both $p = 0.151$) (**Table 2**).

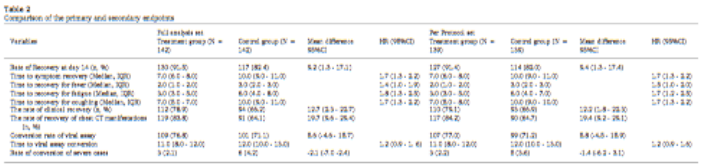

Table 3
Comparison of the adverse events in the full analysis set

Adverse events	Treatment group	Control group	*p* value
Total	65 (45.8%)	77 (54.2%)	0.154
Abnormal liver function	32(22.5%)	32(22.5%)	1.000
Renal dysfunction	8 (5.6%)	11 (7.7%)	0.476
Headache	1 (0.7%)	1 (0.7%)	1.000
Nausea	6 (4.2%)	5 (3.5%)	0.758
Vomiting	2 (1.4%)	3 (2.1%)	0.652
Diarrhea	8 (5.6%)	19 (13.4%)	0.026
Loss of appetite	8 (5.6%)	6 (4.2%)	0.584

Panel 1
The diagnostic criteria of Covid-19

Item	Criteria
Epidemiology	① A recent travel to or residence in Wuhan city or other epidemic regions within 14 days ② Contact with cases from Wuhan city or other epidemic regions or symptomatic cases ③ Clustered cases or having contact with confirmed cases
Clinical manifestation	① Fever ② Radiologic characteristics consistent with pneumonia ③ Normal or decreased leukocyte count at early stages, or lymphopenia
Pathogen	① Positive findings of RT-PCR assay for viral RNA of respiratory tract or blood specimens ② Detection of SARS-CoV-2 or homology of SARS-CoV-2 in the respiratory tract or blood specimens

Diagnostic criteria: At least one criterion in the epidemiology category, plus any two criteria of the clinical manifestations, plus any criterion for pathogen detection

Discussion

To our knowledge, this is the first multicenter randomized clinical trial that demonstrates the safety and efficacy of LH capsule, a Chinese herb product, in patients with Covid-19. Overall, treatment with LH capsules for 14 days resulted in a significantly higher rate of, and a shorter time to, symptom recovery than control group (usual treatment). The rate of recovery of fever, fatigue and coughing was also higher in treatment group. The rate of conversion of viral RNA assay, however, did not reach to statistical significance. LH capsule had a favorable safety profile for the treatment of Covid-19.

SARS-CoV-2 has been shown to result in an injury of the respiratory tract, nervous system (General Office Of The National Health And Health Commission, 2020), the liver (Chai et al., 2020), the heart, esophagus, kidney, urinary bladder and the jejunum (Li et al., 2020; Zou et al., 2020). The susceptibility of SARS-CoV-2 among the whole population might be related to the ability to potently bind with angiotensin converting enzyme 2 (Zhou et al., 2020). SARS-CoV-2 could infect various cells in human with similar biological behaviors of SARS-CoV (Huang and Herrmann, 2020; Zhou et al., 2020). From the perspective of tissue tropism, the pathological features of Covid-19 mainly resembled those of SARS coronavirus infection (Zhao et al., 2020). In light of the high levels of homology of sequence among SARS-CoV-2, SARS-CoV and Middle East Respiratory Syndrome coronavirus, medications that have been proven to be effective for SARS and MERS

might also be adopted to the treatment of Covid-19 (Li and De Clercq, 2020). Unfortunately, no existing antiviral medications are available for the treatment of Covid-19.

Based on a nationwide study with 1099 patients from 552 hospital throughout mainland China, we have recently reported that fever accounted for 88.7% of patients and cough accounted for 67.8% of patients after hospital admission (Guan et al., 2020). In light of the lack of validated effective therapeutic approaches, the medications that could ameliorate fever, fatigue and coughing would be valuable for the clinical management of Covid-19. LH capsules, which is a patented product, have been marketed since the outbreak of SARS in 2003 in China. The latest research showed that LH conferred suppression of the cytopathic effect of SARS-CoV-2 *in vitro* and reduced the viral loads in the cytoplasm and cellular membrane (Li et al., 2020). LH could also suppress the replication of SARS-CoV (Zhu et al., 2003), H_3N_2, H_1N_1 and H_7N_9 *in vitro* (Chinese Academy Of Military Hospital, 2009; Ding et al., 2017; Duan et al., 2011; Mo et al., 2007). The inflammatory cytokine storm has been regarded as an excessive host-defense response to the virus (Xu et al., 2020; Zhe Xu, 2020), which can cause diffuse lung injury and predispose to the development of severe disease. Suppression of excessive release of inflammatory meditators is the Holy Grail for halting the progression to severe disease (Xu et al., 2020). Recent studies indicated that LH could dose-dependently inhibit the release of tumor necrosis factor-α, interleukin-6, macrophage chemokine protein-1 and induced protein-10 (Li et al., 2020), and could also effectively abrogate the expression of tumor necrosis factor-α, interleukin-6, interleukin-1β, interleukin-2, interleukin-4 and interleukin-13 (Mo et al., 2007), thus ameliorating lung injury associated with inflammatory cell infiltration (Cui et al., 2015). These *in vitro* findings have provided with the rationale for clinical application of LH capsules in Covid-19.

In our study, treatment with LH capsules for 14 days markedly improved the rate of symptom recovery (57.7% at day 5, 80.3% at day 10 and 91.5% at day 14). The time to symptom recovery was also significantly shorter in treatment group. Overall, LH capsules have shortened the duration of fever, fatigue and coughing by 1, 3 and 3 days, respectively. The higher rate of clinical cure and recovery of chest CT manifestations could also be associated with the activity against SARS-CoV-2, and probably, the anti-inflammatory effects. There was a lack of statistical significance for the difference in the rate of, and the time to, conversion of viral assays. These findings differed from those in the *in vitro* studies (Li et al., 2020), possibly because of the difference in the model for observation (human vs. cell models) and the study endpoints (changes in the viral loads in human respiratory tract specimens vs. viral loads in the cytoplasm *in vitro*). No serious adverse events were reported, supporting the safety of LH capsules for the treatment of Covid-19.

LH consists of the key components such as *Lonicera japonica* and *Forsythia suspense* which could block the binding of SARS-CoV-2 with the angiotensin converting enzyme (Niu et al., 2020). *Pogostemon cablin* has been shown to ameliorate diarrhea and improve the host-defense of the gastrointestinal tract (Zhou, 2018). *Rhodiola rosea* could ameliorate lung injury via the suppression of oxidative stress and apoptosis (HuangFu et al., 2019) and abrogation of pulmonary inflammation (Yao and Luo, 2020). In addition, *Rheum palmatum* could effectively antagonize the binding of spike protein and the angiotensin converting enzyme (Ho et al., 2007) and suppress the excessive release of inflammatory mediators, thus ameliorating the lung injury (Dong et al., 2017). These observations have provided the evidence regarding the antiviral effects of LH capsules.

The exploration of repurposed Chinese herb product would be valuable to the treatment of Covid-19 because, apart from convalescent plasma, no other medications with proven efficacy exist. Our findings indicated that LH capsules could be recommended to patients with Covid-19 for reducing the symptom burden and improving clinical outcomes. However, there are some limitations of the study design. No blinding was implemented because of the urgency of the outbreak that entailed a timely treatment, and placebo-controlled trial would be unethical in light of the rapid outbreak of communicable diseases such as Covid-19. The duration of treatment was established empirically, and whether a prolonged duration would translate into the greater efficacy warrants further investigation. An extended study would be needed to thoroughly explore the effects of LH capsules on the viral shedding and the resolution of all symptoms.

Conclusion

In summary, LH capsules confer therapeutic effects on Covid-19 by improving the recovery rate of symptoms, shortening the time to symptom recovery, and improving the recovery of chest radiologic abnormalities. In light of the efficacy and safety profiles, LH capsules could be considered for the treatment

of Covid-19. Future double-blind, prospective, randomized controlled trials are needed to fully evaluate the efficacy of LH capsules in a larger patient population.

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Traditional Chinese medicine for treatment of coronavirus disease 2019: a review

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Highlights

Coronavirus disease 2019 (COVID-19) has recently become a public health concern worldwide. The use of traditional Chinese medicine (TCM) may have substantial impact on COVID-19. In this review, we summarize the disease pathogenesis, clinical outcomes, and current applications of TCM for the treatment of COVID-19.

Traditionality

The pathogenesis and clinical symptoms related to severe respiratory disease were described many years ago in TCM texts. The ancient book of TCM *Huang Di Nei Jing* (*Inner Canon of Huangdi*) was written during the Western Han Dynasty of China (dated approximately 99 B.C.E.-26 B.C.E.); the text recorded a plague that could transmit disease from human-to-human with symptoms that were similar to those described for COVID-19. Three additional texts, notably *Shang Han Za Bing Lun* (*Treatise on Cold Damage Diseases*) written by Zhang Zhongjing (200 C.E.-210 C.E.), *Wen Yi Lun* (*Theory of Plague*) and *Wen Re Lun* (*Translated Theory of Warm*) written by Wu Youke (1642 C.E.), recorded therapies and formulas that were effective at treating infectious diseases; among them, the classical prescription Da Yuan Yin and the use of human variolation were considered as means to prevent smallpox. Currently, the use of TCM has resulted in remarkable improvement and alleviation of symptoms in COVID-19 patients.

Abstract

Since late December in 2019, the coronavirus disease 2019 has received extensive attention for its widespread prevalence. A number of clinical workers and researchers have made great efforts to understand the pathogenesis and clinical characteristics and develop effective drugs for treatment. However, no effective drugs with antiviral effects on severe acute respiratory syndrome coronavirus 2 have been discovered currently. Traditional Chinese medicine (TCM) has gained abundant experience in the treatment of infectious diseases for thousands of years. In this review, the authors summarized the clinical outcome, pathogensis and current application of TCM on coronavirus disease 2019. Further, we discussed the potential mechanisms and the future research directions of TCM against severe acute respiratory syndrome coronavirus 2.

Key words: Severe acute respiratory syndrome coronavirus 2 ; Coronavirus disease 2019 ; Clinical outcome ; Angiotensin-converting enzyme 2 ; Traditional Chinese medicine

Background

Coronavirus disease 2019 (COVID-19) has received extensive attention for its increasing incidence and widespread prevalence [1, 2]. On January 31, 2020, World Health Organization declared that the outbreak of COVID-19 had become public health emergency of international concern. According to statistics from National Health Commission of the People's Republic of China, by February 16, 2020, a total of 70,548 confirmed cases and 1,770 fatal cases of COVID-19 had been reported in China. At this writing, cases of COVID-19 infection have been reported in more than 20 countries and in regions worldwide [3].

Given the recent advances in research and biotechnology, a virus believed to be the etiologic agent of COVID-19 was isolated and the sequence of virus genome was revealed using high-throughput sequencing [4]. Currently, this virus is named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [5] and the disease resulting SARS-CoV-2 was officially named COVID-19 by World Health Organization [6]. Bats have been identified as the possible animal host of SARS-CoV-2 [7] and a mechanism for SARS-CoV-2 infection has been postulated. The timeline of important events marking the SARS-CoV-2 outbreak since the report of the first case on December 26, 2019 is shown in Figure 1. Traditional Chinese medicine (TCM) has a thousand-year history of experience with all types of infectious diseases and has been employed previously as effective treatments for severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) [8]. We report here that TCM is currently in wide use for the treatment of COVID-19. In this review, we summarize the clinical outcome, pathogenesis and current application of TCM for the treatment of COVID-19.

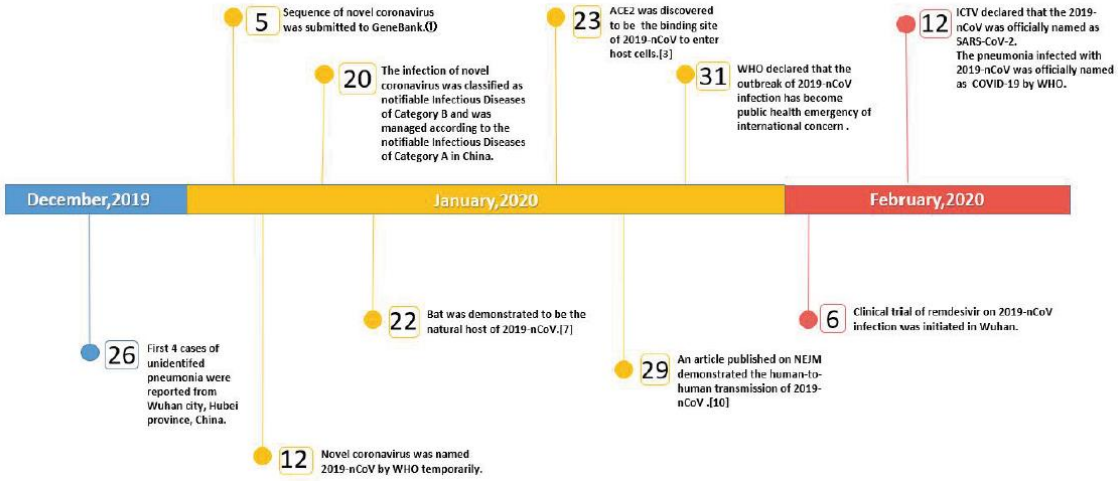

Figure 1

Timeline of important events during SARS-CoV-2 outbreak. COVID-19, coronavirus disease 2019; ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization; ICTV, International Committee on Taxonomy of Viruses ; NEJM, New England Journal of Medicine; 2019-nCoV, 2019 novel coronavirus; ①, https://www.ncbi.nlm.nih.gov/nuccore/MN908947.

Epidemiology of COVID-19

Zhou et al. found that the genome sequence of a coronavirus isolated from a bat showed 96% similarity with the sequence of SARS-CoV-2; these results suggested that bat species might be a host of SARS-CoV-2. In addition, the earliest cases of COVID-19 were all individuals who reported direct contact with the Huanan Seafood Market in Wuhan, China [9]. Many wild animals such as hedgehog, badger, snake and birds are sold for human consumption at this market, although bats are not typically included in this group. Researchers from South China Agricultural University declared the intermediate host of SARS-CoV-2 could be the Chinese pangolin (*Manis pentadactyla*) although this has not been confirmed at this date.

An article published on *New England Journal of Medicine* on January 29, 2020, reported human-to-human transmission of SARS-CoV-2 [10]. Recent epidemiology studies by Guan et al. [11] documented that only a few COVID-19 patients (1.18%) were in contact with wildlife, while 31.8% of COVID-19 patients traveled to Wuhan recently and 71.80% of COVID-19 patients had recent contact with people from Wuhan.

Respiratory and contact transmission are the main transmission routes of SARS-CoV-2. SARS-CoV-2 RNA is also detected in feces of COVID-19 patients, suggesting the possibility of fecal-oral transmission as another potential transmission route [12-14]. Aerosol and transplacental transmission routes are also regarded as among important possibilities to consider, although there is no substantial research supporting this hypothesis at this time.

Clinical symptoms of COVID-19

The asymptomatic incubation period of SARS-CoV-2 is 0 to 24 days, with a median incubation period of 3 days [11]. Once the disease has taken hold, most of the patients report symptoms including fever, cough, dyspnea, muscle soreness and/or fatigue. Some patients also reported sputum production, headache, hemoptysis and/or diarrhea. Patients with mild symptoms develop low-grade fevers and mild fatigue but no symptoms suggestive of pneumonia. By contrast, patients with severe disease experience dyspnea and hypoxemia which can develop into acute respiratory distress syndrome (ARDS), septic shock, severe metabolic acidosis and coagulation disorders [9-11, 15]. The epidemiology of severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2 and clinical features of three different coronavirus syndromes (SARS, MERS and COVID-19) are listed in Table 1 and Table 2, respectively.

Table 1 Epidemiology of SARS-CoV, MERS-CoV and SARS-CoV-2

	SARS-CoV [16, 17]	MERS-CoV [17-19]	SARS-CoV-2 [3, 11, 20]
Date of first detection	November, 2002	June, 2012	December, 2019
Location of first detection	Guangdong province, China	Jeddah, Saudi Arabia	Wuhan city, Hubei province, China
Epidemiological distribution	Large outbreaks of imported cases in South China, Canada and Asia	27 countries and regions in the Middle East, Europe, Africa, Asia and North America	Outbreak in China, imported cases in more than 20 countries and regions such as Japan, Singapore, Thailand and others.
Onset season	Winter	Breeding season of camels	Winter
Natural host	*Rhinolophus sinicus*	Bat (?) [a]	Bat (?) [a]
Intermediate host	Wild mammals (civet in South China)	Camels (Middle East and Africa)	*Manis pentadactyla* (?) [a]
Route of transmission	Droplets, contact	Droplets, contact, airborne (?) [a]	Droplet, contact, fecal-oral (?) [a], aerosol (?) [a], transplacental (?) [a]
Main form of transmission	Human-to-human, animal-to-human	Animal-to-human, human-to-human	Human-to-human, animal-to-human
Incubation period (days)	1.9-14.7	2-14, occasionally up to 21 days	0-24
Age, years (range)	39.9 (1-91)	56 (14-94)	47 (35-48)
Male : female sex ratio	1 : 1.25	3.3 : 1	1.39 : 1
Confirmed cases	8,096	2,494	70,548
Fatal cases	774	858	1,770
Basic reproductive number (R0)	0.3-4.1	0.3-1.3	2.68

The statistical characteristics associated with SARS-CoV-2 were derived from the 1099 patients (as of January 29, 2020) reported by the team of Zhong Nanshan. The number of confirmed cases and deaths was determined by the National Health Commission of the People's Republic of China (information presented as reported through February 16, 2020).

(?) [a], presents the result of current hypotheses but without strong evidence at this time; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 2 Clinical characteristics of SARS, MERS and COVID-19

	SARS [17]	MERS [19]	COVID-19 [11]
Common symptoms	Hyperpyrexia (more than 38 ℃)	Fever, cough, polypnea	Fever, cough, fatigue
Common extrapulmonary symptoms	Diarrhea	Acute renal failure, diarrhea	Diarrhea, emesis
Imaging features	Interstitial ground-glass changes in lung, parenchymal lesions in mediastinum	Interstitial ground-glass changes in lung, parenchymal lesions in lung	Ground-glass opacity in lung, bilateral patchy opacity in lung
Common complications	ARDS	ARDS, renal failure, disseminated intravascular coagulation and pericarditis	Pneumonia, ARDS, shock
Blood routine and serological tests	Decrease of white blood cells, lymphocytes and blood platelets, increase of ALT and AST	Decrease of white blood cells, lymphocytes and blood platelets in early stage and increase of white blood cells and neutrophil during disease progression, increase of ALT and AST and renal dysfunction	Normal or decrease of white cells, decrease in lymphocytes in mild cases, persistent decrease of lymphocytes in severe cases, increase of ALT, AST, LDH, CK-MB, CRP and ESR in mild cases, increase of cTn in severe cases

SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome; COVID-19: corona virus disease 2019; ARDS, acute respiratory distress syndrome; CK-MB, creatine kinase isoenzyme-MB; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; cTn, cardiac troponin.

Angiotensin-converting enzyme 2: the receptor for SARS-CoV-2 on target cells

During the pandemic phases of SARS-CoV in 2003, angiotensin-converting enzyme 2 (ACE2) was identified as the SARS-CoV receptor on target host cells [21]. DNA sequencing revealed that SARS-CoV-2 genome shares 89% similarity with that of SARS-CoV, suggesting that the mechanisms for SARS-CoV-2 infection of target cells may be similar to that already identified for SARS-CoV [9, 22, 23]. Zhou et al. [9] demonstrated that ACE2 was the cell entry receptor for SARS-CoV-2 in in vitro infectivity studies. Specifically, Hela cells that were genetically modified to express ACE2 from different species including human, Chinese horseshoe bats, civets, pigs and mice were infected with SARS-CoV-2 in vitro. The results demonstrated that ACE2 proteins from all species tested except mice could serve as entry receptors for SARS-CoV-2. Results from experiments using Hela cells expressing human ACE2, or other known coronavirus receptors, including aminopeptidase N and dipeptidyl peptidase 4, revealed that only ACE2 was effective as an entry receptor for SARS-CoV-2 in vitro.

ACE2 is a type I transmembrane protein composed of 805 amino acids and is primarily expressed in the gastrointestinal tract, heart, kidney and lung. As a negative regulator of the renin-angiotensin system, ACE2 plays an important role in maintaining homeostasis of cardiovascular system and regulating absorption of

amino acids in kidney and gastrointestinal tract [24]. Genetic studies also reveal the role of ACE2 in preventing stroke [25].

Coronavirus invasion of host cells depends on the actions of the spike protein (S protein) on virus surface. The SARS-CoV S protein includes 2 subunits [26]. The receptor binding domain (RBD) on the S1 subunit interacts with ACE2 to form a virion-ACE2 complex. The virion-ACE2 complex is then transported and enters the endosome of target cells. Subsequently, the structure domain of heptad repeats (HR)1 and HR2 in S proteins interact with one another to form a six-helix bundle core. This core promotes fusion of the viral envelope with cellular membrane. The RNAs of virus are then released into the cytoplasm of target cells (Figure 2) [27, 28]. SARS-CoV virions may also enter the target cell via plasma membrane fusion, via a means similar to that used by human immunodeficiency virus [29].

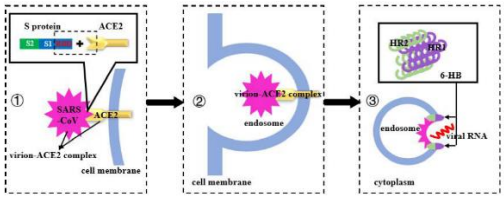

Figure 2

SARS-CoV binds to ACE2 and enters target cell through endosomal membrane fusion. ① The receptor binding domain (RBD) on S1 protein may bind with ACE2 on cell membrane to form virion-ACE2 complex. ② The virion-ACE2 complex is transported and enters the endosome of target cells. ③ The structure domain of heptad repeat (HR) 1 and HR2 in S protein interact with one another to form a six-helix bundle core, which promotes fusion between the viral envelope and the cellular membrane. The virus RNAs are then released into the cytoplasm of target cells.
ACE2, angiotensin-converting enzyme 2; S protein, spike protein; RBD, receptor binding domain; SARS-CoV, severe acute respiratory syndrome coronavirus; HR, heptad repeat; 6-HB, six-helix bundle.

After entering the target cells, the SARS-CoV RNA genome interacts in a complex with the viral RNA replicase and transcription enzymes. The minus strands of viral RNA are transcribed and are ultimately translated into structural proteins [30]. The cytoplasmic viral RNAs and structural proteins are packaged to form new viruses which are released from infected cells and go on to infect other available target cells in the immediate environment.

In vivo studies were also carried out to investigate the role of ACE2 in a respiratory disease model. ACE2 gene-deleted mice exhibited severe lung injury and dysfunction of renin-angiotensin system in a model of lung injury induced by cationic polyamidoamine dendrimer nanoparticles; likewise, treatment with an angiotensin II type I receptor antagonist suppressed the sequelae of nanoparticle-induced lung injury in wild-type mice [31]. Creation of an in vivo infection model for SARS-CoV and SARS-CoV-2 was not straightforward, as neither interacts with mouse ACE2. Perlman and colleagues established a human ACE2 transgenic mice model. With this transgenic mouse model, SARS-CoV could be detected in the pulmonary alveoli. It is conceivable that the human ACE2 transgenic mouse model can be an important tool for conducting SARS-CoV-2 infection experiments and for identifying new drugs that would be effective for the treatment of COVID-19 [32, 33].

Role of TCM on treating COVID-19

In TCM, the term “plague” is used to denote infectious disease. From the earliest times, the Chinese people have understood the severity underlying this disease process and have searched for improved understanding. The ancient book of TCM *Huang Di Nei Jing* (*Inner Canon of Huangdi*) was written during Western Han Dynasty in China (approximately 99 B.C.E.-26 B.C.E.) and recorded that plague,

with symptoms familiar to modern times, could be transmitted from human-to-human. *Shang Han Za Bing Lun* (*Treatise on Cold Damage Diseases*) written by Zhang Zhongjing (200 C.E.-210 C.E.), *Wen Yi Lun* (*Theory of Plague*) and *Wen Re Lun* (*Theory of Warm*) written by Wu Youke (1642 C.E.) recorded therapies and formulae used to treat plague, including Da Yuan Yin and the use of human variolation to prevent smallpox [34, 35]. Dr. Tu Yoyo credited the discovery of artemisinin for the treatment of malaria according to the early records of *Zhou Hou Fang* (*Handbook of Prescriptions for Emergencies*) written by Ge Hong (284 C.E.-364 C.E.) [36]. TCM has provided significant and important therapies for SARS-CoV, influenza A H1N1, influenza A H7N9 and Ebola virus [37-40]. Consequently, TCM is becoming an important means for developing therapies to treat COVID-19.

Pathogenesis of COVID-19 in TCM theory

Ancient Chinese people believed that man is an integral part of nature. According to this theory, environmental factors are critical elements in the pathogenesis of plague. For example, TCM considers that the characteristics of COVID-19 may largely depend on the environment in Wuhan. During the winter of 2019, a large amount of precipitation fell in Wuhan, which resulted in a moist environment and increased the risk of virus infection. This observation implies that a Chinese herb that promotes the elimination of dampness (a kind of pathological product of disease in TCM theory) can be used in the treatment of COVID-19.

TCM treatments for COVID-19

Classical prescription. The fifth edition of "Standard Therapy of COVID-19" (abbreviated Standard Therapy) published on February 9, 2020, recommended that a modification of the integrated Ma Xin Gan Shi decoction with Da Yuan Yin could be used to improve the chest distress, cough and asthmatic symptoms that develop in COVID-19 [12]. The Ma Xin Gan Shi decoction that includes Mahuang (*Ephedrae herba*), Xingren (*Armeniacae semen amarum*), Gancao (*Glycyrrhizae radix et rhizoma*), Shigao (*Gypsum fibrosum*) together with Da Yuan Yin had significant impact on SARS in 2003 [41, 42]. The use of Da Yuan Yin, composed of Binlang (*Arecae semen*), Houpo (*Magnoliae officinalis cortex*), Caoguo (*Tsaoko fructus*), Zhimu (*Anemarrhenae rhizoma*), Shaoyao (*Dioscoreae rhizoma*), Huangqin (*Scutellariae radix*), Gancao (*Glycyrrhizae raadix et rhizoma*), was first recorded in *Wen Yi Lun* (*Theory of Plague*) (1642 C.E.); this decoction has been used to treat plague for thousands of years. The effectiveness of this decoction for the treatment of SARS was evaluated using a molecular docking method; quercetin, kaempferol, 7-methoxy-2-methyl isoflavone, formononetin and baicalein were identified as the five compounds with highest connectivity to the SARS-CoV 3CL protease [43]. A report dated February 6, 2020 from the State Administration of Traditional Chinese Medicine recommended the use of Qing Fei Pai Du decoction that includes Mahuang (*Ephedrae herba*), Shigao (*Gypsum fibrosum*), Banxia (*Pinelliae rhizoma*), Zhishi (*Aurantii fructus immaturus*), Shengjiang (*Zingiberis rhizoma recens*), that was derived from a modification of the integration of Ma Xing Gan Shi, She Gan Ma Huang, Xiao Chai Hu, and Wu Ling San decoctions in *Shang Han Za Bing Lun* (*Treatise on Cold Damage Diseases*) (200 C.E.-210 C.E.); this recommendation was based on previous experience with SARS and the cold and wet weather in Wuhan. The Qing Fei Pai Du decoction has been demonstrated to be 90% effective in treating COVID-19 [44].

Chinese patent medicine. According to the standard therapy, the patent medicine of Huo Xiang Zheng Qi capsule can be used to treat the gastrointestinal symptoms of COVID-19. Huo Xiang Zheng Qi capsule, derived from *Tai Ping Hui Min He Ji Ju Fang* (*Prescriptions People's Welfare Pharmacy*) written by Chen Shiwen and others (1151 C.E.), has the effects of resolving dampness and is used to treat diarrhea associated with virus infection [45]. The usage of Huo Xiang Zheng Qi capsule for COVID-19 was closely related to the cold and wet weather in Wuhan. Likewise, Lian Hua Qing Wen capsules and Fang Feng Tong Sheng pills can be used to treat fever, fatigue and cough associated with COVID-19 [12]. Lian Hua Qing Wen capsule has broad-spectrum antiviral and antibacterial effects, most notably used for respiratory virus infections including influenza, SARS and MERS [46]. According to a recent retrospective analysis, use of Lian Hua Qing Wen capsule might reduce

fever, cough, expectoration, fatigue and difficulty with breathing in COVID-19 patients. Among the findings, the fraction of severe cases was decreased after the treatment of Lian Hua Qing Wen capsule [47, 48].

Other treatments. Other therapies associated with TCM such as acupuncture, moxibustion, and Tai Chi promote health by enhancing the immune system and improving pulmonary function. Although there is no current evidence relating any of these therapies with COVID-19, they may have crucial roles in disease prevention and likewise in promoting recovery of pulmonary function during recuperation from COVID-19. Acupuncture has been shown to relieve the side-effects of hormonal therapy and to alleviate pulmonary injury [49]. *Ben Cao Gang Mu* (*Materia Medica with Commentaries*) written by Li Shizhen (1578 C.E.) recorded that moxibustion could improve digestion, relieve asthma and prevent plague; modern studies reveal that moxibustion can limit the acute inflammatory response in respiratory tract [50]. Tai Chi is a traditional sport in TCM and can enhance recovery of pulmonary function through respiratory training [51]. In addition, Jin Zhi (Gold Juice), first recorded in *Ben Cao Qiu Zhen* (*Truth-Seeking Herbal Foundation*) written by Huang Gongxiu (1769 C.E.), was made from the fermentation of feces from young men. During the Qing dynasty, Jin Zhi was used to reduce fever in patients with plague [52].

Summary and future perspectives

Since the emergence of COVID-19, clinicians and researchers have made great efforts to understand the pathogenesis and clinical characteristics of this infection and to develop effective drugs for its treatment. Currently, there are no effective antiviral available to treat SARS-CoV-2. On February 6, 2020, a clinical trial of remdesivir, a newly-discovered antiviral drug with potential impact on SARS-CoV-2, was initiated in Wuhan. However, given issues related to both safety and efficacy, it will take some time to develop both antiviral drugs and a vaccine to prevent SARS-CoV-2 infection. Western-type antiviral therapies including α-interferon and lopinavir, treatment with antibiotics, and support therapies including oxygen and mechanical ventilation have been used as the treatment of COVID-19. Therapies based on principles of TCM have improved symptoms and enhanced immunity against virus in COVID-19 patients. Positive responses from patients have been noted when efforts are made to combine approaches from TCM and Western medicine on COVID-19. In the future, TCM may also have a role in decreasing the some of the side-effects of Western medicine, notably with respect to recovery of pulmonary function. Finally, we would like to note that many herbs used in these decoctions, including Mahuang *(Ephedrae herba),* Xingren *(Armeniacae semen amarum),* and Chaihu (*Bupleuri radix*) have a bitter taste. Many of the bitter contents of these herbs such as ephedrine and amygdalin are aromatic substances; the hydrophobic properties of these aromatic substances may inhibit the interaction of the virus S protein with ACE2. However, due to the complex targets and multiple contents that are characteristics of TCM decoctions, further studies would be needed to elucidate the detailed mechanisms involved in their impact on COVID-19 using network pharmacology analysis, experimental validation and multi-omics.

The authors declare that they have no conflict of interest.

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Abstract

The epidemic of pneumonia (COVID-19) caused by novel coronavirus (SARS-CoV-2) infection has been listed as a public health emergency of international concern by the World Health Organization (WHO), and its harm degree is defined as a global "pandemic". At present, the efforts of various countries focus on the rapid diagnosis and isolation of patients, as well as to find a treatment that can combat the most serious impact of the disease. The number of reported COVID-19 virus infections is still increasing. Unfortunately, no drugs or vaccines have been approved for the treatment of human coronaviruses, but there is an urgent need for in-depth research on emerging human infectious coronaviruses. Clarification transmission routes and pathogenic mechanisms, and identification of potential drug treatment targets will promote the development of effective prevention and treatment measures. In the absence of confirmed effective treatments, due to public health emergencies, it is essential to study the possible effects of existing approved antivirals drugs or Chinese herbal medicines for SARS-CoV-2. This review summarizes the epidemiological characteristics, pathogenesis, virus structure and targeting strategies of COVID-19. Meanwhile, this review also focus on the re-purposing of clinically approved drugs and Chinese herbal medicines that may be used to treat COVID-19 and provide new ideas for the discovery of small molecular compounds with potential therapeutic effects on novel COVID-19.

In late December 2019, an outbreak of pneumonia of unknown cause began in Wuhan, Hubei Province, China, spreading rapidly around the world [1]. Chinese researchers discovered a previously unknown betacoronavirus through the use of unbiased sequencing in samples from patients with pneumonia [2]. This coronavirus, named SARS-CoV-2, causes a disease called COVID-19 that can be transmitted from person to person [3,4]. COVID-19 may rapidly develop into acute respiratory distress syndrome (ARDS) and in some cases, lead to multiple organ dysfunction or death. In view of alarming levels of spread and severity, COVID-19 was declared a public health emergency of international concern on January 30, 2020 and situated as a pandemic on March 11, 2020 by WHO [5,6]. As of May 3, 2020, there have been more than 3.3 million reported cases and 230,000 deaths in more than 200 countries. Unfortunately, no drugs or vaccines have been approved for combating the virus [7]. Considering the growing threat of COVID-19 pandemic, it is essential to study the efficacy of existing antiviral drugs as well as Chinese herbal medicines against SARS-CoV-2. In this review, we summarized the epidemiological characteristics, pathogenesis, virus structure and targeting strategies of COVID-19, with emphasis on the re-purposing of clinically approved drugs and Chinese herbal medicines that may be used to treat COVID-19 and provide new ideas for the discovery of small molecular compounds with potential therapeutic effects on COVID-19.

1. Genome structure and pathogenesis of SARS-CoV-2

SARS-CoV-2 is a spherical, enveloped, single-stranded positive RNA virus with a diameter of 80 nm–160 nm and a genome size of 29.9 kb [8]. SARS-CoV-2 falls within the subgenus *Sarbecovirus* of the genus Betacoronavirus that shares about 79.6 % identity with genome of SARS-CoV [9,10]. The architecture of virions is composed of nucleic acid and nucleocapsid protein to form the helical nucleocapsid. Lipid envelope which is studded with structural protein including the membrane (M) glycoprotein, the envelope (E) protein, and the spike (S) glycoprotein [11].

Virus infection is initiated by the interaction between S protein and host cell surface receptors. The S protein would be cleaved by the cellular serine proteases TMPRSS2 into S1 and S2 subunits, which are responsible for receptor recognition and membrane fusion [12,13]. The C-terminal domain (CTD) of S1 specifically binds to host cell receptors angiotensin-converting enzyme 2 (ACE2) or CD147, which causes the conformational change of S2. Then two heptad repeats join in S2 forming an anti-parallel six-helix bundle that allows for the mixing of viral and cellular membranes, resulting in release of the viral genome into the cytoplasm subsequently [14,15]. After release, the viral genomic RNA begins to express. The replicase gene encodes two large ORFS, rep1a and rep1b, which express two co-terminal polyproteins, pp1a and pp1ab. They produce 16 unstructured proteins which assemble into the replicase–transcriptase complex (RTC). RTC creates an environment suitable for RNA synthesis and is ultimately responsible for RNA replication and transcription of the sub genome RNAs. After replication and sub genome RNA synthesis, the S, E and M viral structural proteins are translated and inserted into the endoplasmic reticulum (ER), subsequently moved into endoplasmic reticulum-Golgi intermediate compartment (ERGIC) [16]. There, N protein encapsulates viral genome buds into a membrane containing ERGIC to form mature viruses, which are transported to the cell surface in vesicles and released by exocytosis [17].

After SARS-CoV-2 infection, pathogenic T cells are rapidly activated to produce granulocyte macrophage colony stimulating factors, such as GM-CSF and IL-6 [18]. GM-CSF will further activate CD14+/CD16+ inflammatory monocytes to produce a large amount of IL-6 and other inflammatory factors by a positive feedback effect [19,20]. In addition, high levels of neutrophil extracellular traps may also contribute to cytokine release [21]. Ultimately, uncontrolled inflammatory responses may lead to shock and tissue damage in the heart, liver and kidney, as well as respiratory failure or multiple organ failure, causing death in patients with severe COVID-19 [22,23] (Fig. 1).

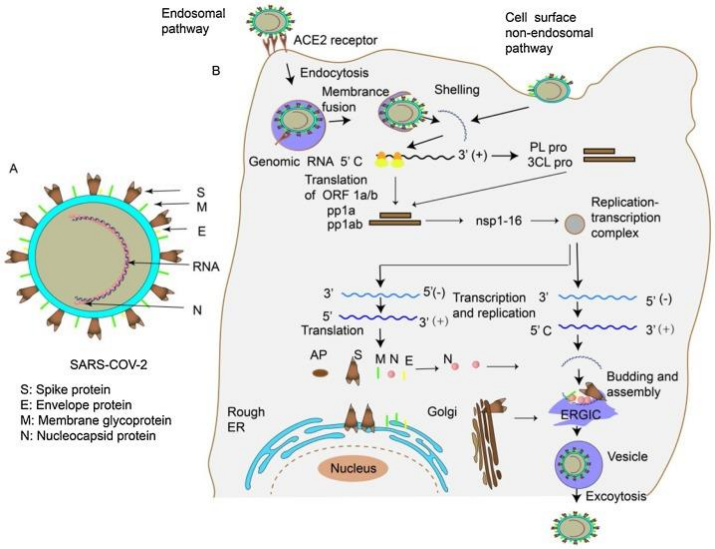

Fig. 1. Life cycle of SARS-CoV-2 in host cells. (A) Structure of SARS-CoV-2. (B) Mechanism of SARS-CoV-2 infection.

2. Key targets and their roles in SARS-CoV-2 infection

Therapeutics with high specificity and efficacy is the ultimate goal of pathogenesis study, while target discovery is the foundation. Based on previous studies, spike protein, ACE2, TMPRSS2, 3CLpro, RdRp and PLpro are considered as major targets for antiviral drugs against SARS and other coronavirus infections [24]. Sharing high conservation of the catalytic site and homology with SARS-CoV [9,25], the above six proteins may be potential targets for the treatment of COVID-19. From the view of virus and cell fusion, Arbidol, a broad-spectrum antiviral drug, as a virus-host cell fusion inhibitor, can prevent virus from entering host cells

to treat COVID-19 [26]. It has also been shown that SARS-CoV-2 depends on Spike proteins on the surface to entry into host cells by binding to Angiotensin-converting enzyme 2 (ACE2) receptors on the host cell surface [12]. ACE2 is the host cell surface receptor, which is the key to the initial stage of SARS-CoV-2 invasion into the host. Therefore, excess soluble forms of ACE2 or ACE2 inhibitors could be a possible methodology to treat COVID 19. In addition, Transmembrane Protease Serine 2 (TMPRSS2) can activate Spike protein and promote SARS-CoV-2 infection of host cells, which plays an important role in the process of SARS-CoV-2 infection of host cells [12]. The existing TMPRSS2 inhibitor Carmustat mesylate, bromhexine hydrochloride may also be an effective treatment for COVID-19 [12,27]. From the view of virus proteases, 3C-like protease (3CLpro) and Papain-like protease (PLpro) are two viral proteases responsible for cleaving viral peptides into functional units for virus replication and packaging in host cells. It has been shown that SARS-CoV-2 3CLpro inhibitors, baicalin and baicalein exhibit strong antiviral activity in cell-based systems [28]; 6-Mercaptopurine (6 M P) and 6-thioguanine (6 TG) are specific inhibitors of SARS-CoV and MERS-CoV papain, deubiquitinated and isg-depleted cysteine proteases [29,30], they may be reasonable candidates. From view of virus replication, Nsp12, an RNA-dependent RNA polymerase (RdRp), is an important enzyme of the coronavirus replication/transcription complex [31]. Currently, inhibitors targeting RdRp are mainly ribavirin, remdesivir, etc., and these drugs mainly compete with physiological nucleotides for the RdRp active site [32].

3. Therapeutic agents for treatment of COVID-19

Detailed insights into viral structure, pathogenesis and host immune responses described above can boost the identification of COVID-19 therapeutics including novel drugs, new application of United States Food and Drug Administration (FDA) approved drugs, even new drug target discovery. Several small molecule drugs are being tested for their efficacy on COVID-19, some of which have reached clinical trials, while others are still in preclinical phase [33]. We grouped potential drugs into structure, mechanism and evidence based on their reported effects in similar viruses, so their impact on SARS-CoV-2 infection could be prioritized to be evaluated. In this chapter, we will specially focus on the research progress of Chloroquine, Hydroxychloroquine, Remdesivi and Lopinavir/Ritonavir.

3.1. Chloroquine and hydroxychloroquine

Chloroquine and Hydroxychloroquine (CQ/HCQ) have a long-standing history as a broad-spectrum antiviral drug in the prevention and treatment of malaria [34]. CQ/HCQ block viral from entering into cells by inhibiting glycosylation of host receptors, proteolytic processing, and endosomal acidification, as well as regulate immunity through attenuation of cytokine production, inhibition of autophagy and lysosomal activity in host cells [35,36]. CQ can inhibit SARS-CoV-2 infection at a low-micro molar concentration and HCQ is more potent than CQ [37,38]. A multicenter clinical trial involving more than a dozen hospitals in China showed that CQ can improve radiologic findings, enhance viral clearance and reduce disease progression in the treatment of patients with COVID-19, so China has included CQ in the recommendations regarding the prevention and treatment of COVID-19 [37,39,40]. At the same time, another clinical trial showed that HCQ can significantly shorten the clinical recovery time and promote the absorption of pneumonia among patients with COVID-19 [41]. Notably, azithromycin reinforced the effect of CQ/HCQ in COVID-19 patients, but the publishing journal's society subsequently declared that the trial did "not meet the Society's expected Standard" [42,43]. Conversely, the higher CQ dosage should not be recommended for critically ill patients with COVID-19 because of its potential safety hazards, especially when taken concurrently with azithromycin and oseltamivir [44,45]. In summary, although CQ/HCQ have shown anti-SARS-CoV-2 efficacy both *in vivo* and *in vitro* trials as well as relatively well tolerated, some clinical trial designs and outcome data have not been submitted or published to peer review [46]. It is not recommended in the use of CQ/HCQ for COVID-19 outside of the hospital or a clinical trial due to lack of reliable efficacy data and potential toxic effects [47,48].

3.2. Remdesivir

Remdesivir (GS-5734), a prodrug of GS-441524 developed by the American pharmaceutical company Gilead Sciences, showed promise at the peak of the Ebola virus outbreak due to its low EC_{50} and host polymerase selectivity against the Ebola virus [49,50]. Subsequently, research about it also showed significant anti-SARS-CoV and MERS-CoV activity [51,52]. As a nucleoside analog with exonuclease resistance, remdesivir is metabolized to active nucleoside triphosphates that effectively prevents the elongation of the RNA chain by inhibiting RNA polymerase, but will not be digested with a viral exonuclease (nsp14) with proofreading activity [53]. Compared with ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine and favipiravir (T-

705), remdesivir has the best efficacy and the lowest toxic side effects on anti-SARS-CoV-2 in Vero E6 cells [32]. The United States first reported the clinical case of remdesivir in the treatment of SARS-CoV-2 associated pneumonia [54]. Currently, a number of clinical trials are ongoing, aiming to verify the safety and antiviral activity of remdesivir in the treatment of COVID-19. Clinical findings of the team of Professor Cao Bin of the China-Japan Friendship Hospital suggested that the remdesivir is adequately tolerated but do not provide significant clinical or antiviral effects in severe patients with COVID-19 [55]. However, the results of the global clinical trial are believed that remdesivir can relieve symptoms and reduce mortality, especially for patients in intensive care who require mechanical ventilation [56]. Meanwhile, the clinical trials in Chicago have suggested that early COVID-19 patients benefit more due to the reduction of lung damage [57]. In conclusion, remdesivir is still in the consideration of one of the most promising drugs for treatment COVID-19 currently [58].

3.3. Lopinavir/Ritonavir

Lopinavir/Ritonavir (LPV/r), also known as Kaletra, is an oral combination agent for treating HIV approved by the FDA, which has shown anti-coronavirus efficacy in studies of SARS and MERS [[59], [60], [61], [62]]. As a new protease inhibitor, LPV/r interrupts viral nucleic acid replication via inhibition of 3CLpro [63]. Xushun Guo's team at Sun Yat-sen University School of Medicine derived a homology modeling to confirm that LPV/r significantly inhibited the function of CEP_C30 to prevent the SARS-CoV-2 reproduction cycle [64]. In addition, two groups in China and Korea have reported LPV/r can improve the clinical symptoms of patients with COVID-19 [65,66]. Besides, LPV/r can achieve better antiviral effects when used with interferon or ninavir than alone [67]. However, the latest evidence suggests that it may cause liver damage and prolong hospital stay in the COVID-19 infected patients [68]. Furthermore, no benefit was observed with LPV/r treatment beyond standard care in hospitalized adult patients with severe COVID-19 [69]. Therefore, whether LPV/r can become an important adjuvant drug in anti-SARS-CoV-2 therapy and improve the clinical outcome of patients remains to be determined (Table 1).

Table 1 (continued)

No.	Drug Candidate	Structural Formula	Potential Mechanism of Action on COVID-19	Anti-SARS-CoV-2 Evidence	Reference
17.	Dipyridamole		Inhibits phosphodiesterase	Clinical Trial	[94,95]
18.	Fingolimod		Modulates sphingosine 1-phosphate receptor	Clinical Trial	[96]
19.	Losartan		Blocks angiotensin II receptor	Clinical Trial	[97,98]
20.	Azithromycin		Inhibits 50S ribosomal protein	In Vitro Assay, Clinical Trial	[42,99]
21.	Ribavirin		Inhibits viral mRNA and protein synthesis	In Vitro Assay, Clinical Trial	[100,101]
22.	Triazavirin		Inhibits RNA synthesis	Clinical Trial	[102]
23.	Tranilast		Inhibits hematopoietic prostaglandin D synthase	Clinical Trial	[103]
24.	Ebastine		Inhibits H1	In Vitro Assay, Clinical Trial	[104]

4. Chinese herbal medicines with the potential to inhibit COVID-19

Previously, we summarized small molecules currently used/planned to treat COVID-19, which may be an important short-term strategy for the treatment of COVID-19, but their efficacy and safety in COVID-19 need to be further confirmed by clinical trials. Drug development against COVID-19 appears to be crucial in the context of a rapidly evolving epidemic, however, the conventional development of new drugs is time-consuming with safety concern. Therefore, it seems unrealistic to synthesize new drugs and perform safety and toxicity tests over a short period of time. Antiviral therapy with Chinese herbal medicines have been recorded for a long time in Chinese history, and previous studies have shown that Chinese herbal medicines have great potential for preventing SARS transmission [105]. Given the low toxicity and availability of Chinese herbal medicines, screening active compounds targeting viral or host targets from Chinese herbal medicines may be a potential strategy for treating COVID-19. In this review, we summarized potential Chinese herbal medicines (Table 2) that may treat COVID-19 by targeting proteins such as Spike protein,

ACE2, 3CLpro, PLpro and RdRp. We also predicted the binding affinities between these compounds and COVID-19 related targets by molecular docking, with a focus on six compounds: quercetin, andrographolide, glycyrrhizic acid, baicalin, patchouli alcohol, and luteolin. And the binding patterns of these six compounds to the key targets of SARS-CoV-2 are shown in Fig. 2.

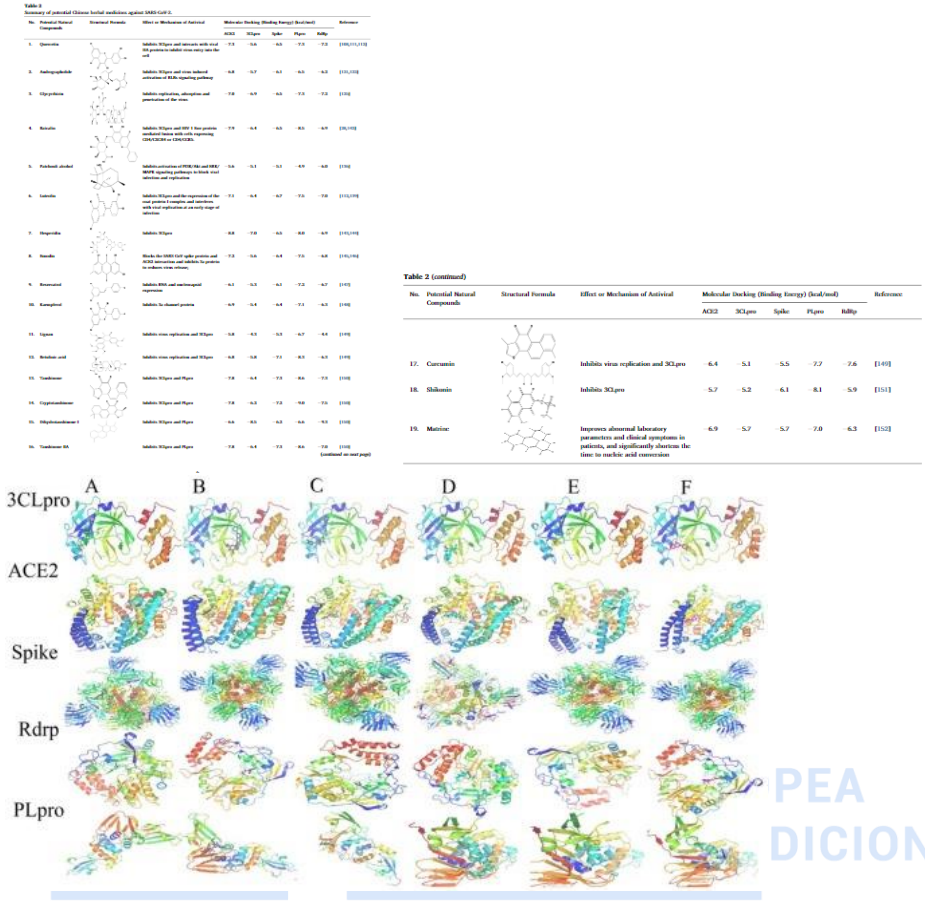

Table 2 (continued)

No.	Potential Natural Compounds	Structural Formula	Effect or Mechanism of Antiviral	Molecular Docking (Binding Energy) (kcal/mol)					Reference
				ACE2	3CLpro	Spike	PLpro	RdRp	
17.	Curcumin		Inhibits virus replication and 3CLpro	−6.4	−5.1	−5.5	−7.7	−7.6	[149]
18.	Shikonin		Inhibits 3CLpro	−5.7	−5.2	−6.1	−8.1	−5.9	[151]
19.	Matrine		Improves abnormal laboratory parameters and clinical symptoms in patients, and significantly shortens the time to nucleic acid conversion	−6.9	−5.7	−5.7	−7.0	−6.3	[152]

Fig. 2. The optimized binding patterns of ligands with key targets of SARS-CoV-2 by molecular docking, including (A) Andrographolide, (B) Baicalin, (C) Quercetin, (D) Glycyrrhizic acid, (E) Patchouli alcohol and (F) Luteolin.

4.1. Quercetin

Quercetin, a flavonoid compound, is widespread in fruit and vegetables. As a dietary source compound, quercetin exerts diverse biological activities including anti-inflammatory, anti-oxidant, anti-viral, anti-allergic, anti-cancer, mood-improving as well as vasoprotective [[106], [107], [108]]. Studies have found that quercetin exhibits antiviral properties against a variety of viruses, including Influenza A Virus (IAV) [108], Hepatitis C Virus (HCV) [109], Enterovirus 71 (EV71) [110], and SARS-CoV, etc [111,112]. It has been confirmed that quercetin showed a good inhibitory effect on SARS-CoV 3CLpro expressed in Pichia pastoris, with an inhibition rate of 82 % [111]. In addition, enzyme inhibition assays *in vitro* also showed that quercetin had inhibitory activity against SARS-CoV 3CLpro [112]. Since the 3CLpro sequence of SARS-CoV-2 is highly similar to that of SARS-CoV [10,25], we speculated that quercetin may also exhibit antiviral effects on SARS-CoV-2. However, it has not been documented whether quercetin inhibits SARS-CoV-2, so we docked quercetin to 3CLpro as well as other key targets, and the docking results showed that quercetin bound well to each target, with a binding energy of -5.6 kcal/mol to 3CLpro. Surprisingly, we found that quercetin bounds better to Spike protein, ACE2, RdRp and PLpro indicating good potential against SARS-CoV-2. In addition, it has a wide range of sources with relatively low cost, so it is worth testing its efficacy against SARS-CoV-2 infection.

4.2. Andrographolide

Andrographolide, the main active component isolated from the extract of the herb *andrographis paniculata*, has a wide range of biological activities including immunity regulation, anti-virus, anti-bacteria, anti-parasite, anti-tumor, and anti-hyperglycemia [113,114]. Previous studies have shown that andrographolide has a broad spectrum of antiviral properties, which inhibits various virus infections including influenza A virus (IAV) [115], human immunodeficiency virus (HIV) [116], Chikungunya virus (CHIKV) [117], dengue virus (DENV) [118,119], and Enterovirus D68 (EV-D68) [120]. Atchara Paemanee et al. suggested that andrographolide may exert broad-spectrum antiviral activity by interfering a variety of cellular pathways (including autophagy, unfolded protein response (UPR) pathway and oxidative stress, etc.). They further found the anti-dengue virus activity by acting on GRP78, a key regulator of unfolded protein response [119]. In addition, andrographolide exerts antiviral activity against H1N1 by inhibiting the activation of RLRs signaling pathways and thereby improving H1N1 virus-induced cell death [121]. To test the anti-viral activity against SARS-CoV-2, we docked andrographolide with key targets, and the results also showed that andrographolide bound well to the key targets including Spike protein, ACE2, 3CLpro, RdRp and PLpro, which indicated that andrographolide has potential efficacy against SARS-CoV-2. Moreover, Enmozhi, S. K. et al. proved andrographolide as a potential inhibitor of SARS-CoV-2 3CLpro through in silico studies [122]. Overall, as a plant-derived compound, andrographolide is widely distributed with low cytotoxicity, but its potent antiviral activity against a variety of viruses calls for further investigation.

4.3. Glycyrrhizic acid

Glycyrrhizic acid is a plant product isolated from the traditional Chinese medicine *licorice* (Chinese name: Gan Cao). Glycyrrhiza uralensis contains active ingredients such as thymol and carvacrol, which have significant antiviral and bactericidal effects [123]. A large number of studies have shown that *licorice* and its chemical components have protective effect on lung inflammation and damage, and it is a promising herbal medicine for treating SARS [124]. Cinatl J et al. compared the effects of conventional antiviral drugs ribavirin, 6-azouridine, pyrazofurin, mycophenolic acid, and glycyrrhizic acid on SARS-CoV, and the experimental results showed that glycyrrhizic acid had a better viral inhibitory effect than the other four drugs in inhibiting the viral adsorption and penetration [125]. Hoever G et al. also showed that glycyrrhizic acid has a good anti-SARS-CoV effect, while SARS-CoV-2 and SARS-CoV belong to different subclasses of coronaviruses with similar structures [126]. In addition, glycyrrhizic acid can promote IFN-γ production by T cells [127]. Recent studies have shown that SARS-CoV-2 and SARS-CoV have the same receptor ACE2, and glycyrrhizic acid can bind to this receptor, suggesting that glycyrrhizic acid may have therapeutic effects on SARS-CoV-2 [128]. To discuss whether glycyrrhizic acid has an anti-SARS-CoV-2 effect, we performed molecular docking of glycyrrhizic acid with the binding energy of glycyrrhizic acid and ACE2 -7.0 kcal/mol (As the shown in Table 2). At the same time, the binding energy of glycyrrhizic acid with other targets: 3CLpro, PLpro, RdRp and Spike is -6.9 kcal/mol, -7.3 kcal/mol, -7.2 kcal/mol, -6.5 kcal/mol, respectively. It can be seen that glycyrrhizic acid also has strong binding affinity to other targets. Given the antiviral effect of glycyrrhizic acid on SARS-CoV, and its potential interaction with ACE2, we speculated that glycyrrhizic acid may have potential to treat SARS-CoV-2. Moreover, glycyrrhizic acid plays an important role in inhibiting immune hyperactivation and cytokine storm factor development [129], therefore, we believe it is worth testing its efficacy against SARS-CoV-2 infection.

4.4. Baicalin

Baicalin, a component of *Scutellaria baicalensis Georgi* (Chinese name: Huang Qin), has a wide range of therapeutic effects, including sensitization and anti-apoptosis [130,131]. Chen et al. have demonstrated the antiviral activity of baicalin against SARS coronavirus, with an EC50 value of 12.5 μg/mL at 48 h, and the activity tended to decrease with incubation time beyond 48 h [132]. Due to the similarities between SARS-CoV-2 and SARS-CoV, it can be speculated that baicalin may also have an antiviral effect on SARS-CoV-2. In addition, Deng et al. used UV spectrophotometry to determine angiotensin-converting enzyme inhibitory activity and found that baicalin could inhibit ACE *in vitro*, with an IC50 value of 2.24 mM [133]. Hansen Chen et al. used molecular docking technology to find that baicalin may have a strong binding effect with ACE2, and the possible binding sites are ASN-149, ARG-273, HIS-505 [128]. Haixia Su et al. showed that baicalin, as a non-covalent inhibitor of SARS-CoV-2 3CLpro, has high ligand binding efficiency and specific binding to proteases by ITC map, native electrospray ionization mass spectrometry (ESI-MS) and its chemical structure [28]. At the same time, we used molecular docking to study the docking of baicalin to other key targets of SARS-CoV-2, in which the binding energy of baicalin to the target PLpro was -8.5 kcal/mol. The results of docking showed that baicalin bounds strongly to other targets of SARS-CoV-2 (Table 2). Therefore, it can be reasonably speculated that baicalin is one of the potential drugs for COVID-19 treatment. In view of the low toxic effect of baicalin, its effect against SARS-CoV-2 warrants further study.

4.5. Patchouli alcohol

Patchouli alcohol (PA), a tricyclic sesquiterpene compound extracted from the traditional Chinese medicine *patchouli*, has a wide range of pharmacological and biological effects including antiviral, immunomodulatory, anti-inflammatory, antioxidative, and antitumor [134]. PA has been found to have anti-influenza A (IAV) effect *in vitro*, while H1N1 virus is the most sensitive to PA [135]. In addition, Yunjia Yu et al. found that intracellular PI3K/Akt and ERK/MAPK signaling pathways may be involved in the anti-IAV effect of PA and PA significantly inhibits the *in vitro* proliferation of different IAV, suggesting that PA may block IAV infection by directly killing viral particles and interfering with some early stages after viral adsorption [136]. Another study showed that PA also has an effect against influenza virus (IFV) *in vivo* and enhances protection against IFV infection in mice by enhancing host immune responses and attenuating systemic and pulmonary inflammatory responses [137]. To investigate the anti-SARS-CoV-2 activity of PA, we investigated the possibility of PA binding to SARS-CoV-2 related targets using molecular docking (Table 2). The docking results showed that the binding effect of PA and Rdrp was satisfactory, which provided some support for the antiviral effect of PA. The above study results showed that patchouli alcohol had antiviral effect and also modulated the levels of inflammatory cytokines, suggesting that PA may be a novel and effective antiviral and anti-inflammatory drug for COVID-19.

4.6. Luteolin

Luteolin, a natural flavonoid extracted from Chinese herbal medicine, displays multiple biological activities, including anti-inflammatory, anti-cancer, antioxidant, antiviral, and heart protective [138]. It was reported that luteolin can interfere with the virus in early virus life cycle, to a certain extent, block the absorption and internalization of influenza virus, thereby inhibited the replication of IAV [139]. The above experiments suggested that luteolin is a potential antiviral drug that inhibits viral replication by regulating host proteins. In addition, Minhua Peng et al. confirmed luteolin inhibited the dengue virus NS2B/NS3 protease activity by analyzing the nucleotide sequence of the luteolin-resistant escape mutant [140]. It also has been documented luteolin has an anti-Epstein-Barr virus (EBV) effect, and in immunoblot analysis, 20 μg/mL of luteolin showed a significant inhibitory effect on EBV lytic cycle [141]. Another study showed that luteolin extracted from *Torreya Nucifera* is an effective SARS-CoV 3CLpro inhibitor [112]. To interrogate the anti-SARS-CoV-2 effect of luteolin, we performed molecular docking of luteolin to key targets of SARS-CoV-2. The docking results showed that luteolin bound well to the key target of SARS-CoV-2. Among them, the binding energy of luteolin to ACE2 was -7.1 kcal/mol (Table 2). Taken together, luteolin has a good antiviral effect, which suggests that luteolin may be a potential drug for the treatment of COVID-19.

The results of molecular docking are shown in Table 2. From the target point of view, the binding effect of ACE2 and PLpro with these natural compounds was more prominent; while from the natural compounds, the lowest binding energy was -9.0 kcal/mol for Cryptotanshinone and PLpro, while the highest was -4.3 kcal/mol for Lignan and 3CLpro, that is to say, the range of binding energy was from -9.0 kcal/mol to -4.3 kcal/mol, which indicated that the natural compounds had a good binding effect with the target. Our aim of docking was to select natural compounds with high potential efficacy against SARS-CoV-2, but it should be pointed out that these compounds cannot be considered to treat COVID-19 only by such a screen which is aimed to provide priority to focus. Furthermore, the 3D structure of the targets we used were based on the reported gene sequences. If the virus mutates during transmission, new screening is recommended. In conclusion, our review summarizes more than a dozen of natural compounds classified as antiviral/pneumonic protectors, which may directly inhibit SARS-CoV-2. However, their actual effect in the treatment of COVID-19 needs to be verified by further studies.

5. Comparison and combination therapy of clinically approved drugs and Chinese herbal medicines

Developing new application of FDA approved drugs is the most effective strategy for sudden new diseases and will rapidly alleviate the current epidemic situation [153]. Compared with new drugs, existing antiviral drugs have the advantages of safety, pharmacokinetic characteristics, clear clinical adverse reactions. And by further verifying the effectiveness of drugs that have completed at least clinical phase I can save preclinical and partial clinical study time and shorten the time cost of drug research and development. However, because existing antiviral drugs are not designed for SARS-CoV-2, they may not be ideal in antiviral efficacy and require larger doses, which may bring more serious side effects.

From the occurrence of the epidemic to date, traditional Chinese medicines also played an extremely important role. Guided by the theory of traditional Chinese medicine, exerting the advantages of overall regulation of traditional Chinese medicine is an important method for the clinical treatment of COVID-19. At present, Qingfei Paidu Decoction is recommended for the treatment of clinically confirmed cases according to Guideline for the Diagnosis and Treatment of Novel Coronavirus (SARS-CoV-2) Pneumonia (On Trials, the Seventh Edition) in China [39]. A study including 98 patients with COVID-19 showed that Qingfei Paidu Decoction has a good clinical effect for the treatment of COVID-19, it can significantly improve the abnormal laboratory test indicators and clinical symptoms of patients, reduce the adverse reactions of patients, and effectively improve the therapeutic effect [154]. In addition, Lianhua Qingwen was also demonstrated to significantly inhibit SARS-COV-2 replication in Vero E6 cells at the mRNA level as well as markedly reduce the production of pro-inflammatory cytokines, suggesting that Lianhua Qingwen may have a potential inhibitory effect on the cytokine storm induced by SARS-COV-2 [155]. At present, symptomatic and supportive therapy is still the key to clinical treatment. Therefore, traditional Chinese medicines have both antiviral and symptom-relieving effects may lead to better therapeutic effects.

At present, western medicine treatment is mainly based on the principles of symptomatic treatment, prevention of complications, treatment of underlying diseases, and prevention of infection [39], while traditional Chinese medicines play an important role in relieving the symptoms of patients and delaying or reducing the development of mild diseases into severe diseases [156], and may also play a role in reducing the side effects of western medicine, especially in the recovery of pulmonary function [157]. Both Chinese herbal medicine and approved western medicine have their own advantages in the treatment of COVID-19. In the course of COVID-19's treatment, the combination of Chinese herbal medicine and approved western medicine has shown obvious effect, which is of great value in alleviating the early clinical symptoms of patients and reducing the incidence of patients from mild to severe then to intensive care [158]. The combination of nefenavir and sinomenine significantly reduced the amount of virus accumulation and shortened the time of virus clearance compared with single use of nefenavir and sinomenine [159]. In short, the combination of Chinese herbal medicine and approved western medicine is worth thinking about in the future treatment of COVID-19.

6. Conclusions and future prospects

COVID-19 poses a great threat to global health and safety. It is an urgent task for us to control the spread of the epidemic and reduce the mortality rate as soon as possible. But so far, the specific mechanism of the virus is still unclear, and no specific drug has been developed for the virus. At present, it is important to control the source of infection, cut off the route of transmission, and make use of existing drugs and means to actively control the progress of the disease. Efforts should also be made to develop specific drugs, promote vaccine research and development, reduce disease morbidity and mortality, and better protect the lives of the people.

At present, the potential therapeutic agents used in COVID-19 come from previous experience in treating SARS, MERS or other new influenza viruses. As broad-spectrum antiviral drugs have long been approved on the market to treat different viral infections, their metabolic characteristics, dosage, potential efficacy and side effects are clear. Re-purposing of clinically approved drugs may be an important short-term strategy for the treatment of novel coronavirus. But the disadvantage is that these treatments are too “broad-spectrum” to specifically treat COVID-19. In addition, its side effects can not be underestimated. A number of clinical trials are under way to evaluate the effectiveness of other treatment options. Active symptomatic support is still the key to treatment. Although stem cells, monoclonal antibodies, polypeptides, interferon or plasma from recovered patients have been shown to be effective in treating COVID-19 patients, their safeties are still being evaluated and the efficacy remains to be further confirmed.

Except the some undergoing small molecules, this paper also focuses on the most promising compounds in traditional Chinese medicine in recent years, which can be used as effective antiviral drugs for the treatment of diseases caused by SARS-CoV-2 based on *in vitro* and *in vivo* studies. In addition, computer molecular docking shows that these monomers have good binding ability to COVID-19 virus and host targets. The low toxicity and availability of active compounds of traditional Chinese medicine should be used as potential drug candidates for COVID-19 treatment.

This review also has limitations. The large and rapidly published literature on COVID-19’s treatment means that the findings and recommendations are constantly evolving as new evidence arises. It is not uncommon that drugs that proved effective at an early stage based on small-scale clinical trials later turned out to be ineffective. We look forward to the cooperation of all scientists around the world to develop effective drugs to treat current and future potential SARS-CoV-2 infections to control the further spread of the epidemic.

Disclosures

All authors have read and approved the final submission.

Declaration of Competing Interest

There is no conflict of interest associated with this article.

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Abstract

Background:
The study aims to evaluate the effectiveness and safety of acupuncture therapy for coronavirus disease 2019.

Methods:
The following electronic databases will be searched from December 2019 to December 2020: Medline, PubMed, EMBASE, Web of Science, China National Knowledge Infrastructure, Wan-fang database, Chinese Scientific Journal Database, Chinese Biomedical Literature Databases, and other databases. All published randomized controlled trials about this topic will be included. Two independent researchers will operate article retrieval, duplication removing, screening, quality evaluation, and data analyses by Review Manager (V.5.3.5). Meta-analyses, subgroup analysis, and/or descriptive analysis will be performed based on the included data conditions.

Results:
High-quality synthesis and/or descriptive analysis of current evidence will be provided from mortality rate, cure rate, the time of negative nucleic acid detection for 2 consecutive times (not on the same day), improvement of chest CT scans, disappearance time of fever and cough, and side effects.

Conclusion:
This study will provide the evidence of whether acupuncture is an effective and safe intervention for coronavirus disease 2019 .

PROSPERO registration number: CRD42020179298.

1 Introduction

1.1 Description of the condition

Coronavirus disease 2019 (COVID-19) is a newly discovered highly contagious respiratory disease, with outbreaks in late December 2019 in Wuhan, China.[1–3] COVID-19 has strong infectivity and pathogenicity, and it has affected 2,344,983 people in 210 countries worldwide and caused 161,191 deaths by April 19, 2020.[4] The main clinical characteristics of COVID-19 are fever, dry cough, fatigue and ground-glass opacities on computed tomography.[5–10] The COVID-19 pandemic represents the greatest global public health crisis of this generation, and no proven effective therapies for this virus currently exist.[11–14]

1.2 Description and function of intervention

Acupuncture is an important part of complementary therapy guide by the theory of traditional Chinese medicine. It treats diseases through the conduction of meridians and acupoints adding certain operations. Acupuncture therapies include many different treatments, such as acupuncture, moxibustion, electroacupuncture, fire needle, acupoint injection, auricular point therapy, etc. Acupuncture has been proved effective in some diseases.[15–18] During the period of COVID-19, the China Association of Acupuncture-Moxibustion recommends acupuncture therapy for the treatment of COVID-19.[19] And some hospitals in China have used acupuncture therapy to prevent and treat COVID-19.[20]

1.3 Why the review is important

According to the published research, there is a lack of high-quality evidence on acupuncture in the treatment of COVID-19. Therefore, this systematic review aims to assess the effectiveness and safety of acupuncture therapy for COVID-19.

2 Methods

This systematic review protocol has been registered in the PROSPERO network (No. CRD 42020179298). All steps of this systematic review will be performed according to the Cochrane Handbook (5.2.0).

2.1 Selection criteria

2.1.1 Types of studies

Randomized controlled trials (RCTs) of acupuncture therapy for COVID-19 without any limitation of blinding or publication language will be included. RCTs that involve at least 1 acupuncture-related treatment to COVID-19, and 1 control treatment (or blank treatment) will be included. The studies of animal experiment, review, case report, meta-analysis, and duplicate publications will be excluded.

2.1.2 Types of patients

Patients who were diagnosed as COVID-19 will be included, without limits on gender, age, race, nationality, and disease classification.

2.1.3 Types of interventions and comparisons

Interventions can be any type of acupuncture therapy: acupuncture, moxibustion, electroacupuncture, fire needle, acupoint injection, auricular point therapy. Multiple control interventions will be included: no treatment, placebo, and other interventions (e.g., standard care, drugs, Chinese medicine). Comparisons contain acupuncture and its relation will be excluded. Interventions of acupuncture combined with other therapies will also be included, only if the other therapies were used as comparisons.

2.1.4 Types of outcomes

Primary outcomes will include mortality rate, cure rate, the time of negative nucleic acid detection for 2 consecutive times (not on the same day), and improvement of chest CT scans. Secondary outcomes will include disappearance time of fever and cough, serum level of TNF-α and IL-6, and side effects of acupuncture.

2.2 Search methods for identification of studies

2.2.1 Electronic searches

The following electronic databases will be searched from December 2019 to December 2020: Medline, PubMed, EMBASE, Web of Science, China National Knowledge Infrastructure, Wan-fang database, Chinese Scientific Journal Database, Chinese Biomedical Literature Databases, and other databases. All published RCTs about this topic will be included. Exemplary search strategy of Medline is listed in Table 1. According to the difference of databases, keywords may combine with free words and comprehensive search will be performed.

Table 1:

Medline search strategy.

2.3 Data collection and analysis

2.3.1 Selection of studies

Two reviewers (SLH and SYW) will independently select the studies. They will check the results with each other. When disagreements occur, a third reviewer (JL) will make the final decision. They will read the full texts of all included studies if necessary. Screening operation will flow the diagram of Figure 1. If the full literatures are unable to obtain or related data is incomplete, we will contact the corresponding author.

Figure 1:

Flow diagram of studies identified.

2.3.2 Assessment and quality of included studies

Two reviewers (MMW and JR) will evaluate quality of included articles and assess the risk of bias based on Cochrane Handbook 5.2.0. Quality assessment of included studies contains randomized method, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, completeness of outcome data, and selective reporting. Divergence of evaluation will also consult a third reviewer (JL).

2.3.3 Data extraction

Two independent reviewers (SLH and WJY) will extract data after selection and quality assessment, they will extract the data using a standardized data extraction form and any differences of opinion between them will be resolved through discussion, if failed, they will discuss with the third reviewer (JL). Data will be recorded onto an electronic form, including the basic information of the article (the title of article, first author, year, and language), inclusion and exclusion criteria, the baseline of the study (the sample size, sex ratio, age, and disease classification), interventions in the observation group and the control group, and outcome measures.

2.3.4 Measures of treatment effect

Two reviewers (SLH and SYW) will perform analysis independently and then cross-check treatment effect with Review Manager 5.3.5. Risk ratio with 95% confidence intervals will be adopted when dichotomous data existence. Continuous data will be presented by mean difference or standard mean difference with 95% confidence interval. Risk ratio form will be changed to analyze when binary data existence.

2.3.5 Dealing with missing data

Due to the possibility of data loss in the literature, we will contact the corresponding author by email or other means. If the missing data is not available, we will analyze the existing data assumed to be lost at random.

2.3.6 Assessment of heterogeneity

The heterogeneity of studies will be evaluated by *Q* test and I^2 statistic with RevMan5.3.5. The heterogeneity will be deemed as low ($I^2 < 50\%$), moderate (50–75%), and high ($I^2 > 75\%$).

2.3.7 Assessment of reporting bias

Publication bias and other reporting bias will be assessed by creating funnel plots. A symmetrical funnel plot indicates a low risk of bias, while an asymmetric funnel plot indicates a high risk of bias.

2.3.8 Data synthesis

Meta-analysis or descriptive analysis will be conducted according to the intervention method, measurement method, and heterogeneity level. If clinical and methodological heterogeneity are low, the fixed-effect model with merger analysis will be used. When heterogeneity is at medium level, the random-effects model with merger analysis will be used. However, if the heterogeneity is significantly high, subgroup analysis or descriptive analysis will be performed.

2.3.9 Subgroup analysis

Subgroup analysis will be performed based on the results of data synthesis, and if heterogeneity is found to be caused by the specific characteristics of the included study (e.g., age, disease classification, the intervention methods and the measurement methods used in the clinical trials), subgroup analysis will be conducted relevant to these categories.

3 Discussion

COVID-19 is a respiratory disease with wide infectivity and strong pathogenicity. It poses great threat to public health and affects social production and life seriously. Acupuncture is an important traditional Chinese medicine treatment with simple operation and low cost. Some Chinese hospitals are using acupuncture therapy to prevent and treat COVID-19. If the evidence could prove acupuncture is useful for COVID-19, it might save much cost and be beneficial to worldwide people. However, no systematic reviews on this topic have been published. In order to give compelling evidence and better guide in clinic practice, all actions of this review will be performed according to Cochrane Handbook 5.2.0.

Author contributions

Conceptualization: Shaolei Huang, Dianhui Yang, Suyao Wang.

Data curation: Mengmeng Wang, Wenjie Yu, Shaolei Huang, Suyao Wang.

Investigation: Jiao Rong, Jing Li.

Methodology: Shaolei Huang, Jing Han.

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Validation: Dianhui Yang, Jing Han.

Visualization: Shaolei Huang.

Writing – original draft: Shaolei Huang, Suyao Wang.

Writing – review & editing: Dianhui Yang, Jing Han.

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Abstract

Objective: To observe clinical effect of heat-sensitive moxibustion on coronavirus disease 2019 (COVID-19) and to discusses the effective moxibustion treatment program.

Methods: A total of 42 patients with COVID-19 (general type) were treated with heat-sensitive moxibustion at the acupoint area of Shenque (CV 8) and Tianshu (ST 25). The treatment was conducted under the standards of heat-sensitive moxibustion manipulation, which were "locating acupoint by feeling, moxibustion by differentiate sensation, dosage varies individually, ending after sufficient dosage". The incidence of *deqi* after first heat-sensitive moxibustion, the reduction of negative emotions, the improvement of chest distress and impaired appetite, and the active acceptance rate of moxibustion before and after treatment were observed.

Results: ① The *deqi* rate of heat-sensitive moxibustion for 20 min、40 min、1 h were respectively 52.4% (22/42), 90.5% (38/42), 100.0% (42/42). ② The incidences of feeling relaxed and comfortable immediately after the first, second, and third heat-sensitive moxibustion were 61.9% (26/42), 73.8% (31/42), and 92.9% (39/42), which were higher than 42.9% (18/42) before heat-sensitive moxibustion treatment (P<0.05). ③ The incidences of chest distress after the first, second, and third heat-sensitive moxibustion were 23.8% (10/42), 16.7% (7/42), and 9.5% (4/42), which were lower than 50.0% (21/42) before heat-sensitive moxibustion treatment (P<0.05); the incidences of impaired appetite after the first, second, and third heat-sensitive moxibustion were 26.2% (11/42), 19.0% (8/42), 9.5% (4/42), which were lower than 57.1% (24/42) before heat-sensitive moxibustion treatment (P<0.05). ④ After the first treatment, the active acceptance rate of patients for heat-sensitive moxibustion was 100.0% (42/42), which was higher than 11.9% (5/42) before heat-sensitive moxibustion (P<0.05).

Conclusion: The heat-sensitive moxibustion can effectively reduce the negative emotions and improve the symptoms of chest distress and impaired appetite with COVID-19. It is generally accepted by patients, and worthy of popularization and application in clinical treatment.

28. Huang XQ, Zhou MY, Cheng YR, Ye L, Wang MW, Chen J, Zhao LJ, Feng ZH. Opportunities and challenges of traditional Chinese medicine going abroad for COVID-19 treatment. Am J Emerg Med. 2020 Jun 6:S0735-6757(20)30489-7. doi: 10.1016/j.ajem.2020.06.008

We appreciate the work that Kai Zhang has done to highlight the treatment of COVID-19 in China via the use of Traditional Chinese Medicine (TCM) [1]. COVID-19 initially occurred in China at the end of December 2019 [2]. The Chinese government began to build many shelter hospitals in Hubei Province to treat patients with COVID-19, and medical workers from all over the country rushed to Wuhan to provide assistance [3]. Among the medical staff supporting Hubei Province, there were more than 4500 members of China's TCM system. In addition, TCM has been approved for the treatment of COVID-19 [4]. As of March 23rd, among the patients with COVID-19, 74,187 patients (about 91.5%), had been treated with TCM; of these, 61,449 people were treated in Hubei Province (Fig. 1A). Clinical efficacy data has shown that the overall effective rate of TCM treatment has reached more than 90% [5]. TCM is heavily involved in the fight against COVID-19, and has played an important role in the overall prevention of the disease, as well as in the treatment and rehabilitation of infected patients [6]. According to a number of US media reports, with the spread of the epidemic in the United States, the demand for TCM to treat colds and improve immunity has increased significantly. Consequently, the scale of the TCM market has grown significantly (Fig. 1B). Although TCM treatment has played an important role in this epidemic, it is also important to note that it is relatively difficult for TCM articles to be published in journals around the world. The reasons for this are believed to be as follows. First, the treatment process of TCM is individualized; not everyone's medication is exactly the same, and it is therefore difficult to evaluate the curative effect of TCM via Western medicine assessment methods. Second, there are many classifications of TCM, and the style and manner of each type of medicine are very different. Third, the origins and curative effects of TCM are different, as can be the effects of the same prescription. Fourth, Chinese doctors generally do not accept Western medical treatment processes, and the writing method of TCM practitioners is also different from that of Western medicine practitioners. Finally, TCM practitioners are trained for a long time, and foreigners have some difficulties in understanding

TCM. We believe that TCM can play an important role in the prevention and treatment of COVID-19 during this epidemic. However, determining how to improve TCM on the international stage going forward remains unclear. We trust that with the joint efforts of practitioners of TCM, it will surely be highlighted by the world.

29. Guan-Yuan Jin, Louis Lei Jin, Jin Zheng, Belinda Jie He. Advantages of anti-inflammatory acupuncture in treating sepsis of novel coronavirus. pneumonia. World Journal of Traditional Chinese Medicine (WJTCM). DOI: 10.4103/wjtcm.wjtcm_12_20

Advantages of anti-inflammatory acupuncture in treating sepsis of novel coronavirus pneumonia

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Abstract

Background: Sepsis is one of the most serious complications and a leading cause of death in patients with novel coronavirus pneumonia (NCP; severe acute respiratory syndrome coronavirus 2). In general, it is the result of an unregulated inflammatory cascade such as a postinfection "cytokine storm." The conventional treatment mainly relies on glucocorticoids, of which curative effects are not ideal, as they come with significant side effects. It is critical to seek or develop other effective therapeutics in dealing cytokine storm to fight NCP with sepsis. Aims and Objectives: Raise awareness of the significance applying anti-inflammatory acupuncture in dealing NCP patients with sepsis and provide an appropriate acupuncture protocol that can be easily integrated into existing medical guideline. **Materials and Methods:** Current evidences from animal experiments and clinical trials about acupuncture in treating infectious sepsis are reviewed, and a detailed discussion on advantages of anti-inflammatory acupuncture is followed, then the rationality on the point selection and stimulation parameters of acupuncture is analyzed to propose an appropriate acupuncture protocol. **Results:** Current experiments have shown that acupuncture can play a significant role to improve inflammation reaction and reduce mortality in infectious animal and patients with sepsis and its mechanisms are mainly achieved by stimulating the vagus-cholinergic anti-inflammatory pathways. Applying acupuncture in treating NCP patients with sepsis has four aspects of advantages. Moreover, a simple and convenient clinical acupuncture protocol including point selection and appropriate stimulation parameters is proposed. **Conclusion:** Acupuncture, especially electroacupuncture, has shown potentials in effectively treating infectious sepsis of animal models and critically ill patients in small sample studies by stimulating the nervous system, but has been largely overlooked in the clinic so far. It is advised that acupuncture should be integrated into the existing medical guidelines in dealing with NCP complicated with sepsis.

Keywords: Acupuncture, anti-inflammation, coronavirus disease-19, electroacupuncture, novel coronavirus pneumonia, sepsis, severe acute respiratory syndrome coronavirus 2

Introduction

As we know, sepsis is defined as a life-threatening organ dysfunction caused by an unregulated host response to infection. To date, it affects more than 30 million people annually worldwide and is one of the major causes of death for terminally ill patients. Any infected person can potentially develop sepsis, and the incidence rate is as high as 1%–2% of all hospitalized patients.[1] Sepsis is also one of the main complications and causes of death in patients with novel coronavirus pneumonia (NCP; severe acute respiratory syndrome coronavirus 2),[2] which is also referred as coronavirus disease 2019. A cytokine storm or unregulated inflammatory cascade following a viral infection is the main cause of sepsis. When it occurs, it is of the utmost importance for the NCP patient to control the spread of inflammation and prevent the development of cytokine storm as soon as possible.

In the treatment of sepsis due to cytokine storm, the conventional therapeutics mainly rely on corticosteroids (glucocorticoids). In most animal studies, corticosteroid administration consistently protected against lethal

sepsis. In contrast, however, clinical trials in sepsis found much less consistency in survival benefits from corticosteroids, though most trials demonstrated faster resolution in shock and organ dysfunction.[3] On the other hand, the side effects of excessive use of glucocorticoids are significant. Therefore, for sepsis, other reasonable therapies have been looking for or combining with. In fact, there is also a promising, simple, and no side effect treatment method, that is, anti-inflammatory acupuncture mediated by reflective central inhibition of the innate immune system,[4] which has been overlooked in the existing medical guidelines so far.[2],[5]

Following a review of recent experimental and clinical evidence and the mechanisms of anti-inflammatory acupuncture in treating sepsis, the authors will delve into the advantages of applying acupuncture in treating the sepsis of NCP patients. A set of acupuncture protocols including point selection and proper stimulation parameters based on previous methods applying in animal studies and clinical trials, combining with the authors' expertise, is proposed herewith. It is advised that acupuncture should be integrated with the existing therapeutics of both conventional medicine and traditional Chinese medicine (TCM), in order to help reduce the incidence of NCP or sepsis, reduce the mortality rate, and speed up the healing process.

Scientific evidences of anti-inflammatory acupuncture for sepsis

Most researchers agree that the degree of inflammatory response heavily impacts the outcome of sepsis, and the elevated level of serum tumor necrosis factor (TNF)-α or interleukin (IL)-6 level is related to the rise of mortality in sepsis patients. A decreased 28-day mortality rate could be found following with decreased concentrations of TNF-α and IL-6 in blood after treatment. So far, there are many laboratory and clinical evidences that show acupuncture or electroacupuncture (EA) may inhibit macrophage activation and the production of TNF, IL-1 beta, IL-6, IL-18, and other pro-inflammatory cytokines via the stimulation of the vagus nerve.[4]

In 2014, Torres-Rosas *et al*. reported that when EA was applied to mice with sepsis, cytokines that help limit inflammation were stimulated as predicted. The results found that half of those mice survived for at least a week, whereas none of those mice that did not receive acupuncture survived. That discovery presented a potential novel approach to use acupuncture for sepsis in humans. They observed that EA at the Zusanli (ST36) of the mice reduced the lipopolysaccharide-induced serum levels of all cytokines analyzed, including TNF, monocyte chemotactic protein-1 (MCP1), IL-6, and interferon-γ (IFN-γ). These results indicated that EA had an inhibition effect and not just merely delayed the production of cytokines. It is also found that the surgical removal of the sciatic nerve (not the common peroneal or tibial nerve) can reduce the anti-inflammatory potential of EA. This suggested that both the common peroneal and the tibial nerves contribute to the anti-inflammatory potential of EA by activating the sciatic nerve and demonstrated for the first time ever the ability of the sciatic nerve to control systemic inflammation in sepsis.[6]

Other researchers have also observed that EA at Zusanli (ST36) and Guanyuan (CV4) of mice (5–8 mm and 3–5 mm depth respectively, then retain the needle for 30 min with continuous wave of 3 Hz, once every 12 h for a total of 3 times) could increase the synthesis and release of vasoactive intestinal peptide in hypophysis and peripheral blood of sepsis rats, and inhibit thymocyte apoptosis through neuro-immune regulation.[7] Furthermore, EA at Zusanli (ST36), Tianshu (ST25), Shangjuxu (ST37), and Xiajuxu (ST39) could significantly improve the level of CD_{14}^{+}/HLA-DR (human leukocyte antigen DR) and immunosuppression in patients with sepsis by EA (continuous wave, frequency 4 Hz, 60 min/time, 2 times/day for 3 days).[8] CD_{14}^{+}/HLA − DR is the antigen expression on the surface of monocyte/macrophage and its decrease is closely related to the degree of immunosuppression in sepsis.

In 2015, there were also small sample clinical trials of acupuncture for sepsis in China. A total of ninety patients with sepsis were randomly divided into a control group, thymosin α1 group, and acupuncture treatment group, thirty cases in each group. The control group received routine treatment according to the guiding

principle of survival activities of sepsis. Thymosin α1 group was injected subcutaneously once a day for 6 days. In the acupuncture treatment group, Zusanli (ST36), Yanglingquan (GB34), Neiguan (PC6), Guanyuan (CV4), and other associated acupoints were needled (e.g., needling in the morning, twirling and toning for about 20–30 s, keeping the needle for 30 min, during which three times of needling were conducted, each time for about 20 s), once a day for 6 days. T cell subsets ($CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$) and immunoglobulins (IgG, IgA, and IgM) were detected. The hospitalization time, readmission rate, and 28-day mortality rate of the three groups were compared. The results showed that after 6 days of treatment, the T cell subsets and Igs were significantly increased in the three groups ($P < 0.01$). The levels of $CD3^+$, $CD4^+$, $CD8^+$, IgG, IgA, and IgM in thymosin α1 group and acupuncture group were significantly higher ($P < 0.01$). Compared with the control group, the length of stay in intensive care unit (ICU) of thymosin α1 group and acupuncture treatment group was significantly shorter, and the readmission rate and 28-day mortality rate were lower ($P < 0.05$, $P < 0.01$). There was no significant difference between thymosin α1 group and acupuncture group.[9] In addition, other researchers observed that EA at Zusanli (ST36) and Guanyuan (CV4) of sepsis patients, with the vertical depth of 5–10 mm and 5–7 mm inserted, respectively, could not only reduce the inflammatory reaction of sepsis, but also shorten the length of stay in the ICU when reducing the level of blood lactate.[10]

As for the main mechanism of acupuncture or EA in the treatment of sepsis, it has been almost clear that it is achieved by strengthening the vagus-cholinergic anti-inflammatory pathways to weaken the cytokine storm. [Figure 1] shows two anti-inflammatory pathways[11] activated by acupuncture (EA) in Hegu (LI4) or Zusanli (ST36) in the treatment of sepsis. The first pathway is the vagus-spleen-cholinergic pathway, in which the efferent signal of vagus nerve is propagated to the celiac ganglia and the superior mesenteric ganglion in the celiac plexus, where the splenic nerve originates. Norepinephrine (NE) released from the splenic nerve interacts with β2-adrenergic receptors (β2) and causes the release of acetylcholine (ACh) from T cells containing functional choline acetyltransferase (T cells). ACh interacts with α7nAChRs on macrophages and suppresses pro-inflammatory cytokine release and inflammation. The anti-inflammatory effect of EA at Hegu (LI4) is through this way.

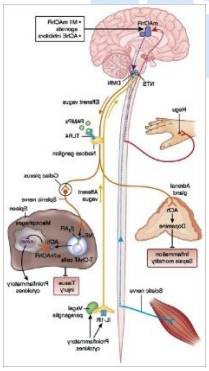

Figure 1: Two vagus-cholinergic anti-inflammatory pathways stimulated by acupuncture at Hegu LI4 or Zusanli ST36(Illustrated by Debbie Maizels, Springer Nature, for Pavlov and Tracey[11]

The second pathway is vagus-adrenal medulla-dopamine pathway, that is, EA at Zusanli (ST36) activates sciatic nerve signals, which by unknown mechanisms convert to efferent vagus nerve signaling to the adrenal medulla (which is usually dominated by sympathetic nerve, now known to also have the distribution of the vagus nerve), resulting in dopamine release. Dopamine suppresses inflammation and improves survival in a model of sepsis.[6]

In addition to the effects on the sympathetic and parasympathetic pathways, acupuncture can activate the hypothalamic–pituitary–adrenal (HPA) axis governing the systemic release of glucocorticoids from the adrenal glands that has an anti-inflammatory role. A typical example of its role is that acupuncture on Huantiao (GB30) inhibits complete Freund's adjuvant-induced paw edema in mice through a mechanism that is prevented by adrenalectomy and glucocorticoid inhibitors. HPA axis stimulation can be a successful strategy to induce the

production and systemic distribution of glucocorticoids to modulate metabolic and immune responses.[12]

Analyzing benefits of using acupuncture to treat sepsis of novel coronavirus pneumonia

According to the preliminary clinical observation, most of the terminally ill patients with NCP showed significant increase of pro-inflammatory cytokines, such as IL-6, TNF-a, and IFN-γ, with the characteristics of cytokine storm.[13] Integrating acupuncture to treat NCP complicated with sepsis has the following four advantages.

First, acupuncture therapy is suitable for all stages of the NCP patients. For mild cases, it can strengthen the immunity of the body and reduce the risk of deterioration. For severe cases, it can also alleviate the disease as much as possible through the rapid neural and bidirectional regulation of the immune function.

The early stage of sepsis is generally thought to be caused by an unregulated production of pro-inflammatory mediators forming a cytokine storm, that numerous cytokines such as TNF-α, IL-1, IL-6, IL-12, IFN-α, IFN-β, IFN-γ, MCP-1, and IL-8 are rapidly produced in body fluids after the body is infected. This stage is characterized by a hyperactivity of the immune system. With the progress of the pathological course, the body exhibits a process of compensatory anti-inflammatory response by releasing a large amount of inflammatory suppressing cytokines. In this stage, immunosuppression is being dominated, which is often the key to determine the prognosis of sepsis patients.[14] Therefore, the production or release of pro-inflammatory cytokines should be controlled or limited as soon as possible in the early stage of sepsis, and the immunosuppression should be relieved or reduced as soon as possible in the later stage of sepsis. Acupuncture therapy has the characteristics of bidirectional regulation of immune function. No matter to control the cytokine storm in the early stage of sepsis or to improve the immunosuppression in the late stage of sepsis, acupuncture shall play an important regulatory role. Moreover, the regulatory direction of acupuncture depends on the functional state before acupuncture, that is, if the production or release of cytokines is excessive, the acupuncture stimulation may weaken the cytokine storm, and if the immunosuppression has occurred, the acupuncture stimulation may decrease the immunosuppression. When applying acupuncture in the treatment of sepsis, those side effects of glucocorticoids would not occur. Animal studies have shown that acupuncture can regulate the secretion of adrenocorticotropic hormone (ACTH) and corticosteroids bidirectionally: increase when it was originally low and decrease when it was originally high. Other studies have observed that ACTH level in the blood of healthy people increased rapidly after acupuncture, reaching 1.5–2 times of that before the treatment, and peaking at 2–5 min. The concentration of cortisol in the blood increased to 1.5–2 times (15%) of that before the treatment.[15]

After 20 min of acupuncture at Hegu (LI4) and Zusanli (ST36), the level of corticosteroids in the blood of healthy people increased significantly and had a longer lasting effect. In patients with appendicitis receiving acupuncture, 17-ketosterol and corticosterone (CORT) were increased in 24-h urine, which indicates the increase of ACTH after acupuncture. In animal experiments, after EA, the content of ACTH in the blood was measured directly, and there was also a significant increase. If the levels of ACTH and corticosteroids had increased before acupuncture, acupuncture could reduce them.

In 2017, when studying the effects of acupoint association on the related hormones of HPA axis in insomnia rats, Wu *et al*. observed that the levels of corticotropin-releasing hormone (CRH) in hypothalamus and ACTH and CORT in serum were significantly higher in the insomnia group. After acupuncture with three different acupoint associations (Baihui + Shenmen, Baihui + Sanyinjiao, and Baihui (GV20) + nonacupoint group), the levels of CRH, ACTH, and CORT of them decreased to some extent, compared with the model group.[16]

In several of these animal studies, sepsis was associated with a significant early increase in ACTH levels, which returned to baseline around 72 h. The clinical studies have found that the ACTH level of critical patients was significantly lower than that of the control group, especially in septic shock.[3] From this, the effect of acupuncture on sepsis seemed related to the bidirectional regulation of ACTH.

Second, the anti-inflammatory or the regulating immunity actions of acupuncture are achieved by stimulating the nervous system. The neural regulation by acupuncture has a rapid and accurate feature, although not lasts long, which has great potential significance in preventing and treating NCP patients, especially in rescuing severe cases complicated with sepsis.

Let us take the acute stress reaction (e.g., fight or flight) as an example to follow the timeline: in response to acute stress, the body's sympathetic nervous system is activated. The sympathetic excitation stimulates the adrenal glands triggering the release of catecholamines, which include adrenaline and noradrenalin (NE). This results in an increase in heart rate (HR), blood pressure, and breathing rate. After the threat is over, it takes between 20 and 60 min for the body to return to its prearousal levels. If the threat is real and the "fight" is unavoidable, the HPA axis is activated after the first surge of adrenaline subsides. The release of cortisol by the adrenal cortex starts later takes place within 20–30 s and thus last longer. Once the danger has passed, the production of cortisol will cease too and consequently the balance between the sympathetic and parasympathetic nervous systems is attained.[17] The elevated endogenous cortisol secretion is generally more suitable for the sake of activating body's anti-inflammation action, as it does not have potential side effects brought on by supplementing exogenous glucocorticoids.

Although it is known that stress can suppress the immune system through the action of adrenaline and cortisol, researches have shown that the HPA axis can actually have a positive effect on the immune system, reversing the effects of cortisol and increasing the killing ability of natural killer cells. ACTH, part of the HPA axis response, has an opposite effect to that of cortisol. These results may be of great significance in the treatment of diseases related to excessive or persistent inflammation, such as autoimmune diseases, as well as in treating NCP.

Time is of the essence for terminally ill patients with sepsis. Once acupuncture effectively stimulates sympathetic nervous system (especially postganglionic fiber), or vagal cholinergic anti-inflammatory pathways, it is possible to quickly calm the cytokine storm and rescue some patients from deathbeds. As for the short duration of action from each acupuncture session, it can be improved by shortening the treatment interval by increasing treatment frequency from daily to even several sessions per day.

Third, acupuncture therapy not only has the effect of regulating immune function or anti-inflammatory role, but also has a beneficial effect on other system functions of the body.[18] For example, the lung is the first target organ damaged by sepsis, which is often manifested as acute lung injury or acute respiratory distress syndrome. Needling the bilateral Zusanli (ST36) of rats (directly inserted the needle at the depth of 7 mm), followed by a pulse current of (3 V, 2 m, 3 Hz) for 12 min, 8 h for 1 time, for 2 days, reduced the inflammatory reaction and the acute lung injury of rats with sepsis after being scalded.[19] In another study, acupuncture reduced the acute lung injury of sepsis patients by regulating the balance of pro-inflammatory and anti-inflammatory cytokines, inhibiting the inflammatory reaction: Needling Zusanli (ST36) and Cheze (LU5), following Deqi (acquire Qi with movement of needle), EA was added with disperse and dense wave, continuously stimulating for 30 min, 1 time a day for 5 days, which improved the oxygenation index of sepsis patients, reduced the APACHE II score and TNF-α in patients' serum and alveolar lavage fluid, and increased the concentration of IL-12.[20]

Another example is that the gastrointestinal tract is often the initial organ of sepsis. In the process of sepsis onset, the free radicals released by inflammatory cytokines first destroy the gastrointestinal function, and then the gastrointestinal mucosa is swollen and eroded, the permeability is increased, and the intestinal bacteria are displaced, thus inducing systemic inflammatory response syndrome and multiple organ dysfunction syndrome. Therefore, regulating gastrointestinal function should be the focus of early treatment of sepsis. Reported by Yu *et al*., needling bilateral Zusanli (ST36), Zhongwan (CV12), Tianshu (ST25), Neiguan (PC6), Shangjuxu (ST37), and Qihai (CV6) at (30 min/time, once a day for 5 days) could effectively improve gastrointestinal symptoms, reduce gastric retention and intra-abdominal pressure, improve serum motilin level, and reduce gastrin level in elderly patients with severe sepsis.[21]

Reported by Wu *et al*., on the basis of routine treatment, acupuncture was used to stimulate Zusanli (ST36), Tianshu (ST25), Shangjuxu (ST37), and Xiajuxu (ST39) in patients with sepsis. After Deqi, EA was applied (continuous wave, 4 Hz, 60 min each time, twice a day for 3 days). It improved the intestinal permeability of patients with sepsis, restored the intestinal function as soon as possible, and achieved the target 20–25 Kcal/kg per day feeding in the early stage of the patients with critical illness.[22] In 2009, Hu *et al*. observed that EA (a constant voltage, 2–100 Hz, 2 mA for 0.5 h) at Zusanli (ST36) significantly lowered the elevated levels of pro-inflammatory factors in the small intestine and alleviate tissue edema and mucosal dysfunction in rats of sepsis caused by cecal ligation and puncture. It was apparent that these were resulted from the activation of vagus-cholinergic anti-inflammatory pathways.[23]

Acupuncture treatment can alleviate some serious life-threatening symptoms, such as for patients in shock, low blood pressure can be improved by enhancing microcirculation; for bleeding coagulation imbalance, acupuncture can also play a regulatory role. As for the treatment of mild cases of NCP, it is more effective. Therefore, when NCP patients are treated with various conventional or TCM therapeutics, the authors highly recommend that acupuncture should be utilized first and foremost.

Fourth, acupuncture seemed to have a faster stimulation effect on the autonomic nervous system (ANS) than moxibustion. Although moxibustion also has the effect of regulating immune function,[24] its effects are not the same as that of acupuncture. In a pilot controlled clinical trial,[25] it was observed that acupuncture and moxibustion at bilateral Zusanli (ST36) and Guanyuan (CV4) had different effects on fatigue by regulating ANS. Acupuncture was more effective in instantaneous changes of HR variability that reflects the activity of vagus nerve and moxibustion in long-term aspects. Both acupuncture and moxibustion improved fatigue in chronic fatigue syndrome (CFS) patients, but moxibustion was more effective. The possible mechanism of the intervention may be through the activation of the vagus nerve and the conclusion drawn was that moxibustion was deemed more effective than acupuncture in the long-term treatment of CFS.

From the view of needling sensations, the pain stemmed from a stronger needle stimulation can be easily recognized as a kind of stressor by the body, thus stimulating the sympathetic nerve and the HPA axis. Previously, we have discussed the characteristics of quick stress response. In addition, the local microtrauma via needling can also induce the anti-inflammatory effect of the body, which cannot be achieved by general moxibustion (unless blistering moxibustion or purulent moxibustion is applied). Therefore, in authors' opinion, moxibustion is more suitable for the treatment of chronic inflammatory diseases or to prevent NCP. However, for severe cases, such as concurrent sepsis, acupuncture intervention may generate faster results than moxibustion, which, of course, needs to be evaluated further to reach a proper conclusion. Of course, as acupuncture is a procedure involving sharp needles penetrating the skin, worries in its usage for such highly contagious and infectious disease such as NCP are reasonable indeed. However, be rest assured that this can be resolved as long as the cleaning needling technique is strictly adhered by the practitioner during the needle operation, while also being trained in self-protection techniques, such as manipulating the needles with gloves. Wearing gloves to perform acupuncture may be a bit inconvenient to the practitioner, especially when begin to manipulate the needles. but it is worth in the long-run when compared with the possible therapeutic effects from acupuncture. As for whether other external treatments (such as massage, cupping, or scraping) can be alternatives of acupuncture in the treatment of sepsis, more comparative studies are needed. At the moment, the anti-inflammatory role of acupuncture is achieved by manual needling or EA at acupoints. Similar effects may also be achieved by transcutaneous electrical stimulation or implantation of electrodes near the vagus nerve trunk *in vivo*. Although the vagus-stimulating action of acupuncture is not as precise and repeatable as implantable electrode stimulation, it is still more simple and feasible, with no need to worry about excessive stimulation.

Anti-inflammatory acupoint selection and appropriate stimulation parameters

Based on the prior clinical studies for sepsis when combining our own expertise, we hereby propose a set of acupuncture protocols (selection of acupoints and stimulation parameters) for preventing and treating NCP

patients complicated with sepsis. These protocols are easy to operate and convenient for clinical application. It is suggested to use them in conjunction with existing integrative therapeutics.

Point selection and needling methods

Any single one or combinations of the following three groups of main acupoints can be selected to treat NCP with sepsis:

1. Bilateral Zusanli (ST36), Shangjuxu (ST37) (or nearby tender/reflex points)
2. Bilateral Hegu (LI4), Shousanli (LI10) (or nearby tender/reflex points).

For the above acupoints, the filiform needle should be directly inserted into the muscle beneath the acupoints, and it is optimal to *Deqi* (attaining the feeling of soreness, distension, heaviness, numbness, or the sensation of "like fish swallowing bait" beneath the needle).
In case that it is difficult to *Deqi*, simply retain the needle for 30 min. Every 5 min, perform "needle-awakening manipulation" (slightly twist the needle) to enhance the stimulation for up to a 1 min.

3. Bilateral ear reflex points/tender points inside the concha (such as the lung/heart points in the cavum concha and the kidney/small intestine points in the cymba concha).

First, detect the tender points either by simple pressure or measurement of low electrical conductance. Use a slightly thick filiform needle (0.25–0.3 mm in diameter) to penetrate the ear point (such as lung point to heart point) to the subcutaneous area of the reflex area. It is optimal to have sharp pain immediately (such as no pain, the needle should be pulled out and pricked again). Retain the needle for 30 min. After the removal of needle, the needling spot can slightly bleed (about 10 drops is proper) or without bleeding.

Other associate points: add Tianshu (ST25), Qihai (CV6) for symptoms of the intestines; add ChiZe (LU5), Neiguan (PC6) for symptoms of the lung, etc.

Electrical stimulation parameters

For patients without contraindications of electrical stimulation, EA should be used as much as possible to ensure sustained stimulation for a certain period with sufficient stimulation input. Low frequency (3–4 Hz), continuous wave or disperse-dense wave, suitable (medium) intensity, at least 30 min each time is recommended.

When applying the above acupuncture protocol, sufficient treatment frequency should be ensured, preferably 2–4 times per day, which can be adjusted according to the patient's condition and sensitivity as well as response to acupuncture. Although needling sensations are not necessary for acupuncture efficacy, it is more appropriate for critical or acute patients to perceive needling sense as a sign of therapeutic information of acupuncture being inputted into the body.

Supporting evidence for the protocol

In the treatment of NCP with sepsis, because the local anti-inflammatory effect of acupuncture is not required, distal acupoints can be chosen. The above-mentioned three sets of main points in the protocol are located in the upper limbs, lower limbs, and auricle, respectively. When those points are stimulated simultaneously, that is referred as a type of point-association method through different afferent pathways:[26] The point association of ear reflex points innervated by cranial nerves with the limb acupoints innervated by spinal nerves, or the point association of Hegu (LI4), Sousanli (LI10) on the upper limb with Zusanli (ST36), Shangjuxu (ST37) on the lower limbs innervated by different spinal nerves.

Although the stimulation of vagus nerve used in previous experiments is often realized by stimulating vagus efferent nerve or certain limb acupoints innervated by spinal nerve, in fact, the auricular branch of the vagus nerve distributed on the auricle (concha area) as the afferent nerve can also be another target of vagus stimulation. It has been confirmed that stimulation of the concha area can activate the vagus nerve. In 2019, Addorisio M*et al*. observed that the use of vibration to stimulate the external ear could inhibit the production of TNF, IL-1 β, and IL-6 in healthy people and improve the symptoms of rheumatoid arthritis patients.[27] Therefore, acupuncture at the ear reflex points found at the concha area is deemed a simple and easy method to stimulate cholinergic anti-inflammatory pathways.[28]

There are also evidences that acupuncture at Hegu (LI4) or Sousanli (LI10) of the hand can stimulate the excitation of vagus nerve.[11],[29] The effect of EA at Zusanli (ST36) on sepsis by stimulating cholinergic anti-inflammatory pathways through sciatic nerve has also been confirmed in animal experiments.[6] Combined use of Shangjuxu (ST37) with Zusanli (ST36) has showed an improvement of immunosuppression and gastrointestinal function in severe cases.[8],[21],[22]
As for the relationship between the degree of needling sensations and perceived acupuncture effects, the experience of classical acupuncture for thousands of years is that "The arrival of Qi (referred as Deqi) equates to be effective." It has been thought that intense acupuncture can raise sympathetic tone, whereas weak acupuncture can cause parasympathetic excitation. Accordingly, from the view of stimulating sympathetic nerve and activating the HPA axis, it should be that the stronger the acupuncture stimulation is, the better the effects. However, from the anti-inflammatory view of stimulating vagus nerve, the requirements for acupuncture sensations or Deqi are not quite that high. In a recent study, Uchida *et al*. studied the effect of needling sensations on transient HR slowing and ANS function during needling. It was observed that in 32 healthy men with deep acupuncture, needling on their hands at Sousanli (LI10) (15–20 mm), even without needling sensations, could significantly reduce their HR. Moreover, regardless of the level of Deqi or perceived pain during needling, their autonomic nerves are still transferred o parasympathetic advantage.[29] This study provides further support for the selection of limb acupoints and stimulation parameters in the above acupuncture protocol that can stimulate cholinergic anti-inflammatory pathways.

As for the benefits of combined electrical stimulation in support of anti-inflammation, some studies have shown that both manual acupuncture and EA can have a significant impact on leukocytes and their related cytokines. However, in subjects with collagen-induced arthritis and inflammation, EA was more effective than manual acupuncture in reducing pro-inflammatory cytokines such as IL-6, IFN-γ, and TNF-α.[30]

The reason why low-frequency electrical stimulation should be selected is that its anti-inflammatory action is different from that of high-frequency electrical stimulation. It was found that the sympathetic nerve stimulation induces both local and systemic catecholamine secretion, depending on the selected frequency of electrical stimulation. A high-frequency EA can activate the preganglionic nerve that innervates the adrenal medulla to induce systemic catecholamine secretion, whereas a low-frequency EA seemed to activate the postganglionic sympathetic nerve to induce local release of NE,[31] so it can achieve a better inflammatory inhibition action.

As a clinical reference, the above acupuncture protocol is designed to enhance the vagus-cholinergic anti-inflammatory pathways when dealing with NCP complicated with sepsis. Actually, aiming at different purpose of prevention and treatments (prevention-based or life-saving centric), there are myriad types of acupuncture or moxibustion protocols. For example, for patients with mild-to-moderate symptoms and/or in recovery period, moxibustion could be selected. For severe patients with sepsis or in shock, acupuncture with an intense stimulation at the extremities or the governor/DU meridian (central reflex area) may be critical to stimulate the sympathetic–adrenal system and HPA axis to save life. Although the effectiveness of these protocols remains to be verified via large sample and high-quality clinical trials, it is clear that acupuncture and moxibustion can bidirectionally regulate the immune system with little or no side effects. This is especially

true when applying acupuncture including EA to treat infectious sepsis by an enhanced stimulation to the nervous system.

Finally, we would like to emphasize that even though it is common knowledge that the body's inflammatory response to the invasion of pathogenic microorganisms is one of the wisdoms of the body, many people are probably not familiar with another related wisdom of the body, that is, neuronal networks to control excessive inflammation and infectious disorders.[6] Both laboratory and clinical evidence have recently shown that there is a negative feedback loop between the ANS and the innate immunity. Electrical stimulation of the vagus nerve inhibits the activation of macrophages and the production of various pro-inflammatory cytokines.[4] Acupuncture, as one of the most convenient and effective natural protocols of TCM, is the best choice to stimulate the neural networks, with little or no side effects. As NCP is now running rampant around the globe, why not give anti-inflammatory acupuncture a chance?

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Conflicts of interest

There are no conflicts of interest.

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Abstract

The Coronavirus Disease 2019 (COVID-19) has been declared as a global pandemic, but specific medicines and vaccines are still being developed. In China, interventional therapies with traditional Chinese medicine for COVID-19 have achieved significant clinical efficacies, but the underlying pharmacological mechanisms are still unclear. This article reviewed the etiology of COVID-19 and clinical efficacy. Both network pharmacological study and literature search were used to demonstrate the possible action mechanisms of Chinese medicines in treating COVID-19. We found that Chinese medicines played the role of antivirus, anti-inflammation and immunoregulation, and target organs protection in the management of COVID-19 by multiple components acting on multiple targets at multiple pathways. AEC2 and 3CL protein could be the direct targets for inhibiting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Quercetin, kaempferol, luteolin, isorhamnetin, baicalein, naringenin, and wogonin could be the main active ingredients of Chinese medicines for the management of COVID-19 by targeting on AEC2 and 3CL protein and inhibiting inflammatory mediators, regulating immunity, and eliminating free radicals through COX-2, CASP3, IL-6, MAPK1, MAPK14, MAPK8, and REAL in the signaling pathways of IL-17, arachidonic acid, HIF-1, NF-κB, Ras, and TNF. This study may provide meaningful and useful information on further research to investigate the action mechanisms of Chinese medicines against SARS-CoV-2 and also provide a basis for sharing the "China scheme" for COVID-19 treatment.

1. Introduction

World Health Organization has declared the Coronavirus Disease 2019 (COVID-19) as a pandemic on March 14th, 2020. [1] As of May 12th, 2020, there were more than 4,218,000 confirmed cases of COVID-19 in 216 countries, areas, and territories, and over 290,000 people have lost their lives [2]. The number of cases and deaths rises continuously and rapidly every day. Even worse, the biggest challenge is that there are no proven therapies or vaccines against COVID-19, and there are significant research gaps in many other essential research and innovation areas. As one of the earliest affected countries, the outbreak in China has been well controlled, and it is nearing completion. Many countries and international organizations affirmed that China took active and effective measures. Chinese counterattacks can be replicated to fight the epidemic [3]. Among them, traditional Chinese medicine (TCM) has played an irreplaceable role and provided unique advantages in the management of this disease. Nevertheless, the underlying action mechanisms of Chinese medicines (CMs) are still unclear. In this paper, the possible action mechanisms of CMs in controlling COVID-19 were reviewed and analyzed. This outcome will help us to understand this disease more, develop effective methods to treat this disease, and benefit the world to control this disease.

2. Etiology of COVID-19

COVID-19 is an acute respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and was first reported at the end of 2019 in Wuhan, China. The clinical symptoms are mainly fever, dry cough, and fatigue. A few are accompanied by nasal congestion, runny nose, sore throat, muscle pain, and diarrhea [4]. Severe patients have high levels of cytokines and chemokines in plasma [5], which can easily lead to cytokine storm. Acute respiratory distress syndrome (ARDS), shock, multiple organ dysfunction syndrome (MODS), and fulminant myocarditis appear in severe and death patients of COVID-19 [[5], [6], [7], [8]].

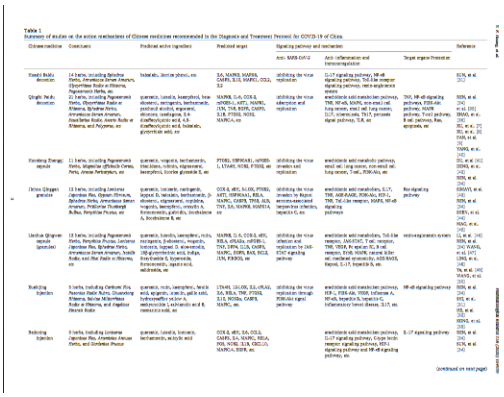
3. Efficacy of CMs in treating COVID-19

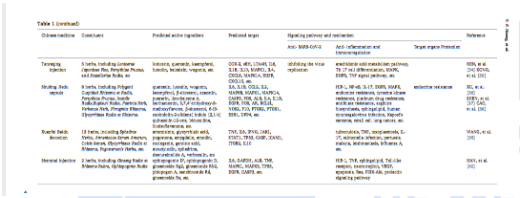
TCM has played an essential role in treating epidemic diseases in the long history of China, and its theories and methods have been recorded in many classic medical books of TCM, such as the *Yellow Emperor's Inner Canon* and the *Treatise on Cold Damage*. The efficacies of these methods were confirmed again in combating SARS in 2003 [9]. Therefore, TCM is a valuable resource for drug discovery against COVID-19 [10].

In China, the treatment protocol of COVID-19 emphasizes the combination of TCM with conventional therapy [11]. The current practice has demonstrated that TCM intervention is essential and effective in the management of COVID-19, showing by the improvement of the cure rate, shortened disease course, delayed disease progression, and reduced mortality rate [12,13]. It was reported that the overall effective rate reached over 90 % in 74187 confirmed COVID-19 cases who received TCM treatment [14]. Lou [15] reported that the change of hematology is positive in COVID-19 patients treated with TCM. A prospective multicenter open-label randomized controlled trial also confirmed the efficacy of Lianhua Qingwen capsule in

ameliorating the clinical symptoms of COVID-19 patients, including fever, fatigue, and cough [16]. Several retrospective and controlled clinical studies have also reported that TCM treatment effectively improved the fever, sweating, cough, headache, shortness of breath, chest distress, nausea, and diarrhea in COVID-19 patients [[17], [18], [19], [20]]. The chest radiogram has been improved significantly as well [21]. The levels of ESR, CRP, and IL-6 were significantly decreased, and the level of IFN-γ was increased in the group received both TCM and conventional treatments in comparison with the group only received conventional treatment (antibiotics and antiviral therapy) [22]. TCM was also helpful to the elderly [23], children [24], and severe COVID-19 patients [25,26].

TCM is a combination of philosophy and ancient disease control and treatment experiences with proved efficacies for different diseases. The overall concept and treatment methods are based on syndrome differentiation, which is the most significant and essential feature. In China, doctors are recommended to use different CMs and strategies for the prevention, treatment, and recovery of COVID-19 at its different stages. The frequently used CMs for COVID-19 are summarized in Table 1 [[27], [28], [29]]. At the press conference on COVID-19 of the State Council of China, three-medicines and three-decoctions of TCM (TMTD), *i.e.*, Jinhua Qinggan granules, Lianhua Qingwen capsule, Xuebijing injection, and Qingfei Paidu decoction, Huashi Baidu decoction, Xuanfei Baidu decoction were emphasized for their remarkable clinical effects in controlling COVID-19 during this epidemic [15]. Especially, Lianhua Qingwen capsule (granules) and Huashi Baidu granules have been officially approved for reliving the fever, cough, and fatigue of mild and moderate COVID-19 patients by the China State Drug Administration recently [30,31]. According to TCM theories, these medications have five different functions, *i.e.*, treating exterior syndromes, resolving dampness, clearing heat, replenishing deficiency, and resolving phlegm, cough, and asthma [32]. According to the medicinal properties of CMs used at different stages of COVID-19, the possible mechanisms of CMs for mild cases could be antiviral and symptom relief, for moderate cases could be anti-inflammation and immune regulation, and for severe and critical cases could be antiviral, inhibiting cytokine storm, and protecting target organs. However, the exact mechanisms must be predicted, analyzed, and validated by network pharmacology analysis, animal studies, and eventually clinical trials. In this article, we mainly summarized and analyzed the possible action mechanisms predicted by network pharmacology analysis from others and us for these CMs that have been used in clinics with reported efficacies recently, including TMTD and others.





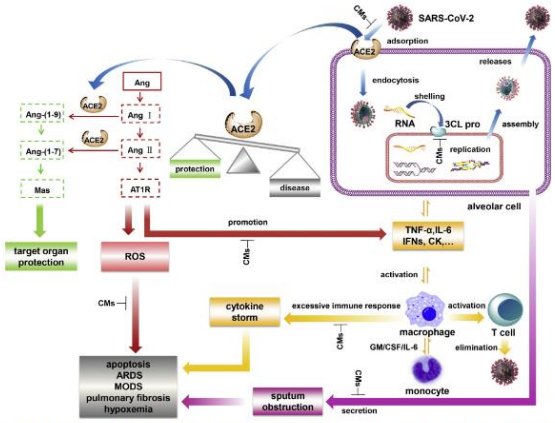
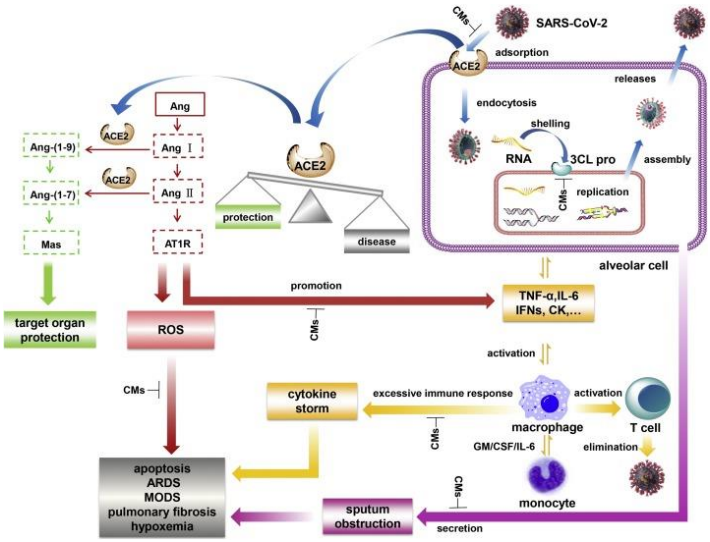


Fig. 1. The potential action mechanisms of Chinese medicines in treating COVID-19. Blue arrows: Chinese medicines (CMs) might directly inhibit SRAS-CoV-2 adsorption and replication by interfering virus's binding to ACE2 and 3CL pro. CMs can also indirectly protect target organs by inhibiting the binding of SRAS-CoV-2 to ACE2, regulating the balance of ACE2 in the body through the Ang / Mas pathway. **Red arrows:** CMs might reduce the production of inflammatory mediators, protect target organs, and relieve the deterioration of COVID-19 by anti-inflammatory and anti-oxidant effects through affecting the Ang / AT1R pathway. **Yellow arrows:** CMs might play an anti-inflammatory and immune regulatory role to prevent cytokine storm. **Purple arrows:** CMs also has an expectorant effect of relieving airway obstruction.

4. Possible action mechanisms of CMs in treating COVID-19

Since the accessibility to SARS-CoV-2 is very limited, the current researches mainly utilized virtual simulation technologies, such as network pharmacology, which can predict the potential bioactive components and action mechanism with a high probability as we have done in a classic Chinese medicine formula Gualuo Xiebai decoction [33]. To understand the current status of researches on the mechanisms of CMs in treating COVID-19, we searched several medical databases, including Web of Science, PubMed, and Chinese National Knowledge Infrastructure (CNKI), from 2019 till now with the subjects of Chinese medicines, mechanism, and COVID-19 or SARS-CoV-2. From the retrieved literature, we further screened the literature by selecting computer-simulated or experimental researches, such as network pharmacology, *in vivo* or *in vitro* studies on COVID-19 or SARS-CoV-2 with recommended formulas or Chinese patent medicines in the Diagnosis and Treatment Protocol for COVID-19 of China [4], and excluding duplicates, reviews, case reports, and studies on other viruses or diseases, *etc.* Finally, two *in vitro* study in English, twenty-five network pharmacology studies in Chinese and one in English were selected and analyzed. The result showed that the action mechanisms of CMs in treating COVID-19 were multi-dimensional (Table 1 and Fig. 1); the detailed mechanisms were described below.



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4.1. Direct action on SARS-CoV-2

According to the literature [[61], [62], [63]], angiotensin-converting enzyme 2 (ACE2) and 3C-like protease (3CL pro) can be the critical targets for antiviral drug design. CMs could target ACE2 to prevent SARS-CoV-2 from entering into host cells or target 3CL pro to inhibit the replication and assembly of the virus in cells.

Li [46] reported that the Lianhua Qinwen capsule significantly and dose-dependently inhibited the replication of SARS-CoV-2 with an IC_{50} value of 411.2 μg/mL in Vero E6 cells that were infected with 100 $TCID_{50}$ of SARS-CoV-2 using cytopathic effect and plaque reduction assay. The number of virions of cells treated with Lianhua Qinwen capsule at 600 μg/mL was significantly reduced compared with that of control cells under the transmission electron microscope. Wang [47], Ling [48] and Ye [49] speculated that quercetin, kaempferol, luteolin, aloe-emodin, rutin, forsythoside E, and hyperoside in Lianhua Qinwen could be the active ingredients in inhibiting SARS-CoV-2 through JAK-STAT signaling pathway by network pharmacology analysis and computer-aided drug design (CADD) of virtual screening.

Wu [35], Xu [37], and Fan [39] reported that the mechanisms of Qingfei Paidu decoction in the treatment of COVID-19 were to inhibit the invasion and replication of virus directly. Patchouli alcohol (*Pogostemonis Herba*), saikosaponin B (*Bupleuri Radix*), ergosterol (*Polyporus*), shionone (*Asteris Radix et Rhizoma*), 23-acetate alisol B (*Alismatis Rhizoma*) might act directly on the SARS-CoV-2 3CL pro to block virus proliferation. In contrast, patchouli alcohol, tussilagone (*Farfarae Flos*), ergosterol, asarinin (*Asari Radix et Rhizoma*), ephedrine hydrochloride (*Ephedrae Herba*), and shionone (*Asteris Radix et Rhizoma*) might act on host cell ACE2 to block the invasion.

By using molecule docking and network pharmacology analyses, Du [41] and Deng [42] screened out licorice glycoside E, naringenin, robinin, kaempferol, (2R)-7-hydroxy-2-(4-hydroxyphenyl) chroman-4-ketone, quercetin, isorhamnetin and irisolidone from Huoxiang Zhengqi as potential 3CL pro inhibitors, which could inhibit SARS-CoV-2 replication by targeting on PIK3CG and E2F1 through PI3K-Akt signaling pathway. Besides, based on traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) and literatures, SHI [51] constructed and analyzed the mechanisms of Xuebijing injection and found rosmarinic acid in this injection could inhibit the virus replication through PI3K-Akt signal pathway as well.

Simayi [43] and Shen [44] predicted the active ingredients of Jinhua Qinggan granules based on network pharmacology and molecular docking. Kaempferol, quercetin, luteolin, baicalein, oroxylin A, licochalcone B, and glyasperin C were proposed to bind with ACE2 and regulate multiple signal pathways, such as PTGS2,

BCL2, CASP3, Kaposi sarcoma-associated herpesvirus infection, hepatitis C, human cytomegalovirus infection, Epstein-Barr virus infection, and measles.
Kong [55] showed the results of Tanreqing injection against SARS-Cov-2 by molecular docking. Kaempferol, quercetin, baicalein, luteolin, and rhubarb wogonin had a good affinity with SARS-CoV-2 3CL hydrolase.
By using molecular docking, Sun [31] found baicalein, licorice phenol, and other twelve main active ingredients in Huashi Baidu decoction could bind to Mpro and ACE2, which could inhibit SARS-CoV-2 replication and block the virus binding sites.

4.2. Anti-inflammation and immunoregulation

Virus-infected alveolar cells release signals to recruit and activate immune cells. These immune cells secrete a variety of cytokines and chemokines to recruit more immune cells to the lesion site. The activated immune cells destroy the virus by releasing inflammatory mediators and phagocytosis. However, the excessive immune responses initiate a "cytokine storm", which causes many immune cells and secretions in the lungs of COVID-19 patients. Cytokine storm alters vascular permeability, blocks airways, causes edema, hypoxia, and target organ damage, *etc.* The occurrence of cytokine storm in patients with COVID-19 is an essential cause of exacerbation and even death [64,65]. CMs can play a regulatory role in the causes, processes, and other aspects of the cytokine storm by modulating the release of cytokines, the functions of macrophages, monocytes and neutrophils, the permeability of pulmonary vessels, and the activities of T cells [67].
Wu [35], Zhao [36], Xu [37], Xu [38] and Fan [39] applied network pharmacology to analyze Qingfei Paidu decoction and found that quercetin, luteolin, kaempferol, naringenin, beta-sitosterol, isorhamnetin, baicalein, and tussilagone could be the main active ingredients. These ingredients could suppress cytokines release, alleviate excessive immune responses and eliminate inflammation by regulating AKT1, MAPK1, MAPK3, MAPK8, MAPK14, IL-6, RELA, STAT1, JUN, and immune-related pathways, such as Th17 cell differentiation pathway, T cells, and B cells pathway, and the function of the cytokines related pathways, such as TNF signaling pathways, NF-κB, MAPK signal pathway, VEGF signaling pathways, HIF-1 signaling pathway and TLRs signaling pathways. Yang [40] also verified that the therapeutic effects of Qingfei Paidu decoction might result from glycyrrhizic acid mediated anti-inflammatory effects by suppressing IL-6 production in macrophage.
Basing on the pharmacophore models, Ren [34] reported that Huoxiang Zhengqi capsules, Jinhua Qinggan granules, Lianhua Qingwen capsules, Qingfei Paidu decoction, Xuebijing injection, Reduning injection, and Tanreqing injection could prevent SARS-CoV-2 *via* regulating cytokines through the arachidonic acid metabolic pathway.
Shen [44] and Mao [45] showed that kaempferol, naringenin, wogonin, neobaicalein in Jinhua Qinggan granules well docked with specific target proteins of SARS-CoV-2. The core target proteins such as AKTI, HSP90AA1, ELA, IL6, TNF, and MAPKs are mainly related to inflammation and immunoregulation.
Li [46] reported that Lianhua Qinwen markedly reduced pro-inflammatory cytokines (TNF-α, IL-6, CCL-2/MCP-1, and CXCL-10/IP-10) production in a concentration-dependent manner in SARS-CoV-2 infected Huh-7 cells at the mRNA levels by real-time quantitative PCR assays. Wang [47] Ling [48] and Wang [50] speculated that quercetin, luteolin, and kaempferol in Lianhua Qinwen could be involved in immune regulation on the targets of COX-2, ILs, CASP3, EGFR, DPP4, CALM1, RELA, and MAPKs through the signaling pathways of Chagas disease (American trypanosomiasis), Toll-like receptor, JAK-STAT, T cell receptor, TNF, AGE-RAGE, Kaposi, IL-17, human cytomegalovirus infection, and hepatitis B. From the constructed component-target-pathway network, Ye [49] showed that formononetin, rutin, emodin 8-O-β-D-glucoside, hyperoside, loganic acid and salidroside in Lianhua Qinwen could improve human immunity through T cell and B cell receptor signaling, natural killer cell mediated cytotoxicity, and anti-inflammatory pathways including Fc epsilon RI, ErbB, and MAPK signaling pathways.
Besides, Shi [51], He [52], and Kong [53] speculated that Xuebijing injection played an anti-inflammatory effect by NF-κB, VEGF, HIF-1, IL17, and PI3K-Akt signal pathway. The active ingredients of Xuebijing injection, such as rosmarinic acid, quercetin, apigenin, luteolin, hydroxysafflor yellow A, chlorogenic acid, and salvianolic acid B, well targeted RELA, TNF, PTGS2, NOS2, PTGS2, MAPK1, and IL6.
Sun [54] reported that quercetin, luteolin, rutin, and isorhamnetin in Reduning injection played the effects of anti-inflammation, antivirus, and immunomodulation for the treatment of lung injury in COVID-19. It could act on CASP3, IL6, MAPK1, CCL2, and other targets through IL-17, C-type lectin receptor, and HIF-1 signaling pathways.

Through enrichment analysis of KEGG signaling pathway, Kong [55] revealed that multiple targets of Tanreqing injection could play a role in reducing overproduced cytokines, alleviating the excessive immune response and eliminating inflammation by regulating immune-related signaling pathways, such as Th17 cell differentiation, MAPK, EGFR, and TNF.

In Shufeng Jiedu capsule, Xu [56], Shen [57], and Cao [58] reported that quercetin, luteolin, wogonin, kaempferol, acacetin, isorhamnetin, 5,7,4'-trihydroxy-8-methoxyflavone, β-sitosterol, and licochalcone A could be the active ingredients. These ingredients might target on IL6, IL1B, CCL2, MAPK8, MAPK1, MAPK14, CASP3, FOS, ALB, CALM1, NOS2, PTGS2, DPP4, and PTGS2 through endocrine resistance, EGFR tyrosine kinase resistance, platinum drug resistance, antifolate resistance, arginine biosynthesis, HIF-1, NF-κB, MAPK, IL-17, and small cell lung cancer signaling pathways.

In addition, Wang [59] screened artemisinin, glycyrrhizic acid, pogostone, and amygdalin as the potential active ingredients of Xuanfei Baidu decoction from the databases of TCM-PTD, ETCM, TCMSP, and SymMap. TNF signaling pathway, IL-17 signaling pathway, and tuberculosis related pathway could be the key for treating COVID-19.

Sun [31] speculated that the anti-inflammatory effect of Huashi Baidu decoction in treating COVID-19 could involve IL6, MAPK3 and MAPK8, IL-17, NF-κB, Toll-like receptor signaling pathway, and the renin-angiotensin system.

By molecular docking and network analysis, Han [60] showed that ophiopogonins, ginsenosides, and sanchinoside Rd might be the main ingredient of Shenmai injection to treat COVID-19 by targeting on IL-6, IL-2, and TNF.

4.3. Target organs protection

ACE2 can catalyze the splitting of angiotensin (Ang) Ⅰ and Ang Ⅱ into Ang (1-9) and Ang (1-7), respectively, and regulate ACE2-Ang (1 -7)-Mas axis to protect the acute lung injury [66]. However, ACE2 is also a cell receptor of SARS-CoV-2 in the human body [68]. The combination of SARS-CoV-2 and ACE2 [69] disrupts the balance between Ang Ⅰ / Ang Ⅱ and Ang (1-9)/Ang (1-7), thereby generate many free radicals, which can change the permeability of cell membranes and lead to the organ damages. ZHOU [70] speculated that SARS-CoV-2 could infect type Ⅱ alveolar epithelial cells through ACE2, thus destroying the lung tissue and air-blood barrier. Then, the virus continued to infect other organs through ACE2, such as the heart, kidneys, and liver by blood circulation. It further triggered an excessive immune response, such as the imbalance of T-helper-1 (Th1) and Th2 cells, which produced numerous inflammatory cytokines, causing a cytokine storm and leading to MODS ultimately. CMs can play a direct or indirect role in protecting target organs by inhibiting the binding of the virus to ACE2 to produce anti-inflammation, anti-oxidation, anti-fibrosis, and expectorant action [71].

Qingfei Paidu decoction targeted lungs [36] and protected multiple organs. Wu [35], Xu [38], and Fan [39] speculated that Qingfei Paidu decoction might protect the lung from injury by regulating TNF, PI3K-Akt, Ras, MAPK, B cell receptor, and apoptosis signaling pathways. Mao [45] found that Jinhua Qinggan granules played a role in regulating apoptosis *via* PI3K-Akt, MAPK, and Ras pathways. Ling [48] reported that 18β-glycyrrhetinic acid, stigmasterol, indigo, β-sitosterol, luteolin, quercetin and naringenin in Lianhua Qingwen Prescription might target on ACE2 and protect the target organs of COVID-19 through the renin-angiotensin pathway. Xu [56] predicted that MAPK8, MAPK1, MAPK14, FOS, CASP3, endocrine resistance, EGFR tyrosine kinase resistance, platinum drug resistance, antifolate resistance, arginine biosynthesis, and MAPK signaling pathways could be involved in the inhibition of cell apoptosis and pulmonary fibrosis by Shufeng Jiedu capsule.

4.4. Common potential active ingredients and action mechanisms of CMs for treating COVID-19

From the above-reviewed researches, we can see that there are some ingredients and action mechanisms in common for these CMs used for treating COVID-19, which could be important for these CMs to exert their effect and drug development. Therefore, we further calculate the frequencies of these ingredients and action mechanisms that appeared in these reports. The results (Table 2) show that quercetin, kaempferol, luteolin, isorhamnetin, baicalein, naringenin, wogonin (the last three are in the same rank) are the top five ingredients; AEC2 and 3CL protein could be the potential direct targets for anti-SARS-CoV-2; COX-2, CASP3, IL-6, MAPK1, MAPK14, MAPK8 and RELA (the last three are in the same rank) are the top five targets; and IL-17, arachidonic acid metabolic pathway, HIF-1, NF-κB, Ras, and TNF (the last four are in the same rank) are

the top five signaling pathways. These ingredients, targets, and signaling pathways could be the major players in the management of COVID-19 by using TCM and should be focused on in future research and drug development.

Table 2
Frequency analysis of the medications recommended in the Diagnosis and Treatment Protocol for COVID-19 of China.

Decoction piece	Frequency*	Active ingredient	Frequency*	Target	Frequency*	Signaling pathway	Frequency*
Glycyrrhizae Radix et Rhizoma	7	quercetin	9	ACE2	13	IL-17	8
Armeniacae Semen Amarum	5	kaempferol	8	3CL pro	13	arachidonic acid metabolic pathway	7
Ephedrae Herba	5	luteolin	7	COX-2	11	HIF-1	6
Gypsum Fibrosum	5	isorhamnetin	6	CASP3	10	NF-κB	6
Pogostemonis Herba	5	baicalein	5	IL6	10	Ras	6
Forsythiae Fructus	4	naringenin	5	MAPK1	9	TNF	6
Lonicerae Japonicae Flos	4	wogonin	5	MAPK14	6	MAPK	5
Artemisiae annuae Herba	3	ergosterol	4	MAPK8	6	PI3K-Akt	5
Atractylodes Rhizoma	3	lonicerin	4	RELA	6	Toll-like receptor	5
Pinelliae Rhizoma	3	tussilagone	4	EGFR	5	hepatitis B related	4
Poria	3	β-sitosterol	4	IL2	5	small cell lung cancer related	4
Scutellariae Radix	3	rutin	3	LTA4H	5	T cell receptor signaling pathway	4
Bupleuri Radix	2	stigmasterol	3	NOS2	5	apoptosis	3
Citri reticulatae Pericarpium	2	7-methoxy-2-methyl isoflavone	2	TNF	5	human cytomegalovirus infection related	3
Descurainiae Semen, Lepidii Semen	2	acacetin	2	12-LOX	4	influenza A related	3
Isatidis Radix	2	chlorogenic acid	2	CCL2	4	non-small cell lung cancer related	3
Magnoliae officinalis Cortex	2	formononetin	2	COX-1	4	AGE-RAGE	2
Menthae haplocalycis Herba	2	hydroxysafflor yellow A	2	IL10	4	B cell receptor	2
Paeoniae Radix Rubra	2	licochalcone A	2	IL1B	4	Chagas disease (American trypanosomiasis)	2
Phragmitis Rhizoma	2	licorice glycoside E	2	IL4	4	EGFR tyrosine kinase resistance	2
Polygonum cuspidatum Rhizoma et Radix	2			PPARG	4	hepatitis C related	2
Rhei Radix et Rhizoma	2			sEH	4	Kaposi sarcoma-associated herpesvirus infection	2
Verbenae Herba	2			STAT1	4	pertussis related	2
Zingiberis Rhizoma	2			TP53	4	Th17 cell differentiation	2
				AKT1	3		
				ALB	3		
				ICAM1	3		
				MAPK3	3		
				mPGES-1	3		

Although the currently available researches have covered the major formulae and medications recommended in the Diagnosis and Treatment Protocol for COVID-19 of China and generated some useful information, these researches only analyzed these formulae and medications individually. The results of these researches could reflect more on the unique features of individual formula and medication and less on the general features of these formulae and medications. To understand the general features of these formulae and medications, we selected TMTD to screen the common components and then analyzed the targets and signaling pathways that these components may act on using networking analysis. The common components of TMTD were generated by using the SymMap database (https://www.symmap.org/) and TBtools software (V0.665). The results (Fig. 2) shows that there are total 401 compounds in TMTD, of which ten compounds are shared by the six formulae of TMTD, they are ursolic acid, thymol, sucrose, rutin, physcion, oleanolic acid, caffeic acid, β-terpinene, β-sitosterol, and β-elemene. We believe that the common action mechanisms of TMTD may exist in these ten compounds. Therefore, we further predicted the possible targets and signaling pathways that the ten compounds may act on by network pharmacology analysis. The results show that the potential targets were FGF2, BCL2L1, MAPK8, IL6, SRC, JUN, PTGS2, IL1B, FOS, CASP3, REAL, VEGFA, CDK2, TP53, NFKBIA, CDKN1A, CSF2, CASP9, CAT, STAT3, CCND1, CASP1, FASLG, CASP8, and INS and the possible signaling pathways are apoptosis, NOD-like receptor, FoxO, Toll-like receptor, p53, MAPK, cytosolic DNA-sensing, RIG-I-like receptor, VEGF, and Jak-STAT signaling pathways. We also constructed a network to demonstrate the relationship among formulae, component Chinese medicines, active compounds, possible targets, possible signaling pathways, and possible action mechanisms with Cytoscape3.8.0 software (Fig. 3). The network shows that the common action mechanisms of TMTD in the treatment of COVID-19 could be related to signal transduction, immune system, and cell growth and death.

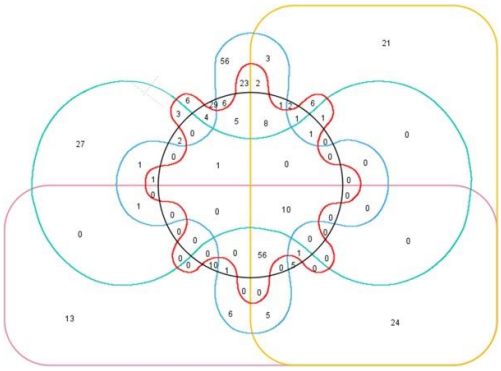

Fig. 2. **The Venn diagram analysis of the three-medicines and three-decoctions. Pink route**: Jinhua Qinggan granules; **Green route**: Xuebijing injection; **Blue route**: Qingfei Paidu decoction; **Red route**: Haushi Baidu decoction; **Black route**: Xuanfei Baidu decoction; **Yellow route**: Liianhua Qingwen capsule.

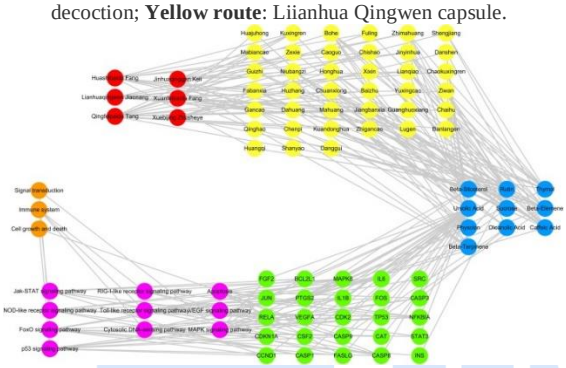

Fig. 3. **The network of three-medicines and three-decoctions in treating COVID-19. Red pies**: the node group of three-medicines and three-decoctions. **Yellow pies**: the node group of Chinese medicines. **Blue pies**: the node group of active ingredients. **Green pies**: the node group of targets. **Purple pies**: the node group of signal pathways. **Orange pies**: the node group of mechanism classifications.

By comparing the results of above two strategies of network pharmacology analysis on the useful formulae and medications of TCM in treating COVID-19, we found that although the common compounds are different, the possible action mechanisms are overlapped with each other, implying that different compounds may act on same or similar targets or pathways. This gives TCM redundant mechanisms to treat diseases and also explains why different TCM formulae with different composition can treat the same disease.

5. Conclusion and prospect

Taken together, the practice in controlling the outbreak in China has proved the clinical efficacy and advantage of integrated TCM and Western medicine in treating COVID-19. Existing clinical data, *in silico* study, and literature analysis confirmed that the possible mechanisms of CMs were antivirus, anti-inflammation, immune regulation, and organ protection through multiple components acting on multiple targets at multiply pathways for the treatment of COVID-19. Nevertheless, the current understanding of the mechanisms of CMs is mainly produced from virtual simulation through molecular docking and network pharmacology analysis. The focus and strategy in different research are somehow different; the ingredients, targets, and pathways predicted in these researches might have certain limitations. To confirm these predicted mechanisms, well designed *in vitro* cell experiments and *in vivo* animal studies based on these predictions are needed. Importantly, the multiple omic technologies, such as proteomics, metabolomics,

genomics, should be applied to analyze the biofluid and tissue samples collected from COVID-19 patients who received TCM treatment in well designed and controlled clinical trials to verify these mechanisms [72].

Author contributions

HZ and YX supervised all research and revised the manuscript. YFH and FH collected information. CB performed networking analysis. YFH and HZ analyzed the data and prepared the manuscript.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Abstract

The Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly become a global pandemic. Up to now, numerous medicines have been applied or approved for the prevention and control of the virus infection. However, the efficiency of each medicine or combination is completely different or still unknown. In this review, we discuss the types, characteristics, antiviral mechanisms, and shortcomings of recommended candidate medicines for SARS-CoV-2 infection, as well as perspectives of the drugs for the disease treatment, which may provide a theoretical basis for drug screening and application.

1. Introduction

The Coronavirus Disease 2019 (COVID-19, 2019 Novel Coronavirus Pneumonia, 2019-NCP) that outbroke in Wuhan, China at the end of 2109 has rapidly become a global pandemic (Li et al., 2020a). Up to 21 Jun 2020, it was estimated that more than 8,708,008 confirmed cases and 461,715 deaths were detected in more than 210 countries, areas, or territories (https://www.who.int/). The common clinical symptoms of the disease are firstly reported as fever, dry cough, dyspnoea, fatigue, sore throat, headache, mild upper respiratory tract illness, severe viral pneumonia with respiratory failure, and even death (Tian et al., 2020; Wang et al., 2020b; Zhou et al., 2020b). Recent studies have found that in addition to the above symptoms, it seems to have a wide range of clinical features, including asymptomatic, diarrhea, myalgia, discomfort, loss of taste and smell, lymphopenia, cytokines storm, etc. (Guan et al., 2020; Li et al., 2020c; Liu et al., 2020a; Tabata et al., 2020; Ye et al., 2020). Surprisingly, it is estimated that the proportion of asymptomatic patients is 18–50 %, or even as high as 87.9 % in some cases (Ji et al., 2020; Lai et al., 2020; Li and Ren, 2020; Luan et al., 2020; Sutton et al., 2020). These results suggest that the disease has strong infectious and pathogenicity, with alarming morbidity and mortality, which needs extensive attention to prevent and control the disease. Moreover, due to the rapid spread of the diseases and the dramatic increase in the number of patients worldwide, effective antiviral medicine is urgently needed.

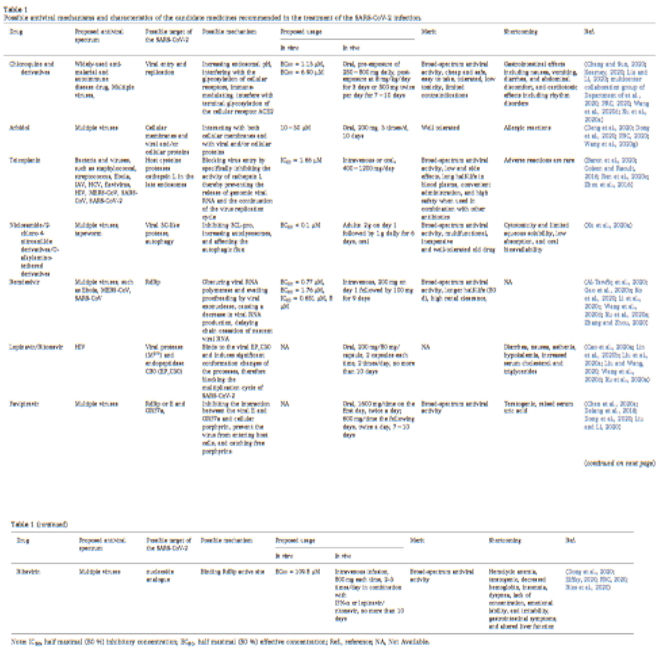
The aetiological agent of COVID-19 has been confirmed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a newly identified virus belonging to the family *Coronavirus*, genus *Betacoronavirus,* and subgenus *Sarbecovirus* (Adhikari et al., 2020; Jiang and Shi, 2020; Li et al., 2020a; Zhou et al., 2020b). The virus contains a single positive-strand RNA of about 30 kb, which encodes at least eleven proteins, including ORF1ab, spike protein (S), ORF3a, envelope protein (E), membrane protein (M), ORF6, ORF7a, ORF7b, ORF8, nucleocapsid phosphoprotein (N), and ORF10 (Chan et al., 2020; Jiang and Shi, 2020; Li et al., 2020a; Zhou et al., 2020b). Among these viral proteins, S protein can recognize and bind the cellular receptor angiotensin-converting enzyme 2 (ACE2), followed by priming of the S protein by host proteases, especially by the serine protease TMPRSS2, resulting in cleavages at the S1/S2 and the S2' sites of the S protein (Hoffmann et al., 2020; Zhou et al., 2020b). Therefore, the S protein is considered as the main antigen of the virus. ORF1ab protein can be cleaved into at least 16 predicted nonstructural proteins (nsp), among which nsp3, nsp5, nsp12, and nsp13 encode putative papain-like viral protease (PLVP), 3C-like protease (chymotrypsin-like or 3C-like protease, $3CL^{pro}$), RNA-dependent RNA polymerase (RdRp), and helicase, respectively (Chan et al., 2020). Besides, the viral ORF1ab, ORF10, and ORF3a proteins can attack heme on hemoglobin 1-β chain synergistically, resulting in decomposing iron to form porphyrin, which will subsequently lead to less and less hemoglobin carrying oxygen and carbon dioxide, produce extremely strong poisoning and inflammation in lung cells, and interfere with the normal heme anabolism pathway (Liu and Li, 2020). Therefore, S protein and proteins derived from the ORF1ab as well as their cellular receptors, such as ACE2 (Zhou et al., 2020b), AGTR2 (angiotensin II receptor type 2) (Cui et al., 2020), TMPRSS2 (Hoffmann et al., 2020), and CD147 (Basigin or EMMPRIN) (Wang et al., 2020c), are the most favorable cellular targets for the development of antiviral compounds.

Since the disease newly emerges, there is no clinically approved antiviral therapy or vaccines for the disease. Hopefully, the virus has highly relationship with bat coronavirus isolate RaTG13 (GenBank No.: MN996532)

base on the full-length genome (Jiang and Shi, 2020; Li et al., 2020a; Zhou et al., 2020b). Thus, many experts and clinicians suggest using anti-coronavirus drugs to prevent and control the disease. Up to now, numerous medicines have been registered or approved for clinical trials for COVID-19 treatment (https://clinicaltrials.gov/ and http://www.chictr.org.cn/). However, the efficiency of each medicine or combination is quite different or still unknown. In this review, we discuss the types, characteristics, antiviral mechanisms, and shortcomings of several recommended candidate medicines for SARS-CoV-2 infection, as well as perspectives of the drugs for the disease treatment. The mechanisms discussed in this article may provide a theoretical basis for drug screening and application.

2. Chemical pharmaceuticals

Repurposed drugs have emerged as attractive sources for the prevention and treatment of the SARS-CoV-2 infection, which could shorten the time and reduce the cost compared to *de novo* drug screen (Baron et al., 2020; Chen et al., 2020c; Liu et al., 2020d; Omar, 2020; Sohini and Narayanaswamy, 2020; Xu et al., 2020a; Zhou et al., 2020c). For example, the antiviral medicines ledipasvir or velpatasvir are particularly attractive candidates to treat COVID-19 with minimal side effects (Chen et al., 2020c). As reported by Chen and colleagues, Epclusa (velpatasvir/sofosbuvir) and Harvoni (ledipasvir/sofosbuvir) could be attractive drugs that can inhibit 3C-like protease of the virus (Chen et al., 2020c). Zhou et al. identified three potential drug combinations (sirolimus plus dactinomycin, mercaptopurine plus melatonin, and toremifene combined with emodin) targeting SARS-CoV-2 by network-based methodologies (Zhou et al., 2020c). Sohini et al. found 20 known candidates, including some synthetic molecules and phytochemicals, which might exhibit antiviral activities by binding to the main protease of SARS-CoV-2 (Sohini and Narayanaswamy, 2020). Among the repurposed drugs (Table 1), remdesivir, chloroquine, hydroxychloroquine, lopinavir/ritonavir, and ribavirin are promising candidates in the development of the antiviral drugs for SARS-CoV-2 infection, which have shown efficacy to inhibit the coronavirus *in vitro* or in animal models (Belhadi et al., 2020; Chen et al., 2020c; Guo et al., 2020; Martinez, 2020; Xu et al., 2020a). Furthermore, according to the 7th edition of the Guideline recommended by the Prevention, Diagnosis, and Treatment of Novel Coronavirus-induced Pneumonia issued by the National Health Commission (NHC) of the People' Republic of China for the treatment of COVID-19, interferon α (IFN-α), lopinavir/ritonavir, ribavirin, chloroquine phosphate, and arbidol are priority recommendation (PRC, 2020). A recent survey showed that the main antiviral drugs are IFN-α (69.5 %), lopinavir/ritonavir (65.0 %), arbidol (60.0 %), and ribavirin (55.7 %) in China (Liu et al., 2020c).



Based on the target of the antiviral medicine, there are mainly two types of antiviral compounds recommended in the treatment of the COVID-19. The first type is targeting host protease or other cellular proteins, such as teicoplanin, arbidol, chloroquine and derivatives, and niclosamide. The second type mainly acts on viral proteins, such as niclosamide, lopinavir/ritonavir, remdesivir, favipiravir, and ribavirin.

2.1. Host protein targeted medicines

Chloroquine (CQ) and its derivatives, especially hydroxychloroquine (HCQ) and chloroquine phosphate, are widely-used anti-malarial and autoimmune disease drugs, which can be distributed throughout the whole body after oral administration (Chang and Sun, 2020; Devaux et al., 2020; Gao et al., 2020a; Kearney, 2020; Li and Liu, 2020; multicenter collaboration group of Department of, S., Technology of Guangdong, P., Health Commission of Guangdong Province for chloroquine in the treatment of novel coronavirus, p., 2020; Wang et al., 2020d). In recent years, CQ is proved to be a broad-acting antiviral effective agent against numerous viruses, including Influenza viruses, Human immunodeficiency virus (HIV), Hand-Foot-and-Mouth Disease (HFMD), Kaposi's sarcoma-associated herpesvirus (KSHV), Chikungunya virus (CHIKV), hepatitis B virus (HBV) and other inflammation-related viruses (Devaux et al., 2020; Khan et al., 2010; Tan et al., 2018; Yang et al., 2016). CQ and its derivatives can increase the pH value of endosome, interfere with glycosylation of cellular receptors, inhibit autophagosome-lysosome fusion, interfere signal pathways, disturb the post-translational modification of viral proteins, modulate the immune responses, and reduce inflammatory reactions (Chang and Sun, 2020; Devaux et al., 2020; Kearney, 2020; Khan et al., 2010; Sinha and Balayla, 2020; Wang et al., 2020a; Yang et al., 2016), and thus blocking the early and late stages of the virus infection. Furthermore, CQ and HCQ can prevent the ORF1ab, ORF3a, and ORF10 of SARS-CoV-2 from attacking heme to form porphyrin, and inhibit the viral ORF8 and surface glycoprotein from binding to porphyrin, thus effectively relieving respiratory distress symptoms (Liu and Li, 2020). Because of its immunomodulatory activities, CQ and its derivatives were also used to control cytokine storms in critical patients with SARS-CoV-2 infection in the late stage (Zhou et al., 2020a). Moreover, hydroxychloroquine is safer and more effective than CQ at inhibiting SARS-CoV-2 *in vitro* and *in vivo* (Gautret et al., 2020; Liu et al., 2020b; Yao et al., 2020b; Zhou et al., 2020a). Therefore, it was recommended to use 400 mg of hydroxychloroquine sulfate twice daily for 1 day, followed by 200 mg twice daily for 4 more days for the treatment (Yao et al., 2020b). Furthermore, a combination of hydroxychloroquine sulfate (200 mg, three times per day during ten days) and azithromycin (500 mg on day1 followed by 250 mg per day, the next four days) can significantly reduce the viral load compared with that of the hydroxychloroquine treatment alone (Gautret et al., 2020). Taken together, the CQ and its derivatives can shorten the course of SARS-CoV-2, reduce the inflammatory responses to infection, inhibit the deterioration of pneumonia, improve lung imaging performance, and promote the negative conversion of the virus (Kearney, 2020; Li and Liu, 2020). However, recent reports show that treatment with HCQ is associated with numerous adverse effects in patients with COVID-19, such as frequent QTc prolongation, erythema multiforme, acute generalized exanthematous pustulosis (Bessiere et al., 2020; Delaleu et al., 2020; Mercuro et al., 2020; Monte Serrano et al., 2020; Ren et al., 2020b; Torjesen, 2020). On Jun 16, 2020, the US Food and Drug Administration revoked the emergency use authorization for chloroquine and hydroxychloroquine due to their lack of efficacy and safety concerns (http://www.chinadaily.com.cn/). Therefore, the safety assessment of CQ/HCQ and the combination of HCQ and other medicine should be conducted for patients with COVID-19.

Arbidol (ARB, also known as umifenovir) is a broad-spectrum antiviral drug that can inhibit numerous DNA and RNA viruses, including influenza virus, Zika virus, West Nile virus and hepatitis C virus (Blaising et al., 2014; Haviernik et al., 2018). It was reported that ARB can interact with both cellular membranes and viral and/or cellular proteins, and thus preventing membrane fusion, blocking trimerization of the spike glycoprotein and virus infection (Blaising et al., 2014; Vankadari, 2020). ARB also has immunostimulating effects, such as stimulating the production of interferon, activating the humoral and cellular immune defense of the body, enhancing the phagocytic activity of macrophages (Blaising et al., 2014; Haviernik et al., 2018). Moreover, ARB exhibits a prolonged antioxidant capacity by reacting with free radicals in two stages (Proskurnina et al., 2020). Therefore, it was supposed that ARB can inhibit SARS-CoV-2 infection at several stages, which is currently used in several clinical trials for the COVID-19 treatment. Furthermore, it was reported that ARB combined with Lopinavir/Ritonavir (LPV/r) is better than LPV/r alone in the treatment of the COVID-19 (Deng et al., 2020). ARB/IFN-α2b Therapy and ARB/adjuvant therapy are helpful to relieve

COVID-19 pneumonia (Chen et al., 2020b; Xu et al., 2020b). However, another group found that arbidol monotherapy has little effect on improving clinical outcomes of mild/moderate patients (Li et al., 2020d). These results demonstrate that a combination of different compounds with ARB targeting different stages of the viral infection might be a promising stream in the antiviral treatment of the COVID-19. Notably, it is not recommended to use three or more antiviral drugs simultaneously (PRC, 2020).

Apart from CQ and ARB recommended by WHO and NHC of China, some compounds also exhibit promising characteristics in the treatment of the COVID-19. The first one is teicoplanin, which is a commonly used glycopeptide antibiotic in the treatment of Gram-positive bacterial infection, especially in *staphylococcal* and *streptococcus* infections (Baron et al., 2020; Ren et al., 2020c). Recently, it was reported that the antibiotic can be used against various viruses such as Ebola, influenza virus, flavivirus, hepatitis C virus, HIV, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV (Colson and Raoult, 2016; Zhou et al., 2016). Teicoplanin can inhibit entry of the pseudoviruses (HIV-luc/2019-nCoV-S) by specifically blocking the activity of host cysteine proteases cathepsin L in the late endosomes, and thus inhibiting the release of genomic viral RNA and the virus replication cycle (Baron et al., 2020; Ren et al., 2020c). Therefore, the half-maximal (50 %) inhibitory concentration (IC_{50}) of teicoplanin is 1.66 u M on the pseudoviruses, and the recommended usage is 400−1200 mg/day intravenously or orally (Baron et al., 2020; Ren et al., 2020c)(Ceccarelli et al., 2020).

Another compound is niclosamide, which is an FDA-approved anthelminthic drug against tapeworm infections for several decades (Xu et al., 2020a). It is a multifunctional drug that can inhibit oxidative phosphorylation and stimulate adenosine triphosphatase activity in the mitochondria, regulate multiple signaling pathways and biological processes, including Wnt/β-catenin, mTORC1, STAT3, NF-κB, Notch, NS2B-NS3 interaction, and pH (Kratky and Vinsova, 2011; Pindiprolu and Pindiprolu, 2020; Xu et al., 2020a). These results suggest that niclosamide has broad-spectrum antiviral activities. Furthermore, the derivatives of niclosamide, such as 2-chloro-4-nitroanilide, O-alkylamino-tethered, or salicylamide derivatives, also exhibit more potent and orally bioavailable for antiviral treatments (Kratky and Vinsova, 2011; Xu et al., 2020a). Moreover, niclosamide may inhibit endocytosis of SARS-CoV-2 by blocking ACE2, and prevent autophagy of SARS-CoV-2 by inhibiting of S-Phase kinase-associated protein 2 (Pindiprolu and Pindiprolu, 2020). However, the antiviral activities of niclosamide and its derivatives in SARS-CoV-2 infection need further research and clinical verification.

2.2. Virus-targeted medicines (direct-acting antiviral drugs)

2.2.1. Viral proteins targeted medicines

The first type of highly recommended medicines direct targeting SARS-CoV-2 in clinical trials is repurposed drugs, which interact with viral proteins, especially viral major proteases.

Lopinavir/Ritonavir (also named as Kaletra or Lopimune, LPV/r) is a fixed-dose combination that is currently used to treat HIV infection. Lopinavir is an inhibitor of HIV protease, which prevents cleavage of the viral Gag-Pol polyprotein and results in the production of immature, non-infectious viral particles (Cao et al., 2020a; Lin et al., 2020b; Liu et al., 2020a; Liu and Wang, 2020; Wang et al., 2020a,2020b; Xu et al., 2020a). Ritonavir works as an inhibitor of cytochrome P450 (CYP450) enzymes, an inhibitor of P glycoprotein, and a glucuronidase inducer, which can inhibit the CYP3A (CYP_{450}, family 3, subfamily A)-mediated metabolism of lopinavir, thereby increasing the plasma levels of lopinavir (Cao et al., 2020a; Sevrioukova and Poulos, 2014). Belhadi et al. reported that LPV/r was associated with the highest total number (2606) in the clinical trials for the COVID-19 treatment (Belhadi et al., 2020), suggesting the combination is promising in the antiviral therapy of the disease. An evaluation based on molecular modeling showed that both ritonavir and lopinavir can suitably bind to the endopeptidase C30 (EP_C30) of SARS-CoV-2 and induce significant conformation changes of CEP_C30, with a stronger efficacy induced by ritonavir (Lin et al., 2020b). However, some groups showed that there was no significant difference between LPV/r group and the standard care group, and LPV/r might results in more adverse events compared with that of the control group (Cao et al., 2020a; Li et al., 2020d). Therefore, the effectiveness and safety of these drugs need further evaluation.

Additionally, many repurposing drugs targeting viral main proteins were recently evaluated using computer-aided protocols, such as molecular docking (Chen et al., 2020c; Liu et al., 2020d; Omar, 2020; Sohini and Narayanaswamy, 2020). As reported by Omar, the binding energy of aliskiren, dipyridamole, mopidamol and rosuvastatin to COVID-19 main protease ($3CL^{pro}$) is relatively high (Omar, 2020). Atazanavir has a potential to

bind to the viral RdRp (Kd 21.83 nM), helicase (Kd 25.92 nM), 3'-to-5' exonuclease (Kd 82.36 nM), 2'-O-ribose methyltransferase (Kd of 390.67 nM), and endoRNAse (Kd 50.32 nM) (Beck et al., 2020), suggesting the drug can be used for COVID-19 treatment by inhibiting simultaneous all the subunits of the SARS-CoV-2 replication complex. Dai and colleagues synthesized two compounds (11a and 11b) targeting SARS-CoV-2 M^{pro} and found that these compounds showed good pharmacokinetic properties *in vivo* with low toxicity (Dai et al., 2020). However, whether these drugs or compounds have antiviral effects against SARS-CoV-2 infection still needs clinical evaluation.

2.2.2. Nucleoside analogues

The second type of highly recommended drugs targeting the virus is nucleoside analogs, which can disturb or halt the replication of the virus genome.

Remdesivir (GS-5734, RDV) is an adenosine analog originally designed for clinical trials against Ebola virus infections (Amirian and Levy, 2020; Martinez, 2020). As reported, remdesivir exhibit antiviral activity against multiple viruses, including Marburg virus, Nipah virus, Hendra virus, measles and mumps, respiratory syncytial virus, as well as human and zoonotic coronaviruses, such as HCoV-NL63, HCoVOC43, HCoV-229ESARS-CoV and MERS-CoV *in vitro* and in non-human primate models (Ko et al., 2020; Martinez, 2020), suggesting it has broad-spectrum antiviral activities. It was reported that remdesivir has obvious clinical benefits in *rhesus macaques* at the early stage of SARS-CoV-2 infection, supporting the early treatment of remdesivir in patients with COVID-19 to prevent the progression of severe pneumonia (Williamson et al., 2020). The active form of remdesivir in peripheral blood mononuclear cells (PBMCs) can obscure viral RdRp and evade proofreading by viral exonuclease, resulting in a decrease in viral RNA production (Al-Tawfiq et al., 2020; Gao et al., 2020b; Ko et al., 2020; Zhang and Zhou, 2020). Further studies showed that the active form of remdesivir, CHEMBL2016761 and GS-441,524, can bind to the viral RdRp binding pocket and replace natural counterpart ATP, causing insertion of remdesivir as well as three additional nucleotides in the extended viral strand (Cao et al., 2020b; Chang et al., 2020; Gordon et al., 2020; Wang et al., 2020d), and thus resulting in a disturbing viral genome. The relative binding free energy of remdesivir to the RdRp is −8.28 ± 0.65 kcal/mol, which is significantly stronger than that of the natural substrate ATP (Zhang and Zhou, 2020). However, in the past few months, remdesivir has been the most controversial drug in the treatment of COVID-19 due to its potential risks on kidney and liver functions (Adamsick et al., 2020; Davies et al., 2020). Moreover, Remdesivir can only reduce the hospitalization time of patients and has little effect on reducing the mortality rate. Several randomized controlled trials are currently ongoing worldwide to assess the safety and efficacy of this medicine in the treatment of COVID-19.

Favipiravir is a modified pyrazine analog, which acts as a prodrug and undergoes ribosylation and phosphorylation in cells by host enzymes to become active ribofuranosyl triphosphate derivative favipiravir-RTP (Delang et al., 2018; Du and Chen, 2020; Furuta et al., 2017). Favipiravir-RTP can bind to and inhibit the viral RdRp activity, and thus prevent viral transcription and replication (Chen et al., 2020a; Delang et al., 2018; Furuta et al., 2017). Further studies showed that favipiravir-RTP incorporates into a nascent RNA strand, leading to a termination of RNA strand elongation and viral proliferation (Furuta et al., 2017). Favipiravir-RTP also acts as a competitor of purine nucleoside binding to the RdRp (Furuta et al., 2017). These results indicate the compound is a promising antiviral drug targeting a broad range of RNA viruses. Recently, it was reported that favipiravir can inhibit the binding of the E and ORF7a proteins of SARS-CoV-2 to porphyrin, prevent the virus from entering host cells and capture free porphyrin (Liu and Li, 2020). Results from a clinical trial showed favipiravir has a higher clinical recovery rate and a more effectively reduced incidence of fever, cough than ARB for COVID-19 (Chen et al., 2020a). Although favipiravir may cause side effects, such as raised serum uric acid, this effect will soon disappear after drug withdrawal (Chen et al., 2020a). It is worth noting that favipiravir has a teratogenic effect, and therefore cannot be used for pregnant women (Delang et al., 2018; Furuta et al., 2017).

Ribavirin is a well-known nucleoside analogue that can inhibit a broad-spectrum of viruses. Results of molecular docking show that ribavirin can bind to SARS-CoV-2 RdRp with a binding energy of −7.8 kcal/mol, forming 13 hydrogen bonds between the RdRp and ribavirin, resulting in the termination of virus replication (Elfiky, 2020). However, ribavirin monotherapy is ineffective for SARS-CoV-2, but it can cause drug-related side effects including gastrointestinal symptoms, anemia, and liver function changes (Li et al., 2020b; Rios et al., 2020). Due to the combination of ribavirin and IFN is effective in treating MERS, combinations of ribavirin

and IFN, and ribavirin and LPV/r are still recommended by the latest edition of the Guideline issued by the NHC, PRC (Du et al., 2020; PRC, 2020). Hung et al. (2020) evaluated a combination of IFN β-1b, LPV/r, and ribavirin for treating patients with COVID-19, and found that triple antiviral therapy using this combination is safe for mild to moderate COVID-19 patients and is superior to LPV/r alone in relieving symptoms, shortening virus shedding and hospitalization time (Hung et al., 2020). These results suggest that a combination of different drugs is feasible in the treatment of COVID-19.

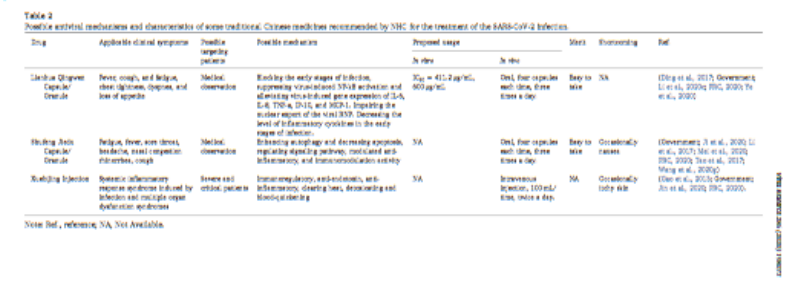
Moreover, other nucleoside analogues, such as IDX-184, Sofosbuvir, and β-D-N4-hydroxycytidine (NHC, EIDD-1931) appear to be promising inhibitors against SARS-CoV-2 (Elfiky, 2020; Sheahan et al., 2020). IDX-184 and Sofosbuvir can bind to the virus RdRp with binding energies of −9.0 and −7.5 kcal/mol, and the numbers of hydrogen bonds are 11 and 7, respectively (Elfiky, 2020). EIDD-1931 produces lethal mutations in the viral genome by increasing the frequency of viral RNA mutations, thus exhibiting a broad-spectrum antiviral activity against SARS-CoV 2, MERS-CoV, SARS-CoV, and Bat-CoVs (Sheahan et al., 2020).

3. Traditional Chinese medicines

Traditional Chinese medicines (TCM) are widely used in China and some Chinese communities outside China, with characteristics of low cost, good curative effect, short duration of treatment, and multiple targets. Numerous facts prove that it is feasible to treat human and animal diseases with TCM, which can effectively relieve symptoms, reduce the development from mild to moderate or severe, improve the cure rate, reduce mortality rate, and promote the recovery of patient by means of “multi-component, multi-target, multi-pathway” (Cantwell, 2010; Huang et al., 2020; Luo et al., 2020; Tong et al., 2020; Yuan et al., 2016).

During the last months, TCMs are widely used in clinical treatments against SARS-CoV-2 infection in China. As a result, TCMs play important roles and have become a highlight of the prevention and control of the COVID-19 (Ang et al., 2020; Ren et al., 2020a; Xu and Zhang, 2020). It was reported that more than 74,187 (91.5 %) of the confirmed cases of the COVID-19 patients in China were treated with TCMs, 61,449 (90.6 %) of which were used in Hubei Province. Clinical observation shows that the total effective rate of TCMs has reached more than 90 % (http://paper.people.com.cn/rmrb, 2020−03-24) (PRC, 2020), indicating the TCMs is effective in the treatment of the COVID-19.

Generally, different therapeutic logic of TCM is used based on the patient's physical condition and different symptoms, as well as the living environmental conditions. During the last six months, numerous prescriptions against COVID-19 were recommended by NHC based on the different symptoms and different patients (Huang et al., 2020; Liu et al., 2020c; Tong et al., 2020). For details dosage, please refer to the latest edition of the guideline issued by the NHC of China (Jin et al., 2020; PRC, 2020; Xu and Zhang, 2020). Among these TCMs, the "three drugs, three recipes" with obvious therapeutic effects, such as Jinhua Qinggan Granules, Lianhua Qingwen Capsules, Xuebijing Injection, Qingfei Paidu Decoction, Huashi Baidu Recipe, and Xuanfei Baidu Recipe, have been screened out and highly recommended to treat SARS-CoV-2 infection (http://paper.people.com.cn/rmrb) (Table 2) (PRC, 2020). For details, please read the review articles by Tong et al. (Tong et al., 2020) and Huang et al. (Huang et al., 2020).



3.1. For medical observation

For medical observation of COVID-19, several prescriptions can be used clinically, including Huoxiang Zhengqi capsules (pills, liquid, or oral solution), Jinhua Qinggan granules/capsules, Lianhua Qingwen capsules/granules, Shufeng Jiedu capsules/granules, Fangfeng Tongsheng pills/granules, and Yupingfeng San (Jin et al., 2020; Liu et al., 2020e; PRC, 2020; Xu and Zhang, 2020).

Huoxiang Zhengqi capsules (pills, liquid, or oral solution, HXZQ) consists of 10 Chinese herbs, with 778–2347 chemical ingredients (Jiang et al., 2020). Among these ingredients, more than 13 ingredients, including protocatechuic acid, chlorogenic acid, caffeic acid, liquiritin, hesperidin, apigetrin, rosmarinic acid, oxypeucedanin hydrate, byakangelicin, apigenin, glycyrrhizin, nobiletin, and 6-gingerol, are recognized as critical chemical marker compounds (Kim et al., 2014). HXZQ is widely used to dissipate cold and eliminate dampness targeting on the clinical features of fatigue and gastrointestinal discomfort (Jin et al., 2020; PRC, 2020). Moreover, this prescription is also used in the treatment of clinical symptoms as hypodynamia accompanied by gastrointestinal upset, as well as the exterior syndrome of cold-dampness (Jin et al., 2020; PRC, 2020). It was reported that HXZQ can regulate the immune response of $CD4^+$ and $CD8^+$ cells and suppress the levels of TNF-α in the intestine (He et al., 2006; Tong et al., 2020). Results of molecular docking showed that PTGS2, HSP90AB1, CAMSAP2, mPGES-1, LTA4H, NOS2 are possible targets of HXZQ in COVID-19 (Huang et al., 2020; Tong et al., 2020). Therefore, HXZQ has anti-inflammatory and immunomodulatory effects in COVID-19 by inhibiting inflammatory factors and regulating immune response (Huang et al., 2020; Tong et al., 2020).

Lianhua Qingwen capsules/granules, Shufeng Jiedu capsules/granules, Fangfeng Tongsheng pills/granules, Jinhua Qinggan granules/capsules, and Yupingfeng San are commonly used to against influenza virus infection (Jin et al., 2020; PRC, 2020; Xu and Zhang, 2020). For example, Lianhua Qingwen capsules/granules (LHQW) composed of 13 herbs, with 733–3084 chemical ingredients (Jiang et al., 2020), has broad-spectrum activities against influenza viruses (IAV) (Ding et al., 2017) as well as other respiratory diseases, such as chronic rhinosinusitis (Lin et al., 2020a), chronic obstructive pulmonary disease (Dong et al., 2014), etc. LHQW can block the early stage of virus infection, impair the nuclear export of the viral RNP, and modulate immune responses during virus infection (Ding et al., 2017). LHQW also can inhibit the replication of SARS - CoV-2, affect virus morphology, improve human immunity, and decrease inflammatory response or even cytokine storm (Hu et al., 2020; Li et al., 2020c; Tong et al., 2020; Ye et al., 2020). Meanwhile, three components of LHQW, Rutin, Forsythoside E, and Hyperoside, can bind the protease of SARS-CoV-2, with the binding energy of −9.1, −9.0 and −8.7 kcal/mol, respectively, forming hydrogen bonds and hydrophobic interactions between the active components and the viral protease (Ye et al., 2020). Moreover, chemical components of LHQW may also target cellular JAK-STAT, EGFR, ACE2, MAPK, PI3K-AKT, and NF-κB, etc (Huang et al., 2020; Tong et al., 2020). These results demonstrate that LHQW seems an effective TCM against SARS-CoV-2 infection.

Shufeng Jiedu Capsule/Granule (SFJD), consisting of eight medicinal herbs, is a widely used TCM for its antiviral, antibacterial, antitumor, and anti-inflammatory activities (Mei et al., 2020). It was reported that SFJD may protect lung injury and neuronal loss by enhancing autophagy and reducing apoptosis in rats with allergic rhinitis (Mei et al., 2020). It improves upper respiratory tract infection induced by *Pseudomonas aeruginosa* through various targets, especially ERK phosphorylation (Li et al., 2017). SFJD effectively regulates anti-inflammatory and immunoregulatory activities during acute lung injury through AKT1 regulation (Tao et al., 2017). Besides, SFJD combined with oseltamivir treatment significantly reduced IAV-induced airway inflammation and pulmonary virus titer (Ji et al., 2020). These results suggest SFJD may prevent and cure diseases, especially infectious diseases, by regulating various signal pathways.

3.2. For mild infection

For mild infection of SARS-CoV-2, Qingfei Paidu Decoction (QFPD), Sangju yin, and Yinqiao san were suggested to use in clinical (Jin et al., 2020; PRC, 2020; Xu and Zhang, 2020). Additionally, QFPD, Huashi Baidu Recipe, and Xuanfei Baidu Recipe are three new prescriptions specifically designed for the COVID-19, which exhibit active and effective roles in the prevention and treatment of the disease.

QFPD has more than 300 active ingredients and can act on more than 790 targets. QFPD might inhibit the invasion and replication of SARS-CoV-2 by directly targeting the viral 3C protein and host ACE2 (Huang et al., 2020). Recent studies showed that QFPD can improve several pathways in patients with COVID-19, such as response to oxygen levels, response to oxidative stress, and blood circulation (Wang et al., 2020e). Moreover, QFPD also can inhibit arachidonic acid metabolic pathway, and thereby regulating inflammatory cytokines (Ren et al., 2020c), suggesting this decoction has a positive effect on COVID-19 treatment. However, the exact biological mechanisms and side effects of these prescriptions need to be further evaluated.

Moreover, Xuanfei Baidu Recipe (XFBD) consists of 13 herbs, which acts on lung meridian by balancing immunity, eliminating inflammation, regulating hepatic and biliary metabolism, and recovering energy metabolism balance (Wang et al., 2020f).

3.3. For severe and critical infection

For severe and critical infection of SARS-CoV-2, Xuebijing Injection, Maxing Shigan tang, and Baihe Gujin tang can be used in clinically (Jin et al., 2020; PRC, 2020; Xu and Zhang, 2020). Xuebijing Injection derived from 5 herbs is suitable for systemic inflammatory response syndrome induced by infection and multiple organ dysfunction syndromes (Government, 2020; Jin et al., 2020; PRC, 2020; Tong et al., 2020). It was reported that the injection has the potential immunoregulatory ability, antibacterial, anti-endotoxin effects (Gao et al., 2013), as well as an anti-inflammatory effect by inhibiting the release of serum pro-inflammatory cytokines (Shen et al., 2013). Therefore, for severe and critical patients of the COVID-19, Xuebijing Injection is recommended for clearing heat, detoxicating, and blood-quickening (Jin et al., 2020; PRC, 2020).

4. Others

Interferon and glucocorticoid are also used in the treatment of the COVID-19. Interferons are well-known agents with broad-spectrum antiviral activities. Recent studies have shown that SARS-CoV-2 is sensitive to type I interferon pretreatment *in vitro* (Lokugamage et al., 2020). During the SARS-CoV-2 infection, phosphorylation of signal transducers and activators of transcription 1 (STAT1) and levels of IFN stimulated gene (ISG) proteins were significantly induced in the IFN-pretreated cells compared with that of the control cells and the cells infected with SARS-CoV but without IFN pretreatment (Lokugamage et al., 2020). Therefore, as recommended by NHC, China, IFN-α is used by atomization inhalation, with 5 million U or equivalent dose each time, 2 times/day for no more than 10 days (Dong et al., 2020; PRC, 2020). Besides, IFN combined with other antiviral compounds is also suggested for the treatment of the COVID-19, such as LPV/r combined with IFN-β, ribavirin plus IFN (Jin et al., 2020; PRC, 2020).

Additionally, glucocorticoids can exhibit anti-inflammatory activity by inhibiting gene transcription or immune response. It was reported that corticosteroid dexamethasone may reduce the mortality of severe COVID-19 patients by about one-third via limiting pro-inflammatory cytokines (Ledford, 2020; Theoharides and Conti, 2020). However, glucocorticoid-induced side effects are complex and frequent, such as inhibiting the protective function of T cells and B cells, blocking the clearance function of macrophages, etc. (Schacke et al., 2002; Theoharides and Conti, 2020). Therefore, glucocorticoid is suggested for a short period with 1–2 mg/kg/d for 3–5 days (Du et al., 2020; Jin et al., 2020; PRC, 2020). Notably, the side effects caused by interferon and glucocorticoid cannot be ignored. Therefore, only appropriate use of interferon and glucocorticoids are recommended, which can significantly improve the clinical symptoms of patients with SARS-CoV-2, reduce disease progression, and accelerate the absorption of lung lesions (Du et al., 2020; Jin et al., 2020; Li et al., 2020b; PRC, 2020).

5. Conclusion and perspective

In conclusion, SARS-CoV-2 is a highly infectious and pathogenic virus. It can be transmitted through contact and air, and may also be transmitted via mother-to-fetus and fecal-oral transmission routes. Although a variety of candidate medicines have been applied in clinical trials, the efficacy of these drugs still needs further research, especially in double-blinded, randomized, placebo-controlled trials.

It is worth noting that SARS-CoV-2 is an RNA virus that is easy to mutate, and some variations in the population were reported in recent studies, which may substantially affect its pathogenicity (Cao et al., 2020b; Wang et al., 2020a; Yao et al., 2020a; Zhao et al., 2020). Drugs against the viral proteins may be ineffective as viral genes continue to mutate, while drugs targeting cellular proteins may have better application prospects. Therefore, it is still one of the most important tasks to screen efficient and safe drugs according to the conserved sequences of the virus or the characteristics of host proteins. Besides, since candidate drugs and therapies are still being screened or in clinical trials, the combination of traditional Chinese medicine and other antiviral drugs as the first choice for the treatment of the disease can achieve better therapeutic effects. For critical patients, reasonable use of drugs should be combined with the actual situation of patients. Moreover, the adverse effects of each drug or therapy need further evaluation and follow-up.

CRediT authorship contribution statement

Chang Li: Writing - original draft, Funding acquisition. **Lin Wang:** Supervision, Funding acquisition, Writing - review & editing. **Linzhu Ren:** Conceptualization, Writing - original draft, Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The author declares no conflict of interest. All authors have approved the final version of the manuscript.

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Report from the Front Line in Wuhan by liu lihong translated by heiner fruehauf at Hankou Hospital No. 8.

On the evening of February 21, carrying the mandate of my teachers Lu Chonghan, Yang Haiying and Yang Zhenhai as well as the expectations of the Tongyou Sanhe community of Chinese medicine practitioners, I arrived in Hankou together with Dr. Lei Ming. The 3rd member of our coronavirus treatment team, Dr. Zhao Jiangbin, arrived the next day.

During the first three days, we underwent intensive training about hospital rules and protective gear In the afternoon of February 24, we entered into the clinical section of the hospital. The inviting unit, the Hemorrhoid Department No. 3 at Hankou Hospital No. 8, had so far received 20+ patients in all stages of COVID-19 pneumonia. Some of them had been there for almost 2 months, while on the shorter end some had been in inpatient care for about 3 weeks. Quite a few of them never tested positive for the virus, but all CT images revealed typical signs of coronavirus-based pneumonia.

Altogether, we have so far diagnosed and treated more than 10 patients with obvious signs of discomfort. The others either didn't exhibit any symptoms, or they did not wish to be treated with Chinese medicine modalities. According to our own observations and those of other practitioners reporting from the front lines of the outbreak, it has become clear that the initial stage of the infection is not at all characterized by typical pneumonia symptoms such as fever and coughing. Many coronavirus patients cough only little or not at all, while their X-ray images show clear evidence of pathological changes due to COVID-19 pneumonia.

• Dampness (shi): Almost everyone agrees that dampness is at the core of this disease. All of the cases we have encountered so far display a thick, white, sticky tongue coating. Since our arrival in Wuhan, every one of us has observed an increase in sticky coating on our own tongues, as well as the onset of incomplete bowel movements.

• Pulse (mai): The biggest common denominator among patients has been the fact that virtually everyone exhibits a slippery pulse in the cun position of the right hand. This phenomenon signifies that turbid damp obstructing the Lung is the main characteristic of this epidemic.

• Absence of Phlegm (wutan) or Low Phlegm Production (shaotan): Typical symptomology includes either a dry cough or no cough. Because on one hand the normal way of phlegm expulsion by coughing is missing and on the other turbid damp pathogens are obstructing the middle burner, the resultant blockage of normal transformative pathways causes turbid phlegm to congeal into a rubbery and glue-like material that severely

interferes with proper airway function and has no way out. This is the most important reason for the lingering "stalemate" quality of the disease, as well as the tendency to take a sudden turn for the worse.

• Complexity Syndrome (hebing) and Dual Affliction (lianggan): According to my own observations, from the very beginning of the epidemic all the way until now at the front line, as well as the opinions shared by my Chinese medicine colleagues working in Wuhan, the etiology of COVID-19 pneumonia is very much a manifestation of Zhang Zhongjing's classical theory of syndrome complexity and dual pathogenesis. In other words, one can confidently say that this particular epidemic from beginning to end bears the characteristics of what is called Complexity Syndrome and Dual Affliction in the Shanghan zabing lun (Treatise on Disorders Caused by Cold and Miscellaneous Disorders). None of the cases we encountered manifested with simple Taiyang Syndrome in the initial stages of the disease, but they all came down right away with Taiyang Yangming Combination Syndrome, or even a situation where all three yang channel systems (taiyang, yangming, shaoyang) were involved. Many patients exhibit signs of dual pathogenesis right from the get-go, by coming down with a rapidly progressing respiratory infection with signs of both taiyang and shaoyang disease. In some instances, the disease lingers at the yangming taiyin dual affliction stage, while others suffer from taiyang shaoyang dual affliction that is further complicated by yangming taiyin issues. Comparatively speaking, shaoyang jueyin dual afflictions are rare. As for the Complexity Syndrome (hebing) variant, which specifically refers to a situation where both the surface and the interior and zang and fu organ systems are involved at the same time, an example would be the simultaneous affliction of the Lung and the Large Intestine. An appropriate prescription needs to take all of these aspects into consideration. Many of the recently publicized anti-COVID-19 formulas display this characteristic of complexity. The Cinnamon Method (Guizhi Fa) approach suggested by my Fire Spirit School mentor Dr. Lu Chonghan, furthermore, represents a typical approach to Taiyang Yangming Complexity Syndrome. Other suggestions, such as Ma Xing Shi Gan Tang (Ephedra, Apricot Seed, Gypsum, and Licorice Decoction) and similar remedies, or Mahuang Tang (Ephedra Decoction) plus Weijing Tang (Phragmites Decoction) are all examples for approaches to more complex syndromes. For dual affliction (lianggan) conditions, moreover, the Aconite Method (Sini Fa) of the Fire Spirit School is also an important method to consider. Especially in situations where "dampness is pronounced and yang is feeble" (shisheng yangwei) the inclusion of aconite containing formulas is particularly appropriate. Aconite, of course, should always be used cautiously—proper differential diagnostics is always the most important prerequisite for any prescription!

• Moisten Dryness and Transform Phlegm, Remove Zang Disease via the Corresponding Fu Organ How is it possible to liberate the airways by expelling the sticky, rubbery, glue-like phlegm that is obstructing the lungs all the way into the alveoli? This question is most relevant for the eventual outcome of the disease process! Why is the typical COVID-19 patient hardly coughing or not coughing at all? I believe that this specific characteristic of the disease is mostly due to the presence of sticky phlegm, which occupies the available airway space. normally required for the generation of a productive cough. From the perspective of Chinese medicine, this phenomenon belongs to the category of dry phlegm (zaotan). This kind of issue requires an approach that involves moisturizing dryness and transforming phlegm (runzao huatan). The herb Dongguaren (Benincasa), for instance, from the afore-mentioned remedy Weijing Tang is a representative herb in this category. Many other seeds possess this type of therapeutic function, i.e. Gualouren (Trichosanthes seed), Laifuzi (Radish seed), Baijiezi (Mustard seed), etc. However, this type of super-sticky phlegm cannot necessarily be completely expelled via the prescription of moisturizing and phlegm transforming herbs. This is where the maneuver of utilizing the zang-fu relationship of Lung and Large Intestine comes into play, by removing zang disease by way of the associated fu organ; by addressing yin disease via its yang counterpart. This approach has often been used within the versatile arsenal of Chinese medicine modalities. Examples can be found in historical case studies. Specifically, this means that the seed varietals of phlegm transforming herbs, if used in sufficient amounts, can expel residual glue-phlegm from the Lung via the Large Intestine. In the arena of acupuncture, the corresponding method would be to needle Taiyuan (LU9) all the way connecting to Yangxi (LI5), or Yangxi all the way to Taiyuan.

II. The Importance of Acupuncture Therapy

Our team began treating every single patient with acupuncture starting on our first day in the coronavirus section. Because of the logistics associated with buying and preparing herbs, our patients started imbibing herbal decoctions only 3 days later. I remember how I felt slightly awkward when approaching my first patient with a needle. For one, the protective gear with its plastic eye goggles blurs the vision. Secondly, three layers of gloves greatly dull sensitivity in the needling hand. And thirdly, I was worried that the patient would trust me, a doctor who had never treated this kind of disease before. I was therefore completely taken by surprise when the patient exclaimed: "This works like a miracle! The stuffy feeling in my chest is completely gone!" And another patient said shortly thereafter: "My throat and chest area used to feel as blocked as a road during rush hour—now it has become like an open road without a single soul on it."

This sort of feedback was a pleasant and unexpected surprise for us, providing us with hope and strength at the same time. Most of the accompanying symptoms, such as stuffiness in the chest, shortness of breath, abdominal discomfort, itchy throat with the urge to cough, dizziness, cold sensation in the upper back, connective tissue pain, sweating, etc, did either decrease with the acupuncture treatments or resolve entirely.

This experience proved that my earlier suggestion to "use acupuncture and herbal medicine together" in the treatment of this epidemic was realistic. Perhaps the greatest benefit of acupuncture is the immediate improvement in the emotional outlook of patients, since they get to experience a noticeable improvement in a short period of time. This aspect cannot be underestimated in the process of curing this disease. Overall, however, the term "cure" needs to be used with extreme caution in the context of this pneumonia epidemic. This is definitely not the kind of situation where one is all better once the fever has broken, or one is OK once the coughing spells come to an end, or when the virus test turns from positive to negative. There are at least 3 additional elements that are prerequisites for a complete cure of COVID-19 related issues: 1) The complete remission of pneumonia signs on CT images; 2) The normalization of all Lung channel abnormalities; 3) The disappearance of sticky tongue coating. Otherwise, the disease may come roaring back for another round!

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Roles and challenges of traditional Chinese medicine in COVID-19 in Hong Kong To the Editor—To date, there are no reported outbreaks of coronavirus disease 2019 (COVID-19) among traditional Chinese medicine (TCM) practitioners and their patients. Traditional Chinese medicine is popular globally, especially in Asian populations such as in Hong Kong. The concept of integrative medicine is appreciated by members of the public.1,2 Patients who do not want to be treated by Western medicine often seek TCM herbal remedies instead. Practitioners of TCM are confronted with infection control issues when they treat patients with mild and vague symptoms. Some TCM practitioners wear personal protective equipment, including mask and gown, to protect themselves during consultations. However, several routine TCM manoeuvres are high-risk. In TCM, the tongue is considered to have many relationships and connections in the body, both to the meridians and the internal organs. It is therefore considered essential and important to inspect the tongue for confirming TCM diagnoses. Pulse diagnosis also provides TCM practitioners with information about the health of their patients. In terms of treatment, many TCM procedures such as acupuncture, cupping, and moxibustion are considered high-risk. Various issues are encountered by TCM practitioners (Table3-5). There are currently over 10 000 TCM practitioners in Hong Kong, compared with 14 600 doctors of Western medicine. These TCM practitioners have an important role to contribute in Hong Kong Med J 2020;26:268–9 https://doi.org/10.12809/hkmj208564 sharing the health burden in the current COVID-19 pandemic, at least in diagnosing and treating mild cases. The role of TCM is now well established and the dispensation, storage, and labelling of Chinese herbal medicines has been regulated since 2003. In addition, TCM practitioners are regulated and there are plans for a Chinese Medicine Hospital in Tseung Kwan O.6 Although there is ongoing

research into TCM treatment of COVID-19, the role of the discipline is limited and needs deliberation and recognition.3-5 In mainland China, the treatment protocol for diagnosis and treatment for novel coronavirus pneumonia has confirmed the integrative role of TCM in the management of COVID-19.7 Treatment is offered based on stages of disease, namely, prediagnosis, confirmed (mild, moderate, severe, and critical), and rehabilitation.7 As with many treatment strategies worldwide, trials are ongoing and there has been no current evidence to support or refute many of the novel treatments, neither in Western nor TCM. The current policy of the Hong Kong SAR government is that all cases are centralised and managed in the public Hospital Authority system, exclusive of private sector or TCM partners. It is recommended that the Hong Kong SAR government may follow the policy in mainland China to provide TCM as a complementary treatment for in-patients with milder disease as part of the healthcare team responding to COVID-19. In addition, TCM can be offered to patients in the pre-diagnosis and rehabilitation periods for health promotion. There is nothing to lose when patients and citizens see that holistic or integrative medicine is provided by the public system. When further evidence of efficacy is established, TCM can be promoted in the other TCM clinics to serve the public. The TCM practitioners in Hong Kong have important roles in treating patients with suspected COVID-19 in the community. Author contributions All authors contributed to the concept of the study, acquisition and analysis of the data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

TABLE. Issues faced by Chinese medicine practitioners in COVID-19 pandemic in Hong Kong

	Issues	Remarks
Clinical	• High-risk manoeuvres of tongue and pulse diagnosis • Common respiratory symptoms of allergic rhinitis and asthma may be difficult to delineate with URTI due to COVID-19 • Documentation and definition of fever • Training for PPE usage	• Wear surgical mask + face shield • For suspected cases, wear surgical mask + face shield + PPE • Observe hand hygiene during pulse diagnosis • Routine body temperature measurement before clinic entry • Clinic setting restructuring
Treatment	• High-risk procedures such as acupuncture, massage, cupping, and moxibustion	• TCM practitioners to wear surgical mask ± PPE • Request patients to wear surgical masks during the whole consultation as well as the treatment
Reporting	• Confusion as to definitions of exposure, close contact, infection, carrier, disease state (asymptomatic vs acute respiratory symptoms vs coronavirus disease) for reporting	• Train and consolidate referral and contact tracing mechanisms for TCM practitioners
Research	• Symptomatic treatment but not evidence-based	• Review existing research of usefulness of TCM[3-5] • Encourage future research

Abbreviations: COVID-19 = coronavirus disease 2019; PPE = personal protective equipment; TCM = traditional Chinese medicine; URTI = upper respiratory tract infection

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Traditional Chinese medicine is a resource for drug discovery against 2019 novel coronavirus (SARS-CoV-2)

Novel coronavirus pneumonia, named as COVID-19 by the World Health Organization, has spread widely since December 2019 [1–3], with more than 40,000 confirmed cases in China and exportations to over 20 countries [4]. On January 30, 2020, the World Health Orga- nization declared the epidemic to be a public health emergency of international concern in the second meeting of the Emergency Com- mittee [5]. It was recommended that potential vaccines and antiviral medicines should be developed. However, the development of these therapeutics will take months, even years. For this specific indica- tion, rapid performance of traditional Chinese medicine (TCM) can contribute as an alternative measure.

In 2003, patients with severe acute respiratory syndrome (SARS) who were treated with TCM benefited from shorter hospitalization, decrease in steroid-related side effects, and improvement of symp- toms [6]. Notably, genomic and in silico structural characterization of novel coronavirus revealed that it is closely related to the SARS coronavirus, further suggesting that TCM may have potential use in the current outbreak [7]. Indeed, the China government is advising doctors to consider combining Western antiviral drugs with TCM remedies in combating novel coronavirus pneumonia. However, there were few studies to help select suitable herbal drugs before costly biological experiments and clinical trials.

Classically, whether a TCM remedy can be clinically used for viral infections depends on two aspects: 1) clinical symptoms and signs of the patient, and 2) the type of TCM remedy and its traditional indi- cations. TCM formulas have been used in China over 2000 years. According to their effectiveness, TCM remedies are divided into var- ious types, each corresponding to a group of diseases. On the other hand, research has shown that many TCM remedies have antiviral ingredients. Selecting specific TCM formula through integrative methods based on both disease symptom and pathogen-directed cause will greatly increase the clinical potential. However, it is still a challenge to experimentally screen many TCM remedies for the treatment of novel coronavirus pneumonia in a short time.

In this issue of the Journal ofIntegrative Medicine, Zhang et al. [8] provided in silico methods to narrow down TCM remedies that may directly inhibit the coronaviral reproduction. Two principals for selection were proposed: oral effectiveness to inhibit viral infec- tion and compatibility of patient manifestation. The identified TCM remedies should contain anti-novel coronavirus chemicals that meet the requirement for orally administered medical drugs. Meanwhile, the identified TCM remedies should be of the types of TCM remedy that have activity against virus-caused pneumonia. To this end, the authors conducted a series of in silico analyses. A number of natural compounds were selected, which were experi- mentally validated for their potential activity against SARS or Mid- dle East respiratory syndrome coronavirus. These chemicals were then evaluated for their suitability for oral administration. Most importantly, the molecular structures of these natural compounds were evaluated for their ability to interact, or dock, with the main proteins of the novel

coronavirus. Positive docking suggested their ability to inhibit the novel coronavirus infection. In order to comply with patient manifestation, the authors conducted another three rounds of screening. First, TCM herbs that contained at least two of the above natural compounds were selected from the Traditional Chinese Medicine Systems Pharmacology (TCMSPT) database [9]. These medicinal plants were classified by the types of diseases they are used to treat. Only those belonging to the types that have been classically used to treat viral pneumonia were selected for further studies. Next, comprehensive evaluation of the effectiveness of these TCM herbs was performed. The authors downloaded its documented chemical constituents of each herb and analyzed their cellular protein targets for network pharmacological analysis. All these processes found that at least 26 TCM herbs have potential in vivo anti-novel coronavirus effects and can simultaneously regulate host inflammation responses.

This work highlights the prospect of computer-aided, structure-based TCM drug discovery for the novel coronavirus pneumonia. These approaches helped to narrow down the large libraries of compounds into a subset in a relatively short time with limited resources; they also provided guidance for the future clinical use of TCM formulas. Although the potential is great, at the same time, we need to be fully aware of challenges and limitations faced by these tools. Computational prediction is a bridge between theory and experiment, and further research is needed. Inhibitory assays and crystallography should be performed to confirm the interac- tion of the herbal compounds with viral proteins and structures. It is worth noting that in a recent study to identify potent ebola- virus inhibitors, only two of the eight compounds selected by in silico screening showed inhibitory properties, reflecting the limited reliability of the computational scoring functions [10]. The decoction of the selected 26 TCM herbs should be tested for their effectiveness and safety in both cell cultures and animal models. Eventually, TCM remedies should be evaluated in carefully tal research and clinical use of these remedies, especially in those countries, territories or areas with reported and confirmed cases of COVID-19. Although the difficulties and challenges are fully recognized, we are looking forward to increasing the contribution and benefits from TCM professionals that will provide treatment to many patients with pneumonia caused by 2019 novel coronavirus (2019-nCoV), a new virus also named as SARS-CoV-2 by the Inter- national Committee on Taxonomy of Viruses.

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Abstract

Acupuncture and moxibustion has a wealth of experience in the prevention and control of epidemic disease since ancient times, which was used for all kinds of acute infectious diseases in modern times and its efficacy has been clearly and reliably reported. This article proposes the theoretical feasibility and reliability of acupuncture and moxibustion interventional prevention and treatment by discussing the recognition of coronavirus disease 2019 (COVID-19) from the perspective of acupuncture and moxibustion. The unique "acupuncture and moxibustion program" for COVID-19 is presented including treatment in different stages, selecting acupoints by distinguishing meridians, applying needle technique by various methods. The article also proposes a new understanding of acupuncture and moxibustion at related acupoints on the surface of the body that can directly affect the "*moyuan*" to treat the disease.

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Since December 2019, the SARS-CoV-2 infection has worldwide affected thousands of people and is life-threatening to patients who progress to the severe/critical condition, especially in the elderly. Governments and scientists all over the world are urging for new treatment measures to combat this world epidemic. Ever since the start, most agreements on the measures against COVID-19 rely on early RT-PCR diagnosis, proper quarantine, and vaccination; while treatment strategies rely on the use of multiple anti-viral drugs and cytokine inhibitors. However, when facing of this pandemic, it takes time to develop specific drugs and vaccines. To save life, it is indeed necessary to use the existing experience and knowledge of traditional Chinese medicine (TCM).

Historically, there have been not less documents recorded in the classical TCM literatures about the use of TCM to treat epidemics. In ancient times, there was no Western medicine, while TCM was used on the main historical infectious disease battlefields. Many TCM formulas and products have been widely application in treating infectious and non-infectious diseases. The accumulated clinical experiences and effective prescriptions are believed to be used effectively today. In this urgent period, TCM should be taken into reference as a treatment option because most patients are still suffering. Importantly, there are also academically skilled TCM experts in China who have rich clinical experiences and can properly treat the novel coronavirus pneumonia to achieve curative outcomes.

In this issue of *Chinese Herbal Medicines*, Professor Chang-xiao Liu, the academician of Chinese Academy of Engineering and Editor-in-Chief of this journal, provides his wonderful insights on the outbreak and characteristics of COVID-19 to us, as well as particularly highlights all advantages of the therapeutic TCM principles and formulas for treating this new coronavirus epidemic. His insightful views are able to guide us to use TCM properly to fight this century virus.

Since the outbreak of SARS-CoV-2 infection, Chinese Government initiated a policy that the COVID-19 patients should be treated with integrated Chinese and Western medicine because it is believed that such integrative approach would achieve better objective curative effects than that of using any one mono medicine alone. According to the news reports in China, TCM treatment has been applied in over 90% of COVID-19 patients, and some Chinese herbal formulas showed significant potencies of relieving patients' symptoms, shorting the time of fiver and reducing the cases being conversed from the mild cases into the severe and even the critical ill.

The drug action mechanisms of TCM are basically both similar and different from the conventional medicine. For the similarity, although COVID-19 is a new infectious disease, there have been reports to show that certain chemical components contained in a Chinese herbal formula are actually targeting on the pathological and molecular targets of COVID-19 to produce therapeutic effects, such as 3CL Pro, ACE2 and IL6 etc. On the contrary, the curative effects of Chinese herbal formula sometimes are not necessarily by directly inhibiting or killing the virus, but through the integration of various aspects such as relieving cytokines storm syndrome, protecting human tissues and organs, relieving immunological injury and enhancing the body's ability. With quick development of research programs on this new infection, we believe that solid scientific data would be provided to support widely application of TCM against this epidemic.

For further implantation of TCM treatment on the novel coronavirus pneumonia worldwide, we would suggest that "full coverage" and "full process" should be ensured in treating COVID-19 patients, together with highly quality assurance of TCM intervention throughout the process. Especially, TCM must be prescribed early, and the sooner the better, so as to prevent the disease from progressing to a serious condition. However, for the severe and critical cases, incorporating TCM into conventional treatment methods are also recommended so as to generate synergistic effect by the combinational therapy of Chinese and Western medicine.

Moreover, TCM is able to play very important roles in rehabilitation of COVID-19 patients. Although some patients have reached discharge standard such as negative viral load and relief of observable syndromes, the patients are usually still suffered from the syndrome of *qi* and *yin* deficiency or other clinical symptoms and pathological alterations like pulmonary fibrosis and multiple organ functional destruction after immunological injury, which may not be completely resolved during their hospitalization. All these above manifestations, we may call them as a "Post-COVID-19 Syndrome" which is indeed needed for further treatment after discharge; while TCM is an indispensable method that should be promoted to use in such a stage of COVID-19 patients.

In the near future, we should further perform comprehensive drug screening based on the reported anti-viral molecular targets with our existing TCM chemical database, as well as the existing marketed drugs. Because scientific data of TCM treatment have not yet been solicited enough, more efforts should be made to expand deeply research on TCM for treating COVID-19 patients. And, we hope that we can enforce the contribution of the TCM treasure box together with modern technological investigation to combat the SARS-CoV-2 infection powerfully, together with internationals.

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Abstract

Corona virus disease (COVID-19) has now spread to all parts of the world and almost all countries are battling against it. This study aimed to assess the efficacy and safety of Integrated Traditional Chinese and Western Medicine (Hereinafter referred to as "Integrated Medicine") to COVID-19. We searched six major Chinese and English databases to identify randomized controlled trials (RCTs) and case-control studies (CCSs) of Integrated Medicine on COVID-19. Two reviewers independently screened, identified studies, and extracted data. Cochrane Risk of Bias tool and the Newcastle-Ottawa Scale were used to assess the quality of included RCTs and CCSs, respectively. Stata (version 13.0; StataCorp) was used to perform meta-analyses with the random-effects model. Risk ratio (RR) was used for dichotomous data while the weighted mean difference (WMD) was adopted for continuous variables as effect size, both of which were demonstrated in effect size and 95% confidence intervals (CI). A total of 11 studies were included. Four were RCTs and seven were CCSs. The sample size of including studies ranged from 42 to 200 (total 982). The traditional Chinese medicine included Chinese medicine compound drugs (QingFei TouXie FuZhengFang) and Chinese patent medicine (e.g. Shufeng Jiedu Capsule, Lianhua Qingwen granules). Compared with the control group, the overall response rate [RR = 1.230, 95%CI (1.113, 1.359), *P* = 0.000], cure rate [RR = 1.604, 95%CI (1.181, 2.177), *P* = 0.002], severity illness rate [RR = 0.350, 95%CI (0.154, 0.792), *P* = 0.012], and hospital stay [WMD = -1.991, 95%CI (-3.278, -0.703), *P* = 0.002] of the intervention group were better. In addition, Integrated Medicine can improve the disappearance rate of fever, cough, expectoration, fatigue, chest tightness and anorexia and reduce patients' fever, and fatigue time (*P* < 0.05). This review found that Integrated Medicine had better effects and did not increase adverse drug reactions for COVID-19. More high-quality RCTs are needed in the future.

Abbreviations

COVID-19 Corona Virus Disease 2019

NCP Novel Coronavirus Pneumonia

SARS-CoV-2 Severe Acute Respiratory Syndrome CoronaVirus-2

α-INF alpha-interferon

TCM Traditional Chinese Medicine

WBC White blood cell

CRP C-Reactive Protein

TNF-a Tumor Necrosis Factor-α

RCTs Randomized Controlled Trials

CCSs Case-Control Studies

RoB Risk of Bias

NOS Newcastle-Ottawa Scale

RR Risk Ratio

WMD Weighted Mean Difference

CI Confidence Intervals

1. Introduction

As a member of coronavirus subfamily Coronaviridae, the coronavirus can infect human beings, many kinds of mammals and birds. Some coronavirus can spread between humans, livestock, and poultry. In December 2019, many cases of Novel Coronavirus Pneumonia (NCP) patients have appeared in Wuhan, Hubei, China [1]. The cause is possibly related to contact with a local fish and wild animal market (Huanan Seafood Wholesale Market). WHO named it as Coronavirus Disease 2019 (COVID-19), and the International Classification Committee named the virus as Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) [2], [3]. So far, although transmission in China has been gradually controlled, the rate of infections outside China is rising rapidly, especially in the United States, Italy, and Spain. Till 27 March 2020, about 531,806 cases of COVID-19 and 24,073 deaths have reported [4]. The COVID-19 has posed significant threats to international health. So the effective prevention and treatment of COVID-19 are a very urgent task.

1.1. Western medicine for COVID-19

China has accumulated a lot of experience in prevention, diagnosis, and treatment of COVID-19. So far, China has issued seven versions of COVID-19 clinical guidelines (Trial Version). According to the latest seventh edition, the treatments of COVID-19 still don't have specific medicine [5]. The treatment of COVID-19 involves multiple disciplines, and the current recommendations are mainly based on Western Medicine including supportive care, respiratory assisted ventilation, anti-infection (mainly antiviral agents), and glucocorticoid therapy [5]. Suggested antiviral agents are alpha-interferon (a-INF), lopinavir, ribavirin, chloroquine phosphate, and abidol. At present, there is no evidence to support the

general or routine use of Western Medicine, nor is there any evidence to prove the risks and benefits of Western Medicine for COVID-19.

1.2. Integrated Traditional Chinese and Western Medicine for COVID-19

Traditional Chinese Medicine (TCM) has a history of thousands of years and has saved the Chinese from major infectious diseases on many occasions. Now, TCM has been practiced worldwide. During the SARS epidemic in 2003, TCM played a huge role [6], [7], [8]. COVID-19 belongs to the category of "Pestilence" in TCM. Its main clinical manifestations are fever, fatigue, dry cough, and the disease is situated in the lung and related to the spleen, stomach, and heart. Like the SARS period, TCM played a major role in the "Fight against the Pestilence in China", saving many people's lives [5], [9], [10]. Existing evidence showed that compared with the simple treatment of Western Medicine; Integrated Traditional Chinese and Western Medicine (Hereinafter referred to as "Integrated Medicine") for COVID-19 may have better effects [11], [12], [13], [14], [15]. However, these studies have small sample sizes, and no convincing evidence is available to demonstrate the benefits and risks of Integrated Medicine for COVID-19.
This study summarized controlled trials and methods of Integrated Medicine treatment of COVID-19, including the changes of clinical symptoms. The secondary objective is to investigate the changes of laboratory indicators and the safety of Integrated Medicine of COVID-19.

2. Materials and Methods

This meta-analysis was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [16]. In addition, we also performed this study according to some other methodological study on meta-analysis [17], [18], [19]. The protocol for this study has been registered in the International Prospective Register of Systematic Reviews (PROSPERO, CRD42020177097).

2.1. Literature search

A pre-developed search strategy was used to identify all relevant clinical trials, regardless of languages or types of publication (excluded unpublished trials). We searched the following six databases: PubMed, Embase.com, Cochrane Library, CNKI (China National Knowledge Infrastructure), WanFang and CBM (Chinese Biomedical Database). The search time was limited from December 01, 2019 to March 24, 2020. Search terms included "traditional Chinese medicine", "Western medicine", "Integrated traditional Chinese and Western medicine", "novel coronavirus pneumonia", "2019-nCoV", "COVID-19", "SARS-CoV-2" and "NCP". The search strategy of the PubMed database is presented in Appendix Table 1.

2.2. Inclusion criteria and study selection

Inclusion criteria: (1) Patients: confirm diagnosed COVID-19 patients (Age≥18 years) by laboratory; (2) Intervention: patients in treatment groups were given TCM therapy in addition to the baseline medication similar to the control group (the TCM therapy included Chinese medicine compound drugs, Chinese patent medicine); (3) Comparison: the patients of the control group were given modern Western conventional treatments; (4) Outcomes: a. clinical efficacy (e.g. overall response rate, cure rate, hospital stay), b. clinical symptoms (e.g. fever, cough, expectoration, fatigue, myalgia), c. laboratory indicators (e.g. lymphocyte

percentage, white blood cell (WBC) count, C-reactive protein (CRP), tumor necrosis factor-α (TNF-a)), d. adverse drug reactions (e.g. nausea and vomit, diarrhea, liver damage); (5) Study types: randomized controlled trials (RCTs) and case-control studies (CCSs) were included. This followings were excluded: review, abstract, letter, case reports, case series reports, and animal experiments.

Two reviewers independently screened the title/abstract of each record by the inclusion criteria. For the indistinguishable record of the title/abstract, we retrieved the full text for further assessment. Finally, resolve any disagreements through discussion between two reviewers or consultation with a third reviewer.

2.3. Data extraction and quality assessment

A pre-designed data form was used to extract the relevant information, including the author, journal, study type, study location, study time, interventions, the dose of drugs, and outcomes. Primary outcomes including the clinical efficacy and the changes of clinical symptoms, such as cure rate, total effective rate, nausea disappearance rate, fever disappearance rate, and fatigue disappearance rate. Second outcomes including the changes of laboratory indicators and the safety of Integrated Medicine of COVID-19, such as CRP, TNF-α, WBC count, liver damage and diarrhea. The Risk of Bias (RoB) assessment tool from the Cochrane Handbook was used to assess the methodological quality of RCTs [20], and the Newcastle-Ottawa Scale (NOS) was used to assess the quality of CCSs [21]. Each RCT was assessed at low risk, high risk, or unclear risk relating to the following items: sequence generation, allocation concealment, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias. The NOS assesses the quality of CCSs with eight questions in three broad categories: (1) patient selection; (2) comparability of study groups; (3) assessment of the outcome. The total score is 9, the higher the score, the better the quality of the study. Two reviewers independently completed the data extraction and quality assessment. Any disagreements between reviewers were resolved by discussion or consultation with a third reviewer.

2.4. Statistical analysis

Stata (version 13.0; StataCorp) was used to perform the statistical analysis. Risk ratio (RR) was used for dichotomous data while weighted mean difference (WMD) was adopted for continuous variables as effect size, both of which were demonstrated with effect size and 95% confidence intervals (CI). Considering heterogeneity of drugs used in different trials, we calculated all results based on the random effect model. We assessed statistical heterogeneity in each pairwise comparison with I^2 statistic, and value of < 25%, 25-50%, and > 50% considered as low, moderate, and high level of heterogeneity, respectively [22]. We would perform subgroup analyses and sensitivity analyses to explore sources of heterogeneity if enough data were available. The Egger's test and funnel plots were used to detect the potential publication bias if the number of included trials was larger than ten for an outcome. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Eligible studies

Fig. 1 showed the study selection process. A total of 11 studies were included in our study. All the articles were published by Chinese, among them, four studies were RCTs [12], [23], [24], [25] and seven were CCSs [11], [12], [26], [27], [28], [29], [30].

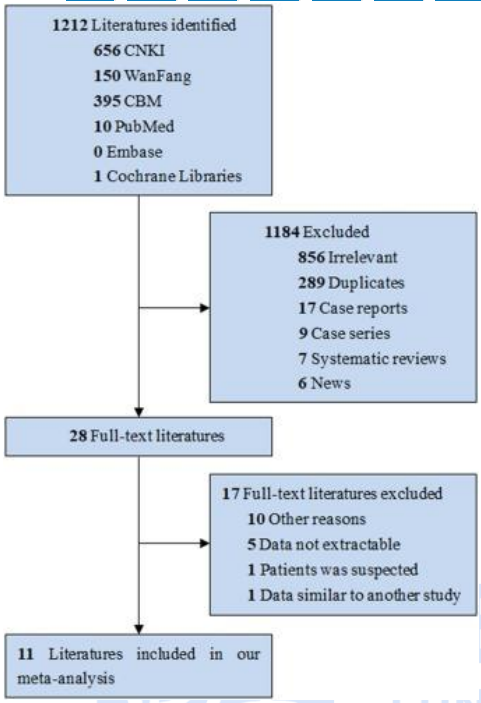

Fig. 1. Flow Diagram of Literature Search and Trial Selection.

The detail of included studies is shown in Table 1. Except for two studies that did not provide the range of study time [23], [24], the study time was from January 01, 2020 to March 02, 2020. The sample size of the included studies ranged from 42 to 200 (total 982). The duration of treatment ranges from 5 to 30 days, with an average of 13.55 days. Two studies did not provide specific Chinese medicine compound drugs and Chinese patent medicine [11], [13]. One studies intervention groups were Chinese medicine compound drugs (QingFei TouXie FuZhengFang) [24]. And the other studies were Chinese patent medicine (such as Shufeng Jiedu Capsule, Lianhua Qingwen granules). The drugs used in the control group were Lopinavir, Ribavirin, Arbidol and et al. In addition, both groups of patients received basic treatment, such as oxygen inhalation and nutritional support.

Table 1. Characteristics of studies included in the meta-analysis.

First author	Type of study	Location of study	Rang of time (2020)	Type of disease	Samples(male)		Treatment		Duration
					Intervention	Control	Intervention	Control	
Zhou WM [23]	RCTs	ChangSha		Light	52(32)	52(28)	Diammonium glycyrrhizinate enteric coated capsules (150 mg,tid) + Lopinavir tablets(500 mg,bid)	Lopinavir tablets(500 mg,bid)	14d

First author	Type of study	Location of study	Rang of time (2020)	Type of disease	Samples(male)		Treatment		Duration
					Intervention	Control	Intervention	Control	
Ding XJ [24]	RCTs	WuHan		Light/Common/Severe/Critical	51(39)	49(39)	Qingfeitouxie fuzhengfang(150 ml,bid) + ifn-α(5 million U,bid) + Ribavirin(0.5 g,bid)	ifn-α(5 million U,bid) + Ribavirin(0.5 g,bid)	10d
Qu XK [26]	CCSs	HaoZhou	01.31~02.11	Light/Common	40(25)	30(16)	Shufeng Jiedu Capsule(2.08 g,tid) + Arbidol(0.2 g,tid)	Arbidol(0.2 g,tid)	10d
Xia WG [11]	CCSs	WuHan	01.15~02.08	Common/Severe/Critical	34(17)	18(6)	Integrated Traditional Chinese and Western Medicine	Western Medicine	23d
Yao KT [27]	CCSs	WuHan	01.11~01.30	Common	21(16)	21(12)	Lianhua Qingwen granules(6 g,tid) + Western Medicine	Western Medicine	19d
Xiao Q [28]	CCSs	WuHan	01.24~01.30	Light/Common	100(64)	100(66)	Shufeng Jiedu Capsule(2.08 g,tid) + Arbidol(0.2 g,tid)	Arbidol(0.2 g,tid)	14d
Cheng DZ [29]	CCSs	WuHan	01.01~01.30	Light/Common	51(26)	51(27)	Lianhua Qingwen granules(6 g,tid) + Western Medicine	Western Medicine	7d
Shi J [13]	CCSs	ShangHai	01.01~02.01	Light/Common/Severe	49(26)	18(10)	Traditional Chinese Medicine	Western Medicine	30d
Yang MB [30]	CCSs	GuangZhou	01.21~03.02	Light/Common	26(16)	23(9)	Reyanning mixture(10-20 ml,bid) + Lopinavir(100 mg,bid) + ifn-α(5 million U,bid) + abidol(0.2 g,tid) + ribavirin(0.5 g,bid)	Lopinavir(100 mg,bid) + ifn-α(5 million U,bid) + abidol(0.2 g,tid) + ribavirin(0.5 g,bid)	7d
Fu XX [12]	RCTs	GuangZhou	01.20~02.23	Common	37(19)	36(19)	Tongjiequwen granule formula(150 ml,bid) + Arbidol(0.2 g,tid)	Arbidol(0.2 g,tid)	10d
Duan C [25]	RCTs	WuHan	02.01~02.05	Light	82(39)	41(23)	Jinhua Qinggan granules(10 g,tid) + Western Medicine	Western Medicine	5d

3.2. Study quality

The quality of the included RCTs is shown in Table 2 [12], [23], [24], [25]. Four RCTs described the adequate random sequence generation process, but only one RCT [25] described the methods used for allocation concealment. Only one RCT [25] described the blinding of participants and personnel and blinding of outcome assessment (High risk), and none described how the incomplete outcome data were processed and reported selective outcome reporting. Overall, the quality of the included RCTs was low.

Table 2. The risk of bias of included Randomized Controlled Trials.

Study	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias

Study	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Fu XX [12]	L	U	U	U	U	U	U
Zhou WM [23]	L	U	U	U	U	U	U
Ding XJ [24]	L	U	U	U	U	U	U
Duan C [25]	L	L	L	H	U	U	U

H: High risk, L: Low risk, U: Unclear risk

Seven CCSs were assessed for quality by the NOS [11], [13], [26], [27], [28], [29], [30]. The maximum quality score is 9 and the range of scores was 3 to 7 (Table 3), with a median of 6 (5.4 ± 1.4). Only one study did not report the case definition [13], and all study reported the definition of controls and comparability of cases and controls [11], [13], [26], [27], [28], [29], [30]. None of the studies reported representativeness of the cases and selection of controls [11], [13], [26], [27], [28], [29], [30]. The reporting for exposure was better, but only one study reported non-response rate [30]. These studies showed a moderate quality.

Table 3. The quality of included Case-Control Studies.

Study	Is the Case Definition Adequate?	Representativeness of the Cases	Selection of Controls	Definition of Controls	Comparability of Cases and Controls	Ascertainment of Exposure	Use the same method to determine case and control exposure factors	Non-Response Rate	Total
Qu XK [26]	★			★	★★	★	★		6
Xia WG [11]	★			★	★★	★	★		6
Yao KT [27]	★			★	★★				4
Xiao Q [28]	★			★	★★	★	★		6
Cheng DZ [29]	★			★	★★	★	★		6
Shi J [13]				★	★★				3

Study	Is the Case Definition Adequate?	Representativeness of the Cases	Selection of Controls	Definition of Controls	Comparability of Cases and Controls	Ascertainment of Exposure	Use the same method to determine case and control exposure factors	Non-Response Rate	Total
Yang MB [30]	★			★	★★	★	★	★	7

3.3. Clinical Efficacy

Four studies demonstrated the overall response rate: Integrated Medicine was better than Western Medicine alone [RR = 1.230, 95%CI (1.113, 1.359), *P* = 0.000] (Fig. 2). Four studies compared the cure rate of the COVID-19 between Integrated Medicine and Western Medicine. The outcome indicated the cure rate in Integrated Medicine was higher than Western Medicine (Fig. 3). The difference was statistically significant [RR = 1.604, 95%CI (1.181, 2.177), *P* = 0.002]. Besides, Integrated Medicine can reduce the severity of illness rate [RR = 0.350, 95%CI (0.154, 0.792), *P* = 0.012] (Fig. 4). And compared with Western Medicine treatment, the Integrated Medicine treatment can shorten the hospital stay [WMD = -1.991, 95%CI (-3.278, -0.703), *P* = 0.002] (Fig. 5).

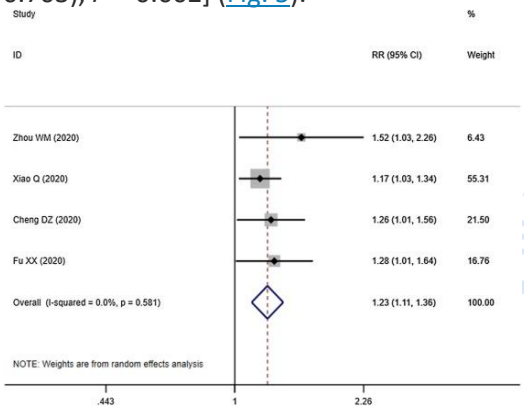

Fig. 2. Overall response rate of the COVID-19 between Integrated Medicine and Western Medicine.

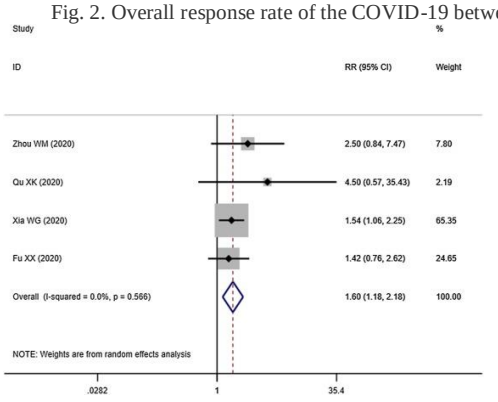

Fig. 3. Cure rate of the COVID-19 between Integrated Medicine and Western Medicine.

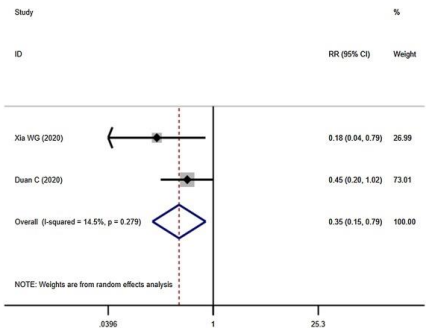

Fig. 4. Severity illness rate of the COVID-19 between Integrated Medicine and Western Medicine.

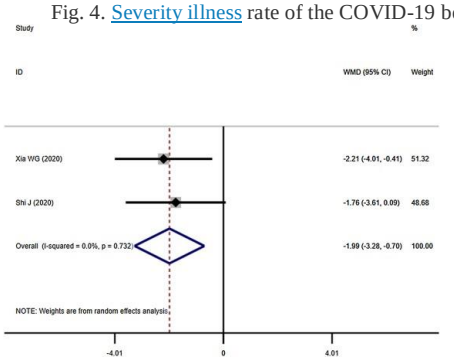

Fig. 5. Hospital stay of the COVID-19 between Integrated Medicine and Western Medicine.

3.4. Symptoms disappearance rate or time

We compared the effects of Integrated Medicine and Western Medicine on the clinical symptoms. The results showed that Integrated Medicine can better improve the symptoms disappearance rate and reduced the symptoms disappearance time than Western Medicine (Table 4). Except for the difference in myalgia and nausea is not statistically significant, Integrated Medicine significantly increased the disappearance rate of fever, cough, expectoration, fatigue, chest tightness and anorexia in patients ($P < 0.05$). In addition, Integrated Medicine can reduce patients' fever, and fatigue time ($P < 0.05$).

Table 4. Comparison of the symptoms disappearance rate or time between integrated Chinese and Western medicine.

Outcome measure	No. of studies	Samples			Statistical method	Effect estimate	*P-value*
		Total	Events/Intervention	Events/Control			
Fever disappearance rate	4	324	157/181	97/143	RR (Random) 95%CI	1.320(1.048,1.663)	0.018
Cough disappearance rate	4	283	113/157	55/126	RR (Random) 95%CI	1.590(1.122,2.253)	0.009
Expectoration disappearance rate	3	126	49/68	16/58	RR (Random) 95%CI	2.549(1.390,4.678)	0.003
Fatigue disappearance rate	3	175	69/101	30/74	RR (Random) 95%CI	1.532(1.137,2.065)	0.005
Myalgia disappearance rate	3	63	24/33	12/30	RR (Random) 95%CI	1.783(0.812,3.913)	0.149
Chest tightness disappearance rate	3	81	25/36	11/45	RR (Random) 95%CI	2.587(1.506,4.444)	0.001

Outcome measure	No. of studies	Samples			Statistical method	Effect estimate	*P-value*
		Total	Events/Intervention	Events/Control			
Nausea disappearance rate	3	38	16/23	9/15	RR (Random) 95%CI	1.132(0.717,1.787)	0.596
Anorexia disappearance rate	2	57	12/19	4/38	RR (Random) 95%CI	5.043(1.116,22.783)	0.035
Diarrhea disappearance rate	3	38	9/22	12/16	RR (Random) 95%CI	0.681(0.199,2.332)	0.541
Fever disappearance time	5	425			WMD (Random) 95%CI	−1.319(-1.842,-0.796)	0.000
Cough disappearance time	3	307			WMD (Random) 95%CI	−0.993(-2.397,-0.532)	0.212
Fatigue disappearance time	3	301			WMD (Random) 95%CI	−1.129(-2.221,-0.037)	0.043
Nasal congestion disappearance time	2	270			WMD (Random) 95%CI	0.033(-0.281,0.348)	0.835
Runny nose disappearance time	2	270			WMD (Random) 95%CI	−0.800(-2.515,0.915)	0.360

3.5. Laboratory indicators

Meta-analyses revealed that the Integrated Medicine was more beneficial to the recovery of laboratory indicators. We found that Integrated Medicine was beneficial for IFN-a [WMD = -3.13, 95%CI (-4.23, -2.04), *P* = 0.000] and lymphocyte percentage [WMD = 1.59, 95%CI (0.61, 2.58), *P* = 0.002] to return to normal. And these differences were statistically significant (*P* < 0.05). Besides, as for the CRP and WBC count, there were no significant differences between Integrated Medicine and Western Medicine (*P* > 0.05) (Table 5).

Table 5. Comparison of the laboratory indicators between integrated Chinese and Western medicine.

Outcome measure	No. of studies	Samples	Statistical method	Effect estimate	*P-value*
CRP	4	326	WMD (Random)95%CI	−1.16(-6.96,4.65)	0.695
TNF-α	2	204	WMD (Random)95%CI	−3.13(-4.23,-2.04)	0.000
Lymphocyte percentage	2	273	WMD (Random)95%CI	1.59(0.61,2.58)	0.002
WBC count	2	273	WMD (Random)95%CI	0.66(-0.03,1.34)	0.060

CRP, C - reactive protein; TNF-a, Tumor Necrosis Factor-α; WBC, White blood cell; CI, Confidence Intervals

3.6. Adverse Drug Reaction

The common adverse drug reactions of Integrated Medicine were nausea and vomiting, diarrhea, liver damage, and reduced blood cell count. As showed in Table 6, there was no significant difference in the adverse drug reactions caused by the two different interventions (*P* > 0.05).

Table 6. Comparison of the adverse drug reaction between integrated Chinese and Western medicine.

Outcome measure	No. of studies	Samples			Statistical method	Effect estimate	*P-value*
		Total	Events/Intervention	Events/Control			
Nausea and vomiting	2	172	5/92	5/80	RR (Random) 95%CI	0.915(0.267,3.138)	0.888
Diarrhea	2	225	32/134	3/91	RR (Random) 95%CI	5.598(0.267,166.774)	0.320
Liver damage	2	202	3/103	12/99	RR (Random) 95%CI	0.281(0.046,1.706)	0.168

RR, Risk Ratio; CI, Confidence Intervals

3.7. Subgroup analysis of the primary outcomes

Results of subgroup analyses of the Chinese medicine compound drugs and Chinese patent medicine for the primary outcome were shown in Appendix Tables 2 and 3. When the Integrated Medicine included *Diammonium glycyrrhizinate enteric coated capsules*, it can improve the cure rate compared with Western Medicine (*P* = 0.036). *Lianhua Qingwen granules* can improv the total effective rate (*P* = 0.037), fever disappearance rate (*P* = 0.003), fatigue disappearance rate (*P* = 0.032), myalgia disappearance rate (*P* = 0.025), expectoration disappearance rate (*P* = 0.004), and chest tightness disappearance rate (*P* = 0.007). In addition, *Lianhua Qingwen granules* shorten the fever (*P* = 0.01), fatigue (*P* = 0.02), and cough (*P* = 0.038) time. *Shufeng Jiedu Capsule* can improvthe total effective rate (*P* = 0.02) and shorten the fever (*P* = 0.003) time. *Tongjiequwen granule formula* can improve the total effective rate (*P* = 0.044). *Jinhua Qinggan granules* can improve fever disappearance rate (*P* = 0.02), cough disappearance rate (*P* = 0.023), and expectoration disappearance rate (*P* = 0.003). *Qingfeitouxie fuzhengfang* can improve cough disappearance rate (*p* = 0.032) and chest tightness disappearance rate (*P* = 0.025).

3.8. Publication bias

Since the number of studies in any comparative analysis did not exceed ten, we did not assess the publication bias.

4. Discussion

Our study systematically evaluated the effect of Integrated Medicine for COVID-19. After a comprehensive search of six databases, we included four RCTs and seven CCTs. The study results showed that the Integrated Medicine had better effects and fewer adverse drug reactions compared with Western Medicine. This study is not the first to find that Integrated Medicine has a greater effect on acute infectious diseases. Similar studies have shown that it has positive effects on lung infiltrate absorption in SARS patients [31]. Facing such a severe epidemic situation in the world, the Western countries should pay attention to the therapeutic effect of TCM. We think it is necessary to hire TCM experts to participate in the treatment of COVID-19 in Western countries.

TCM has served the Chinese people since ancient times and has played an important role in today's medical care. And it especially has a very systematic understanding of the etiology and pathogenesis of acute infectious diseases. And in TCM, the dosage, composition, treatment time, withdrawal and follow-up criteria,

and treatment plan of the compound Chinese herbal medicine can be adjusted according to the situation of the patient. In the included studies, eight different herbal medicine or Chinese patent medicine were used. This means that in terms of treatment, TCM can make more choices to make the best treatment. In addition, TCM was involved in the treatment of COVID-19 with different severity from light to critical [11], [24]. However, the use of these traditional herbs has been controversial due to unclear composition and lack of scientific evidence [32]. In our study, we found that the quality of these studies was low. In the treatment of many diseases, TCM is only used as adjuvant therapy [33], [34]. So, standard treatment and outcome index need to be developed. In this way, the best evidence can be systematically reviewed, summarized and disseminated to better provide evidence-based TCM decision-making.

TCM is superior to western medicine in improving the symptoms and quality of life of patients. This study found that Integrated Medicine can improve the disappearance rate of fever, cough, expectoration, fatigue, chest tightness and anorexia and reduce patients' fever, and fatigue time. This is related to TCMs can affect immune cells and cytokine production associated with immune responses [35]. Immune regulation maintains the homeostasis of the immune system, protects the body from sources of infection or other harmful substances, thereby alleviating the clinical symptoms. It is essential for normal health. However, we found that the outcome indicators were not uniform in the included studies. This situation is dangerous and increases the waste of research resources, may cause some ineffective or adverse interventions to be applied clinically [36]. The diversity of outcome indicators also exists in the laboratory indicators and the adverse reaction indicators. Although we found that the Integrated Medicine may change the inflammation index and have fewer adverse drug reactions than western medicine. But these are not enough, we found that many important indicators cannot be analyzed due to outcome indicators were not uniform. Such as erythrocyte sedimentation rate, each interleukin type, macrophage ratio [13], [24].

As a new kind of respiratory disease, COVID-19 has many unknown factors to be solved. We found that included studies had a short duration, the ranges from 5 to 30 days. COVID-19 is likely to require a longer period of follow-up. In this way, the efficacy and possible adverse drug reaction of COVID-19 can be better observed. Besides, adverse events should be monitored through standardized and effective reporting systems, and some serious adverse events should be observed through epidemiological studies [37], [38].

However, this study also has the following limitations. The TCM and Western Medicine used in the intervention group and the control group is different. But we did not perform subgroup analysis or sensitivity analysis. And many merger statistical analysis studies have more heterogeneity. In addition, most of the included trials had flaws in the methodological design, including randomization, concealment of allocation, and inadequate reports on blinding, withdrawal, and sample size estimates. We also tried to contact the authors who participated in the trial for detailed information; however, we did not get a response at the end. And we did not perform a subgroup analysis according to the severity of the disease in patients withCOVID-19. In COVID-19 patients with different syndromes, the treatment effect may be different.

Above all, COVID-19 is a sudden outbreak disease. There are difficulties for clinicians to conduct RCTs, especially in the acute or critical period. So we included both RCTs and CCTs in this study. Therefore, some high-quality RCTs are needed to evaluate the effect of Integrated Medicine for COVID-19.

5. Conclusion

The study results showed that compared with Western Medicine, the Integrated Medicine for COVID-19 has better effects and did not increase adverse drug reactions. However, due to the low number of included studies, low quality, and inadequate methodologies, high-quality RCTs are needed to evaluate the effect of Integrated Medicine for COVID-19 in future.

Ethical approval

Ethical approval and patient consent are not required since this is an overview based on published studies.

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Conflicts of Interest

The authors declare that they have no competing interests.

Author Contributions

Jinhui Tian, and Junhua Zhang designed this study; Ming Liu and Ya Gao ran the search strategy; Ming Liu and Yuan Yuan collected data, Shuzhen Shi and Ya Gao re-checked data; Ya Gao performed analysis and Jinhui Tian re-checked; Ming Liu and Kelu Yang assess the quality of studies, Shuzhen Shi and Junhua Zhang re-checked; Ming Liu wrote the manuscript, Ya Gao, Jinhui Tian and Junhua Zhang edited. All listed authors reviewed and revised the manuscript.

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Understanding of Guidance for acupuncture and moxibustion interventions on COVID-19 (Second edition) issued by China Association of Acupuncture-Moxibustion 中国针灸学会发布的《新型冠状病毒肺炎针灸干预的指导意见 第二版）》解读 Corresponding author.

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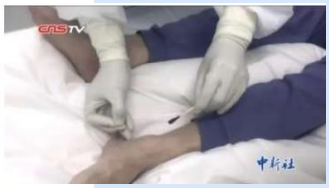
ABSTRACT At present, the situation of global fight against COVID-19 is serious. WHO (World Health Organization)-China Joint Mission fully confirms the success of “China’s model” against COVID-19 in the report. In fact, one particular power in “China’s model” is acupuncture and moxibustion of traditional Chinese medicine. To better apply “non-pharmaceutic measures”—the external technique of traditional Chinese medicine, in the article, the main content of Guidance for acupuncture and moxibustion interventions on COVID-19 (Second edition) issued by China Association of Acupuncture-Moxibution is introduced and the discussion is stressed on the selection of moxibustion device and the duration of its exertion. Keywords COVID-19, Non-pharmaceutic measures, External therapy, Acupuncture, Moxibustion.

Background Novel coronavirus pneumonia was renamed by World Health Organization (WHO) to be “2019 coronavirus disease” (COVID-19) recently. It is the infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and it is a kind of atypical pneumonia. On 1 December 2019, the first case of COVID-19 was confirmed in the city of Wuhan, Hebei province, China and the virus that started the pneumonia outbreak spreads in the country. The major source of infection is the patients with COVID-19 and asymptomatic SARS-CoV-2 carriers seem also a potential source of infection. It is mainly transmitted by respiratory droplets, contact, digestive tract and aerosol transmission. This disease is characterized as highly contagious and is susceptible to humans of all ages. On 8 January, 2020, the first case of COVID-19 was confirmed in Thailand, which is the earliest confirmed case outside China [1]. On 20 January, the first confirmed case was identified in the Republic of Korea [2]. Since then, a number of cases were confirmed in Singapore, Italy, Iran, the United States, Russia, etc.. Thus far, this disease has spreaded globally.

On 31 January 2020, WHO declared this epidemic outbreak a public health emergency of international concern (PHEIC) [3]. On 2 March 2020, Tedros Adhanom Ghebreyesus, the Director-General of WHO, pointed in the opening remarks at the media that outside China, a total of 8739 cases of COVID-19 have been reported to

WHO from 61 countries, with 127 deaths [4]. The epidemics in the Republic of Korea, Italy, Iran and Japan are of greatest concerns. The fight against COVID-19 gets more serious globally. From 16 to 24, February 2020, 25 international and Chinese experts of the WHO-China Joint Mission traveled to Beijing, Hubei, Guangdong and Sichuan, China to investigate the fight against the epidemic. On the second day after the end of investigation, Dr. Bruce Aylward, the head of the international expert panel of the WHO-China Joint Mission, the senior adviser to the WHO's Director-General, stated at the press briefing at WHO Headquarters in Geneva, that faced with the unknown pathogen, China has taken ambitious, flexible and aggressive efforts in responding to the epidemic. In the report, WHO-China Joint Mission has confirmed that China has played a crucial role in protecting the international society, buying precious time for countries to adopt active prevention and control measures and providing them with worthwhile experiences. The report also points out specifically the high effective role of non-pharmaceutic measures [5]. The report said that China, as the country with the greatest knowledge on COVID-19, should further enhance the systematic and real-time sharing of epidemiologic data, clinical results and experience to inform the global response.

With regard to "non-pharmaceutic measures" proposed in the report of WHO-China Joint Mission, besides active surveillance, timely detection, voluntary quarantine and rigorous tracing, actually, there is still a mysterious power, that is various kinds of external therapeutic approaches of traditional Chinese medicine (TCM). By the time for the authors submitting the manuscript, it is known that many therapeutic methods of TCM, e.g. Chinese herbal decoction, acupuncture, moxibusiton, acupoint plaster, auricular acupuncture and cupping have adopted in the treatment of COVID-19. In the regions where TCM therapeutic methods were highly utilized, the curative rate was increased, the number of severe case decreased and the hospital discharge rate improved remarkably [6]. The utilization of TCM therapeutic interventions block effectively the continuous spreading of COVID-19 in China. Note:



Picture from www.chinanews.com

In application of TCM techniques, e.g. acupuncture and moxibustion in treatment of COVID-19. Main content of Guidance Three sections are included in Guidance, named the principle of acupuncture-moxibustion interventions, the methods of acupuncture-moxibustion interventions, and the self-interventions of acupuncture and moxibustion at home under the instruction of physician. Regarding the principle of acupuncture-moxibustion interventions, Guidance emphasizes: The rigorous quarantine and disinfection are required. No matter for the confirmed cases or the convalescent cases, they can be treated in the same room respectively and every suspected one should be isolated in a single room for treatment. During the clinical treatment stage, acupuncture can be combined with western medications and Chinese herbal decoction to achieve the collaborative effect. For the cases at the recovery stage, the core role of acupuncture-moxibustion should be played in the rehabilitation. It is recommended to set up acupuncture-moxibustion based COVID-19 rehabilitation clinic. In reference to the clinical stage identification of TCM suggested in Diagnosis and treatment plan of corona virus disease 2019 (tentative seventh edition) issued by National Health Commission (NHC) of the PRC and State Administration of Traditional Chinese Medicine (SATCM) of the PRC, three stages are included in the treatment with acupuncture and moxibustion, e.g. medical observation stage, medical treatment stage and recovery stage. The therapeutic regimens of each stage are introduced as follows.

Acupuncture-moxibustion interventions at the medical observation stage (suspected cases)

Objective: To motivate the antipathogenic qi of human body and the functions of lung and spleen and scatter epidemic pathogens so as to strengthen the defensive capacity of internal organs.

Main acupoints: Group 1: Fēngmén (风门BL12), Fèishū (肺俞 BL13), and Píshū (脾俞BL20). Group 2: Hégǔ (合谷 LI4), Qūchí (曲池 LI11), Chǐzé (尺泽 LU5) and Yújì (鱼际LU10). Group 3: Qìhǎi (气海CV6), Zúsānlǐ (足三里 ST36) and Sānyīnjiāo (三阴交 SP6). One or two acupoints are selected from each group in one treatment. Symptomatic acupoints: For fever, dry throat and dry cough, Dàzhuī (大椎GV14),Tiāntū (天突CV22) and Kǒngzuì (孔最 LU6) are added. For nausea, vomiting, loose stool, swollen tongue with sticky coating and soggy pulse, Zhōngwǎn (中脘CV12), Tiānshū (天枢 ST25) and Fēnglóng (丰隆 ST40) are added. For fatigue and anorexia, CV12 and the four points around the umbilicus (1 cun bilateral, directly above and below the center of the umbilicus), BL20 are added. For clear nasal discharge, soreness of the shoulders and the back, pale tongue with white coating and slow pulse, Tiānzhù (天柱BL10), BL12 and GV14 are added.

Acupuncture-moxibustion interventions at the clinical treatment stage (confirmed cases)

Objective: To propel the antipathogenic qi of lung and spleen, protect internal

organs, reduce damage, eliminate the epidemic pathogens, cultivate the earth to generate the metal, block the development of illness, ease the emotions and strengthen the confidence on conquer the pathogens.

Main acupoints: Group 1: LI4, Tàichōng (太冲 LR3), CV22, LU5, LU6, ST36 and SP6 Group 2: Dàzhù (大杼BL11), BL12, BL13, Xīnshū (心俞BL15) and Géshū (膈俞 BL17). Group 3: Zhōngfǔ (中府 LU1), Dànzhōng (膻中 CV17), CV6, Guānyuán (关元CV4) and CV12. For the mild case or the ordinary case, 2 or 3 acupoints are selected from group 1 and group 2 in each treatment. For the severe case, 2 or 3 acupoints are selected from group 3. Symptomatic acupoints: For consistent fever, GV14 and LI11 are added, or bloodletting at Shíxuān (十宣EX-UE11) and Ěrjiān (耳尖HX6). For chest oppression and shortness of breath, Nèiguān (内关 PC6) and Lièquē (列缺 LU7), or Jùquē (巨阙 CV14), Qīmén (期门 LR14) and Zhàohǎi (照海 KI6) are added. For cough with expectoration, LU7, ST40 and Dìngchuǎn (定喘 EX-B1) are added. For diarrhea and loose stool: ST25 and Shàngjùxū (上巨虚 ST37) are added. For cough with yellow and sticky sputum and constipation: CV22, Zhīgōu (支沟TE6), ST25 and ST40 are added. For low fever or feverish sensation and discomforts in the body, or fever absence, nausea, vomiting, loose stool, pale or slightly red tongue with white or white sticky coating: BL13, ST25, Fùjié (腹结 SP14) and PC6 are added.

Acupuncture and moxibustion interventions at the recovery stage Objective: To clear away residual toxins, restore the primary qi, promote the repair of internal organs and recover the functions of lung and spleen. Main acupoints: PC6, ST36, CV12, ST25 and CV6.

(1) Qi deficiency of lung and spleen The main symptoms are shortness of breath, fatigue, anorexia, nausea, vomiting, fullness in the epigastric region, weakness in defecation, loose stool, incomplete bowel movement, pale and swollen tongue with white and sticky coating. For the cases with marked symptoms of lung system, e.g. chest oppression and shortness of breath, CV17, BL13, LU1 are added. For the cases with marked symptoms of spleen and stomach dysfunction, e.g. poor appetite and diarrhea, Shàngwǎn (上脘CV13) and Yīnlíngquán (阴陵泉 SP9) are added. (2) Qi and yin deficiency.

The main symptoms are fatigue, dry mouth, thirst, palpitation, profuse sweating, poor appetite, low fever or fever absence, dry cough with little sputum, dry tongue and lack of moisture, thready or weak pulse of deficiency type. For the cases with marked fatigue and shortness of breath, CV17 and Shénquè (神阙CV8) are added. For the cases with marked dry mouth and thirst, Tàixī (太溪 KI3) and Yángchí (阳池 TE4) are added. For the cases with marked palpitation, BL15 and Juéyīnshū (厥阴俞 BL14) are added. For the cases with profuse sweating, LI4, Fùliū (复溜KI7) and ST36 are added. For the cases with insomnia, Shénmén (神门HT7), Yìntáng (印堂 EX-HN3), Ānmián (安眠EX-HN22) and Yǒngquán (涌泉 KI1) are added. (3) Insufficiency of lung and

spleen, phlegm stagnation and collateral blockage The main symptoms are chest oppression, shortness of breath, dislike to speak, lassitude, sweating on exertion, cough with sputum, difficulty in expectoration, coarse skin, mental fatigue, loss of appetite, etc. BL13, BL20, BL15, BL17, Shènshū (肾俞 BL23), LU1 and CV17 are added. For difficulty in expectoration, ST40 and EX-B1 are added.

Guidance points out specifically that either acupuncture or moxibustion is optioned corresponding to the individual conditions at each stage of COVID-19. Additionally, the combination of these two interventions or the combination with acupoint application, auricular therapy, acupoint injection, scraping therapy, infantile tuina or acupoint massage is adopted accordingly. The even needling technique of acupuncture is used and the needle is retained for 20 to 30 min at each acupoint. Moxibustion is exerted for 10 to 15 min at each acupoint. The treatment is given once daily. The manipulation is implemented in reference to the national standard, GB/T21709 Standardized manipulations of acupuncture and moxibustion and clinical experiences. The third section of Guidance is the most characteristic: the self-interventions of acupuncture and moxibustion at home under the instruction of physician.

Moxibustion therapy: Moxibustion is applied by the patient him/herself at ST36, PC6, LI4, CV6, CV4, SP6, etc., about 10 min at each acupoint.

Acupoint application therapy: The plaster, e.g. moxibustion-thermal plaster or moxibustion-like plaster, is used at ST36, PC6, CV6, CV4, BL13, BL12, BL20, GV14, etc.. Tuina therapy at meridian and acupoints: The different tuina methods are exerted at the acupoints on the lung meridian and the heart meridian, the acupoints located below the knee on the spleen meridian and the acupoints on the stomach meridians, such as finger-pressing method, kneading method, palm pressing method, kneading-pressing method, tapping method or knocking method. Each manipulation is exerted for 15 to 20 min till the patient feels soreness and distention in the local area. Traditional physical exercise: The traditional physical exercise is optional according to the individual recovery conditions, including Yijinjing (Exercise for muscle and tendon strengthening), Taijiquan (Taiji boxing), Baduanjin (Eight-section exercise), Wuqinxi (Five-animal exercise), etc.. Each physical exercise is applied once daily, 15 to 30 min each time. Emotional counseling: The attention is paid to emotional regulation. Auricular points, moxibustion, tuina, herbal diet, herbal tea, medicated bath and music are applicable in combination for physical and mental relaxation, anxiety relief and sleep assistance.

Foot bath and fumigation-washing therapy: the herbs for expelling wind, clearing heat and eliminating pathogen are selected, i.e. Jīngjiè (荆芥 Herba Schizonepetae), Àiyè (艾叶Folium Artemisiae Argyi), Bòhe (薄荷 Herba Menthae), Yúxīngcǎo (鱼腥 草 Herba Houttuyniae), Dàqīngyè (大青叶 Folium Isatidis), Pèilán (佩兰 Herba Eupatorii), Shíchāngpú (石菖蒲 Rhizoma Acori Tatarinowii), Làliǎocǎo (辣蓼草 Polygonum lapathifolium L.), Yùjīn (郁金Radix Curcumae) and Dīngxiāng (丁香 Flos Caryophylli), 15 g for each, as well as Bīngpiàn (冰片 Borneolum Syntheticum) 3g. The decocted Chinese herbal liquid is poured into a foot tub and an appropriate amount of warm water is added. When the water is ready at 38 to 45℃, foot bath is exerted for around 30 min. All of the interventions above are the dominant techniques of health care in TCM. Their utilization fully embodies the idea of “disease prevention” in TCM, meaning, preventing from illness before suffering, preventing from the progress of illness after suffering and preventing from recurrence after cured. They play the crucial role in reducing the incidence of COVID-19 and preventing from its recurrence. Suggestions It is observed that the regimens in Guidance recommended are on the base of the ancient literature research, modern clinical research and experimental research of acupuncture and moxibustion and in reference to a series of achievements obtained in the effect mechanism research of acupuncture and moxibustion in recent years. Firstly, the regimens recommended in Guidance are in agreement to the staging of TCM treatment in Diagnosis and treatment plan of corona virus disease 2019 (tentative seventh edition) issued by NHC and they focus specially on the characteristics of acupuncture-moxibustion therapies. Secondly, the implementation of various therapeutic methods is in compliance with “being convenient, safe and effective”. Thirdly, Guidance determines its efforts for the contribution of acupuncture-moxibustion therapies to each stage of the diseases,

points out the combination of acupuncture with western medication and Chinese herbal decoction, plays the coordination effect of acupuncture and moxibustion and believes the crucial effect of acupuncture and moxiustion at the recovery stage of COVID-19.

COVID-19 is the seriously epidemic disease. TCM and acupuncture-moxibustion have not been adopted as the first option in treatment. Besides the limited understanding in the effectiveness of them, the other key reason is for TCM therapy, especially acupuncture-moxibustion, the physician has to very closely contact with patient during treatment, which highly increases the infectious incidence of medical staffs. Therefore, the protection to medical staffs must be in the top priority when exerting acupuncture, moxibustion, seed-pressure of auricular acupuncture, cupping, scraping, etc.. The acupuncture physicians who had participated in the treatment of COVID-19 responded that it is very inconvenient to operate acupuncture with three-layer protective gloves. In case the gloves are broken, infection may occur. The authors believe that for moxibustion interventions, the mild moxibustion with hand-holding moxa stick is not suggested. The moxibustion device with the function of smoke abatement or smoke discharge should be optioned to avoid the stimulation of moxa smoke to the respiratory tract of patient. But, such mild moxibustion with hand-holding moxa stick can be applicable for the home nursing care. Regarding the effectiveness of moxa smoke, the consensus has not been met yet in academic field. But, in reference to the records of ancient medical works and the nowadays popular method of moxibustion in the folk, moxa smoke is applicable for the prevention of infectious diseases. For example, it is recorded in Zhǒuhòu Bèijífāng 《肘后备急方》 (Emergency Formulas to Keep Up One's Sleeve), written by Hong GE, in the Jin Dynasty(317年一420AD) that smoking with moxa around the patient's bed, one moxa cone on each side of the bed is optimal to prevent from epidemic infection. The medical masters in the later generations had inherited this idea. The same prevention method is also recorded in Tàipíng Shènghuìfāng (《太平圣惠方》 Formulas from Benevolent Sages Compiled during the Taiping Era) and Pǔjìfāng (《普济方》Formulas for Universal Relief). This moxibustion intervention is the earliest-recorded measure of air disinfection in history. The modern research discovers that moxa smoke acts on anti-bacteria, anti-fungus, anti-virus and anti-pathogen [8]. Therefore, on the base of individual tolerance, the appropriate use of moxa smoke in room brings a certain effect of disinfection. In Guidance, the duration of moxibustion at each acupoint is 10 to 15 min. But, in clinical practice, the moxibustion is seldom exerted on aucpoints one by one. Instead, the special device, moxa box or moxa holder is used to cover several acupoints simultaneously in one moxibustion intervention. The duration of treatment is over 30 min generally and it will be even longer if the heat-sensitive moxibustion is exerted. Therefore, the authors believe that the duration of moxibustion intervention should be longer to achieve a better effect if the patient is in a comfortable posture and has strong endurance.

Note: Picture from CCTV Compared

Compared with Guidance of the first edition, the content of Guidance of the second edition is much richer and more practical and instructive. With the development of COVID-19, the people are getting deep understanding of the disease and more and more experiences in treatment will be accumulated gradually. It also reflects that the nature of medical development is the process of constant understanding, rectification and conquering disease. At present, COVID-19 is spreading in many countries of the world. China's experiences in fight against

COVID-19 have been recognized and advocated by WHO. Of them, the application of Chinese herbal medication, acupuncture and moxibustion have their unique characteristics. Undoubtedly, the modern medicine measures give priority to treatment and salvage of COVID-19. But, no matter which medical theoretic system is adopted, faced with the epidemic, every measure should aim to treating disease and saving lives. More weapons available in the fight against the disease do bring more benefits to patients.

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Abstract

Objective: To evaluate the effectiveness and safety of Jinhua Qinggan granules in the treatment of patients with novel coronavirus pneumonia (COVID-19).

Methods: Eighty cases of COVID-19 diagnosed from January 24 to February 17, 2020 in Beijing YouAn Hospital Affiliated to Capital Medical University were retrospectively analyzed. All 80 patients received symptomatic and supportive treatment. Among them, 44 patients took Jinhua Qinggan granules (treatment group) within 24 h of admission, and the remaining 36 patients either did not take Jinhua Qinggan granules or took the granules for less than 2 d (control group). In this study, we compared the duration of viral nucleic acid detection and of pneumonia absorption improvement between the two groups.

Results: Among the 80 cases, 37 were male (46%) and 43 were female (54%) with age ranging from 15 to 86 years, with an average age of 51.19 years. The average duration of viral nucleic acid detection was (7 ± 4) d in the Jinhua Qinggan administration group and (10 ± 4) d for the control group (P = 0.010), following which, nucleic acid tests were negative. Of the two groups, 56.82% in the Jinhua Qinggan treatment group and 27.78% in the control group demonstrated negative nucleic acid tests within 7 d or less. The 7-day viral clearance rate was significantly higher in the Jinhua Qinggan group compared with the control group (P = 0.009). Furthermore, the pneumonia recovery time indicated by chest CT was (8 ± 4) d in the Jinhua Qinggan

group, which was significantly shorter than the control group, at (10 ± 5) d (P = 0.021). No adverse reactions were found in the treatment group after taking this medicine.

Conclusion: In patients with COVID-19, Jinhua Qinggan granules can effectively shorten the duration of nucleic acid detection and promote the absorption of pneumonia inflammatory exudate without obvious adverse reactions.

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Abstract

Background: There is currently no effective treatment against coronavirus disease 2019 (COVID-19). The optimal selection of interventions targeting the virus is unknown. Therefore, evidence from randomized controlled trials (RCTs) to support specific treatment against COVID-19 is urgently needed. The use of Chinese herbal medicines (CHMs) might have a role in the treatment and symptomatic management of patients with COVID-19. It was aimed at providing an overview of the available evidence and ongoing trials concerning the effects of CHMs for the treatment of COVID-19. ***Methods:*** This is a narrative review of relevant studies. Searches were conducted to identify documents published till April 22, 2020. Electronic databases, evidence-based collections, websites of relevant organizations, and trial registries were consulted. ***Results:*** A total of 25 guidelines on the treatment of patients with COVID-19 were identified. Four guidelines provided recommendations on the use of CHMs; these guidelines were developed in China and South Korea and were based on the consensus of experts exclusively. The remaining 21 guidelines provided no guidance on CHMs. No finished RCTs of CHMs for the treatment of patients with COVID-19 was found. According to the evidence evaluated in this review, a Cochrane review of CHMs for severe acute respiratory syndrome and five uncontrolled observational studies of the effects of CHMs in patients with COVID-19, the effects of CHMs for COVID-19 are unknown. A total of 52 ongoing clinical trials of CHM interventions for the treatment of COVID-19 were found. These trials will be carried out mostly in China (*n* = 51). Forty (77%) of the ongoing trials will be randomized, whereas 12 (23%) have an unclear sequence generation procedure. Forty-seven trials (90%) will have a sample size <400 participants. ***Conclusions:*** To the authors' knowledge, only the Chinese and the South Korean guidelines recommend CHMs as a treatment option for patients with COVID-19. These guidelines base their recommendations on the consensus of experts. Clinical guidelines or health authorities from other countries do not provide advice on CHMs. Due to the absence of RCT, there is currently no reliable evidence on the effects of any specific CHM intervention for the treatment of patients with COVID-19. A high number of clinical trials of different herbal products are being currently conducted in China.

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Abstract

Background: Corona Virus Disease 2019 (COVID-19) has caused a worldwide epidemic since its discovery. The outbreak of virus infection has aroused great concern of the World Health Organization (WHO). COVID-19 is highly infectious and has a high infection rate. So far, no specific drug has been found to cure it. China as one of the first countries attacked by epidemic has shown outstanding in fighting against the COVID-19. The contribution of traditional Chinese medicine can not be ignored. As a kind of representative of

traditional Chinese medicine, the Chinese patent medicine injection has significant effect in reducing the clinical symptoms of patients and preventing the deterioration of the disease. However, there is no systematic review of its efficacy and safety. The purpose of this study is to evaluate the efficacy and safety of Chinese patent medicine injection in the treatment of COVID-19.

Methods: All randomized controlled trials of Chinese patent medicine injection for COVID-19 will be included. The following electronic databases will be searched: PubMed, Web of Science, the Cochrane Library, EMBASE, China National Knowledge Infrastructure, Wanfang Database, Chinese Scientific Journal Database, Chinese Biomedical Literature database and some clinical trial registration websites. Two researchers will independently screen titles, abstracts, full texts, and extract data, then assess the bias risk of each study. We will conduct meta-analyses to assess all the available evidence of the efficacy and safety.

Results: Systematic review of current evidence will be provided from the indexes of efficacy and safety.

Conclusion: Evidence regarding the efficacy and safety of Chinese patent medicine injection in the treatment of COVID-19 will be provided to clinicians.PROSPERO registration number: CRD42020182725.

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Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19): an empirical study from Wuhan, Hubei Province, China. Chin Med. 2020 Apr 15;15:34. doi: 10.1186/s13020-020-00317-x. eCollection 2020.

Abstract

Background

A novel coronavirus was identified in December, 2019 in Wuhan, China, and traditional Chinese medicine (TCM) played an active role in combating the novel coronavirus pneumonia (NCP) caused by this fast-spreading virus COVID-19. Thus, we aimed to explore TCM characteristics of clinical efficacy to NCP, as well as to optimize Qingfei Paidu decoction (QFPDD) and the recommended formulas to NCP by National Health Commission (NHC).

Methods

Chinese medical sciences theory and clinical application of TCM were analyzed. A total of 54 NCP patients were observed in a hospital from Wuhan, whose clinical characteristics and utilization of Chinese Medicines (CMs) were described. Paired t test was used to measure the change of patients' hemogram during hospitalization period, indicating the effect of CMs. Multiple linear regression analysis was applied to explore the factors affecting the length of hospital stay. Network pharmacology analysis was applied to figure out the performance of NHC-recommended formulas of five disease stages at levels of compounds, targets and pathways.

Result

The average length of hospital stay was 8.96 days. Patients over 45 stayed 9.79 days in hospital in average, longer than 7.64 days of patients under 45. Comparing the hemograms between admission and discharge of hospital, the number of leukocytes, neutrophil, lymphocyte and platelet increased, while the numbers of erythrocytes, hemoglobin concentration and hematocrit decreased. According to the standard coefficients of regression, the factor affecting the length of stay for the most was CMs in category of invigorating spleen

and removing dampness (ISRD), followed by administrating CMs, male, and cough. Thirty-two CMs were screened after deleting duplication from QFPDD and NHC-recommended formulas. Compound quercetin, luteolin, kaempferol, acacetin etc., were all involved in the treatment of various disease stages on the compound level both in generality and individuality.

Conclusion

TCM has a systemic theoretical understanding on the pathological evolution and a positive clinical efficacy on NCP. The CMs of ISRD improved patients' recovery, suggesting the importance of regulating intestinal function and keeping microenvironmental balance in TCM treatment of NCP. The active compounds from QFPDD and NHC-recommended formulas contribute to recovery of varied disease progresses during TCM treating NCP.

Background

A novel coronavirus named COVID-19 was identified in December 2019 in Wuhan, China, which caused infectious pneumonia and spread rapidly. However, there has been no consensus on the nomenclature of novel coronavirus pneumonia (NCP) from the perspective of traditional Chines Medicine (TCM) so far. Academician Tong Xiaolin suggested that the disease should be named as cold-dampness pestilence (寒湿疫) [1], and academician Wang Qi called the disease pulmonary pestilence (肺瘟) in the Manual for Traditional Chinese Medicine diagnosis and treatment of NCP [2]. In general, there is an agreement on the opinion that NCP belongs to the category of epidemic disease (疫病) in TCM. As the special climate of Wuhan, where the local temperature in last winter was higher than that in previous winter, and the rainfall was more frequent than snowfall, the syndromes of NCP patients often presented the characteristics of dampness pathogen (湿邪) in TCM. Integrating the analysis resulted from Professor Liu Qingquan and Dr. Xiang Qiong [3, 4], we consider that NCP (COVID-19) should be defined as dampness toxin pestilence (湿毒疫). Dampness toxin (湿毒) runs through the comprehensive pathology of NCP. Even in Gansu, a region with dry climate, the researchers found that the characteristics of dampness pathogen from NCP patients were similar to those in Wuhan [5].

Chinese Medicine (CM) has accumulated abundant clinical experiences and effective formulas on the prevention and treatment of epidemic diseases. In Ming dynasty, Wu Youke, a famous Chinese medicine doctor, believed that the pathogen of epidemic disease was different from the six excesses (六淫), but was a kind of pestilent Qi (疠气) that had high contagious and powerful toxic features. Pestilent Qi is prone to encroaching specific organs and involving multiple organs failure, and commonly breaks out in populated large cities. In 2004, a clinical study including 524 patients with severe acute respiratory syndrome (SARS) showed that the duration of major symptoms in the group of patients treated by integrated Chinese and western medicines was significantly shorter than those in the group treated by western medicine alone [6]. The satisfied therapeutic effects of TCM in preventing and treating SARS suggested the superiority of TCM on severe infectious diseases.

In March 2020, the Diagnosis and Treatment Guideline of Novel Coronavirus Pneumonia (Edition 7) was released by the National Health Commission (NHC) of People's Republic of China [7], in which Qingfei Paidu decoction (QFPDD) and other TCM formulas were recommended to treat NCP. Although it is necessary to consider the real pathological evolutions of patients based on local climatic features and individual physical characteristics of patients, the inconsistency of syndrome types (证型) is prone to producing cluttered Chinese medicine formulas. In the view of this, the TCM symptom types in this study complied with those in the guideline from treatment to recovery period.

Therefore, this study aimed to figure out efficacy of TCM in treating NCP, to explore the relationship of the TCM's influence factors with patient's individual characteristics, and to optimize QFPDD and NHC-recommended formulas corresponding to the treatment and recovery period of NCP.

By using both statistics analysis and network pharmacological technology, this study can not only partially reveal the therapeutic mechanisms of TCM through the corresponding relationships among formula, medicine, and syndrome, but also provide scientific evidence for screening and optimizing TCM formulas for the treatment of NCP.

Methods

Study population

Data of 54 patients with NCP, namely SARS-CoV-2 pneumonia (COVID-19) undergoing CMs treatment originated from the department of infectious disease in Jihe Hospital from Wuhan during January 24 to February 17. The information about patients' age, gender, symptoms, temperature, use of TCMs and results of laboratory examinations during hospitalization were collected through hospital information system (HIS).

Statistical analysis

The clinical characteristics of patients and frequency of CMs use were described. Independent t test was used to measure the differences of clinical characteristics among patients in varied demographic groups, and paired t test was used to measure the differences of patients' blood test results between admission and discharge of hospital, which could indicate the effect of CMs. Correlation analysis was applied to investigate the relevance among various symptoms and TCM clinical features. Multiple linear regression analysis was applied to explore the factors affecting the length of hospital stay.

Network pharmacology analysis

The compound information of all the 21 Chinese medicines in QFPDD were collected. There were one to three herbs selected as the sovereign medicinal (君药) to represent the main effect of the recommended formulas of five disease stages: mild, moderate, severe, critical and recovery stages.

For target prediction and active compound (C)—target (T) network construction, we input the SMILES of the compounds into online tools Similarity Ensemble Approach (SEA) to predict the putative targets. The software Cytoscape (version: 3.7.0) was used to construct active compounds-targets network, and the network parameters of each element were analysed based on the plug-in Network Analyzer for further analysis.

A web-based gene set analysis toolkit was applied for the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of putative targets. Parameter settings were as follows: Homo sapiens in Organism of Interest, Over-Representation Analysis (ORA) in Method of Interest and KEGG in Functional Database.

Results

Theoretic base of TCM comprehension on NCP

TCM comprehension on pathological evolution of NCP and medication paradigm were analyzed based on syndrome differentiation. As NCP belongs to the category of epidemic disease, the pathogen is generally attributed to dampness toxin according to the main symptom characteristics of this disease. TCM believes that NCP locates in lung, and is closely related to spleen and stomach, and its pathological changes involve in heart, liver and kidney in the later stages. Dampness pathogen can change into cold-dampness (寒湿)

pathogen following the Yin body constitution (阴性体质), and also become the dampness-heat (湿热) pathogen following the Yang body constitution (阳性体质). Clinical observation shows that dampness toxin can directly invade into middle energizer (中焦) in partly NCP patients, and leads to the dysfunction of Qi movement. If the treatment method is appropriate and sufficient healthy Qi (正气) gradually recovers, the pathogen will be driven out, consequently the patient will enter into the recovery period. At the same time clinical manifestations appear some symptoms of Qi and Yin deficiency (气阴两虚证). Thus, the pathological evolution of NCP in TCM can be summarized as dampness toxin invading defense exterior (卫表) in early stage, and then enters the lungs and influences spleen function, eventually involves heart, liver and kidney, which causes Yang Qi collapse (阳气外脱) by excess pathogen and Yin and Yang separates from each other (阴阳离决). If the treatment is timely and suitable, sufficient healthy Qi can eliminate the pathogen, syndromes with deficiency of Qi and Yin in lung and spleen will be manifested (Fig. 1).

Fig. 1

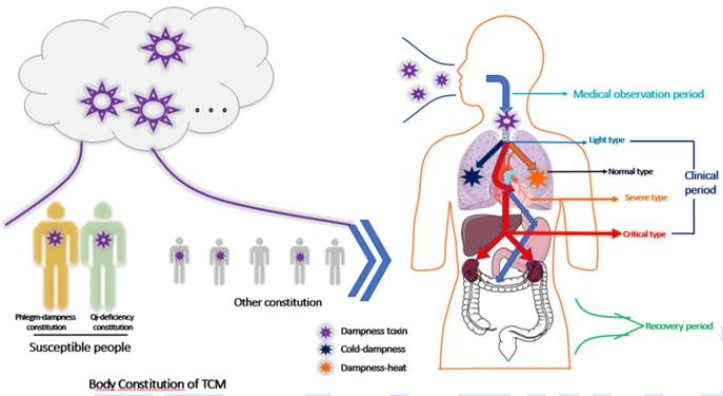

Diagram of pathological evolution of TCM

Although the pathogen between NCP and SARS have certain similarities in virus origination, the TCM pathogenesis of NCP and SARS appear to be different (Table 1).

Table 1 Characteristics of TCM Pathogenesis in NCP and SARS

	NCP (COVID-19)	SARS
Original area	Wuhan, Hubei	Foshan, Guangdong
Onset time	Nov, 2019	Nov, 2002
TCM pathogen	Dampness toxin	Epidemic toxin (疫毒)
Main symptoms	Low fever or no fever, some patients only felt fatigue, or dry cough, nausea, diarrhea. Severe dyspnea occurs 1 week later, which can lead to multiple organs failure.	The typical symptom is fever (frequent high fever), following fatigue, head and muscle pain. Some patients present dry cough, less sputum after 3 to 6 days, as well as chest discomfort and wheezing. In severe stage, the occurrence of gasp and acute respiratory distress.
Location of disease	Lungs, involving spleen and stomach, eventually affecting heart, liver and kidney	Mainly in lung
Pathological evolutional periods	1 Medical observation period: fatigue and gastrointestinal dysfunction or fever 2 Clinical period when diagnosed 2.1 Mild type: syndrome of cold dampness obstructing lung (寒湿郁肺), syndrome of lung with dampness heat retention (湿热蕴肺证) 2.2 Moderate type: syndrome of lung with dampness toxin retention (湿毒郁肺证), syndrome of cold dampness blocking lung (寒湿阻肺证) 2.3 Severe type: syndrome of pestilent toxin blocking lung (疫毒闭肺证), syndrome of dual blaze of Qi and blood (气血两燔证) 2.4 Critical type: syndrome of internal block and external collapse (内闭外脱证) 3 Recovery period: syndrome of Qi and Yin deficiency in lung and spleen (肺脾气阴两虚证)	1 Early stage: syndrome of epidemic toxin invading lung (疫毒犯肺证) 2 Progressive stage: syndrome of epidemic toxin obstructing lung (疫毒壅肺), syndrome of blocking lung with severe dyspnea (肺闭喘憋), syndrome of internal block and external collapse (内闭外脱证) 3 Recovery period: syndrome of Qi and Yin deficiency (气阴两虚证), syndrome of phlegm and blood stasis blocking collaterals (痰瘀阻络证).

Except using CMs treatment according to TCM different syndromes, the application of achievements from modern pharmacological research on CMs, targeting the pathological changes of NCP at different periods will also improve therapeutic effects. For example, *Ephedra sinica* Stapf, *Schizonepeta tenuifolia* Briq., Perillae Folium and Lonicerae Japonicae Flos have antipyretic and analgesic actions, and Ma Xing Shi Gan Decoction (麻杏石甘汤), *Arctium lappa* L. and *Poria cocos* (Schw.) Wolf. are able to regulate immune function and suppress inflammatory cytokine storm, suggesting that TCM plays a comprehensive beneficial regulating role in the treatment of NCP through multi-level and multi-pathways.

Characteristics of patients and TCM utilization

Demographic characteristics of patients were shown in Table 2. The average age of all the 54 patients was 55.07 years old. Male patients (52.03 years old) were younger than female patients (60.25 years old) in average (t = 2.128, P = 0.028). The average length of hospital stay was 8.96 days. Patients over 45 years old stayed 9.79 days in hospital in average, which was longer than 7.64 days of patients under 45 years old (t = 2.232, P = 0.034).

Table 2 Demographic characteristics and TCM features of patients

Characteristic	Population
Gender	
Male	34
Female	20
Age	
< 45	11
[45, 60)	21
[60, 75)	18
≥ 75	4
Symptoms	
Fever	16
Cough	24
Shortness of breath	18
Abnormal digestion	5
Anorexia	8
Tongue manifestation	
Red tongue with white coating (舌红苔白)	24
Red tongue with yellow coating (舌红苔黄)	11
Others	16
Pulse manifestation	
Deep pulse (沉脉)	19
Slippery pulse (滑脉)	12
Others	20

The common symptoms were correlative with TCM clinical features (Table 3). Fever was negatively related to cough and shortness of breath, and was in positive correlation to abnormal digestion, Red tongue with white coating (舌红苔白) and deep pulse (沉脉). Cough was in negative correlation to shortness of breath and abnormal digestion. Shortness of breath was negatively related to deep pulse.

Table 3 Relevant of Symptoms and TCM clinical features

Table 3 Relevant of Symptoms and TCM clinical features

From: Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19): an empirical study from Wuhan, Hubei Province, China

	Fever	Cough	Shortness of breath	Abnormal digestion	Anorexia	Red tongue with white coating	Deep pulse
Fever	1	− 0.299[a]	− 0.411[b]	0.346[a]	0.057	0.316[a]	0.450[b]
Cough	− 0.299[a]	1	− 0.203	− 0.311[a]	− 0.083	− 0.056	− 0.024
Shortness of breath	− 0.411[b]	− 0.203	1	0.032	0.02	− 0.15	− 0.406[b]
Abnormal digestion	0.346[a]	− 0.311[a]	0.032	1	0.039	− 0.016	0.234
Anorexia	0.057	− 0.083	0.02	0.039	1	− 0.17	− 0.175
Red tongue with white coating	0.316[a]	− 0.056	− 0.15	− 0.016	− 0.17	1	0.639[b]
Deep pulse	0.450[b]	− 0.024	− 0.406[b]	0.234	− 0.175	0.639[b]	1

[a]Correlation significant, at 0.05 level; [b]at 0.01 level

NCP patients were prescribed with 87 kinds of CMs in total, and the most frequently used CMs were listed according to their classification (Table 4).

Table 4 The most frequently used CMs for NCP patients

Classification of TCM	Top 3 used TCMs	Frequency
Category of clearing heat and drying dampness and removing toxin (清热燥湿解毒类)	*Scutellaria baicalensis* Georgi (黄芩)	29
	Lonicera japonica Thunb (金银花)	11
	Forsythia suspense Vahl (连翘)	10
Category of aromatic herbs resolving dampness (芳香化湿类)	*Amomum villosum* Lour (砂仁)	28
	Amomum compactum (豆蔻)	12
	Pogostemon cablin (藿香)	9
Category of eliminating dampness with bland medicinal (淡渗利湿类)	*Poria cocos* (茯苓)	40
	Alisma orientalis (泽泻)	34
	Coix lacryma-jobi (薏苡仁)	36
Category of invigorating spleen and removing dampness (ISRD, 健脾祛湿类)	*Atractylodes macrocephala* (白术)	30
	Astragalus membranaceus (黄芪)	5
	Dolichos (白扁豆)	2
Category of power appetite and digestant medicinal (开胃消食类)	*Massa Medicata Fermentata* (神曲)	19
	Hordeum vulgare (麦芽)	18
	Gallus gallus domesticus (鸡内金)	13
Others	*Prunus armeniaca* (杏仁)	33
	Paeonia suffruticosa (丹皮)	27
	Platycodon grandiflorum (桔梗)	27

Clinical effect of TCMs treatment

Multiple linear regression was employed with the length of hospital stay as dependent variable, and patients' age, gender, symptoms and CMs they administrated as independent variables (Table 5). The method of backward elimination was chosen to get the optimal fitting degree of regression model (Table 6).

Table 5 Assignment of independent variables in multiple linear regression

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From: Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19): an empirical study from Wuhan, Hubei Province, China

Variables	Assignment
X1: Age	Continuous variable
X2: Gender	Male = 1, Female = 0
X3: Cough	Yes = 1, No = 0
X4: Short of breath	Yes = 1, No = 0
X5: Administrate category of clearing heat and drying dampness and removing toxin	Yes = 1, No = 0
X6: Administrate category of aromatic herbs resolving dampness	Yes = 1, No = 0
X7: Administrate category of ISRD	Yes = 1, No = 0
X8: Administrate other CMs	Yes = 1, No = 0

Table 6 Regression coefficients in multiple linear regression

Table 6 Regression coefficients in multiple linear regression

From: Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19): an empirical study from Wuhan, Hubei Province, China

Variable	Unstandardized coefficient		Standardized coefficient	*T*	*P*	Collinearity	
	B	S.E.	Beta			Tolerance	VIF
(Constant)	− 4.999	3.956		− 1.264	0.224		
X1:	0.106	0.054	0.446	1.963	0.067	0.522	1.914
X2:	4.538	1.565	0.727	2.899	0.010*	0.428	2.336
X3:	− 3.474	1.430	− 0.580	− 2.430	0.027*	0.474	2.110
X4:	− 2.665	1.311	− 0.436	− 2.033	0.059	0.586	1.707
X5:	1.925	1.442	0.309	1.335	0.201	0.504	1.983
X6:	− 1.510	1.409	− 0.242	− 1.072	0.300	0.529	1.892
X7:	7.864	2.291	1.051	3.433	0.003**	0.288	3.474
X8:	9.771	2.781	0.885	3.514	0.003**	0.425	2.355

*$P < 0.5$ **$P < 0.01$

The regression equation of length of hospital stay was:

Y=−4.999+0.106X1+4.538X2−3.474X3−2.665X4+1.925X5−1.510X6+7.864X7+9.771X8,withR2=0.569andadjustedR2=0.353.Y=−4.999+0.106X1+4.538X2−3.474X3−2.665X4+1.925X5−1.510X6+7.864X7+9.771X8,withR2=0.569andadjustedR2=0.353.

According to the standard coefficients, the factor affecting the length of hospital stay for the most was administrating category of ISRD, followed by administrating other CMs, male, and cough.

Patients received blood test both when admitted to hospital and discharge from hospital (Table 7).

Table 7 Values of blood test when admission and discharge of hospital

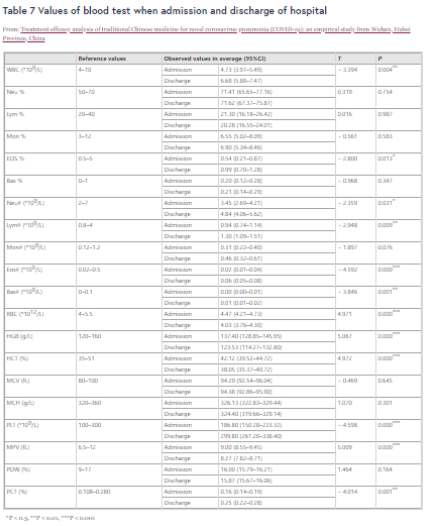


Table 7 Values of blood test when admission and discharge of hospital

From: Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19): an empirical study from Wuhan, Hubei Province, China

	Reference values	Observed values in average (95%CI)		T	P
WBC ($*10^9$/L)	4–10	Admission	4.73 (3.97–5.49)	− 3.394	0.004**
		Discharge	6.68 (5.88–7.47)		
Neu %	50–70	Admission	71.41 (65.55–77.26)	0.319	0.754
		Discharge	71.62 (67.37–75.87)		
Lym %	20–40	Admission	21.30 (16.18–26.42)	0.016	0.987
		Discharge	20.28 (16.55–24.01)		
Mon %	3–12	Admission	6.55 (5.02–8.09)	− 0.561	0.583
		Discharge	6.90 (5.34–8.46)		
EOS %	0.5–5	Admission	0.54 (0.21–0.87)	− 2.800	0.013*
		Discharge	0.99 (0.70–1.28)		
Bas %	0–1	Admission	0.20 (0.12–0.28)	− 0.968	0.347
		Discharge	0.21 (0.14–0.28)		
Neu# ($*10^9$/L)	2–7	Admission	3.45 (2.69–4.21)	− 2.359	0.031*
		Discharge	4.84 (4.06–5.62)		
Lym# ($*10^9$/L)	0.8–4	Admission	0.94 (0.74–1.14)	− 2.948	0.009**
		Discharge	1.30 (1.09–1.51)		
Mon# ($*10^9$/L)	0.12–1.2	Admission	0.31 (0.22–0.40)	− 1.897	0.076
		Discharge	0.46 (0.32–0.61)		
Eos# ($*10^9$/L)	0.02–0.5	Admission	0.02 (0.01–0.04)	− 4.592	0.000***
		Discharge	0.06 (0.05–0.08)		
Bas# ($*10^9$/L)	0–0.1	Admission	0.00 (0.00–0.01)	− 3.846	0.001**
		Discharge	0.01 (0.01–0.02)		
RBC ($*10^{12}$/L)	4–5.5	Admission	4.47 (4.21–4.73)	4.971	0.000***
		Discharge	4.03 (3.76–4.30)		
HGB (g/L)	120–160	Admission	137.40 (128.85–145.95)	5.067	0.000***
		Discharge	123.53 (114.27–132.80)		
HCT (%)	35–51	Admission	42.12 (39.52–44.72)	4.972	0.000***
		Discharge	38.05 (35.37–40.72)		
MCV (fL)	80–100	Admission	94.29 (92.54–96.04)	− 0.469	0.645
		Discharge	94.38 (92.86–95.90)		
MCH (pg/L)	320–360	Admission	326.71 (322.83–329.44)	1.070	0.301
		Discharge	324.40 (319.86–329.14)		
PLT ($*10^9$/L)	100–300	Admission	186.80 (150.28–223.32)	− 4.598	0.000***
		Discharge	299.80 (267.20–332.40)		
MPV (fL)	6.5–12	Admission	9.00 (8.55–9.45)	5.009	0.000***
		Discharge	8.27 (7.82–8.71)		
PDW (%)	9–17	Admission	16.00 (15.79–16.21)	1.464	0.164
		Discharge	15.87 (15.67–16.06)		
PCT (%)	0.108–0.280	Admission	0.16 (0.14–0.19)	− 4.014	0.001**
		Discharge	0.25 (0.22–0.28)		

*P < 0.5, **P < 0.01, ***P < 0.001

The indicators of blood test included white cell count (WBC), percentage of neutrophils (Neu %), percentage of lymphocytes (Lym %), percentage of mononucleosis (Mon %), percentage of eosinophils % (EOS %), percentage of alkaline granulocytes (Bas %), number of neutrophils (Neu#), number of lymphocytes (Lym#), number of single-core cells (Mon#), number of eosinophils (Eos#), number of alkaline granulocytes (Bas#), red cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), average red blood cell volume (MCV), average red blood cell hemoglobin concentration (MCH), platelet number (PLT), average platelet volume (MPV), platelet distribution width (PDW) and platelet pressure (PCT).

(Table 7 Values of blood test when admission and discharge of hospital)

By comparing the parameters of blood test between admission and discharge of hospital, the values of WBC, EOS %, Neu#, Lym#, Eos#, Bas#, PLT and PCT increased, while values of RBC, HGB, HCT, and MPV decreased. The result indicated that TCM treatment significantly ameliorated the immune ability against SARS-CoV-2 in patients.

Network pharmacology of QEPDD and NHC-recommended formulas

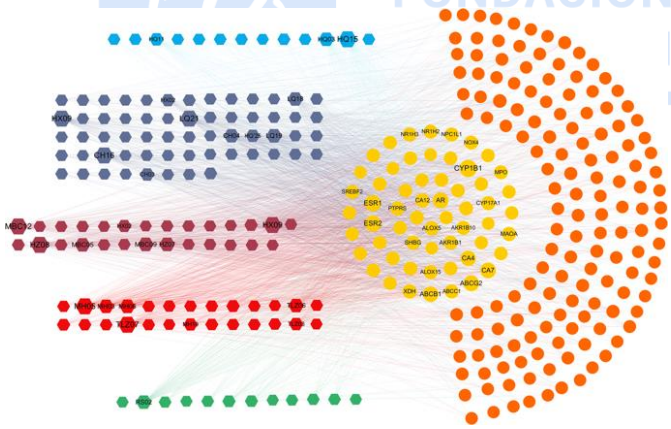
The five disease stages consist of nine syndromes and nine formulas. The sovereign medicinal of mild disease stage contained *Pogostemon Cablin (Blanco), Atractylodes Lancea (Thunb.)Dc., Scutellariae Radix, Chaihu Radix Bupleuri, Forsythiae Fructus*; the moderate stage contained gypsum, *Atractylodes Lancea (Thunb.)Dc., Polygoni Cuspidati Rhizoma Et Radix, Pogostemon Cablin (Blanco), Verbenae Herb*; the severe stage contained *Ephedra Herba,* gypsum*, Lepidii Semen Descurainiae Semen*, buffalo horn; the critical stage contained *Panax Ginseng C. A. Mey., Aconiti Lateralis Radix Praeparata*; and the recovery stage contained *Hedysarum Multijugum Maxim., Ophiopogon japonicus (Linn. f.) Ker-Gawl, Panacis Quinquefolii Radix*.

There are 21 CMs in the QFPDD, and 16 CMs in recommended formulas of five disease stages. After deleting duplication, 32 different CMs were selected in total. As the *Ophiopogon japonicus (Linn. f.) Ker-Gawl.*, gypsum, and buffalo horn were not found in TCMSP database, finally 29 CMs were picked in this study. There are 201 compounds in recommended formulas of five disease stages and 288 compounds in the QFPDD after screening. Comparing with the compound in sovereign medicinal, we discovered kaempferol, beta-sitosterol, Stigmasterol, quercetin, luteolin, Genkwanin, diop, isorhamnetin participated in three or more disease stages. At the same time, these compounds were not unique to a single Chinese herbal medicine, but many common CMs. Comparing the compounds in QFPDD with those in recommended formulas of the first four disease stages, seven of the eight compounds (except isorhamnetin) were found to be representative in QFPDD and in recommended formulas of three or four disease stages.

The recommended formulas included 164 types of putative targets in mild disease stage, 147 types in moderate stage, 150 types in severe stage, 88 types in critical stage and 112 types in recovery stage. Totally, there were 204 types of different targets in recommended formulas, and 240 targets in QFPDD. After comparing the putative targets of five disease stage, it was found that 169 of the 204 targets were common targets, among which 58 were involved in the treatment of all five disease stages. By comparing QFPDD with recommended formulas of the first four stages, it was found that only 9 of 248 targets have nothing to do with QFPDD, and 60 targets were common targets.

According to the compounds (C)-putative targets (T) network (C-T network) related to the five stages, the degree value of quercetin, luteolin, kaempferol, acacetin and genkwanin were all greater than 30 and exist in a variety of CMs, which acted on different disease stages (Fig. 2). And the degree value of a total of 25 targets was greater than 30, among which the first five were CYP1B1, ESR1, ESR2, AR, and ABCG2, respectively.

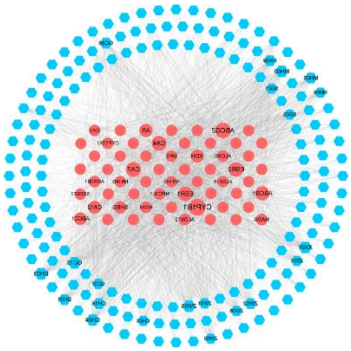
Fig. 2



Active compounds (C)-putative targets (T) network of five disease stages

Then a C-T network of QRPDD was constructed similarly. By considering the large number of targets, only 60 common targets in QFPDD and recommended formulas of the first four disease stages were selected for visualization (Fig. 3). For the compounds, kaempferol, quercetin, luteolin, galangin, luteolin, isorhamnetin and the degree value were all greater than 30. For the targets, the first five were CYP1B1, ABCG2, CA7, CA4 and ESR2, respectively.

Fig. 3

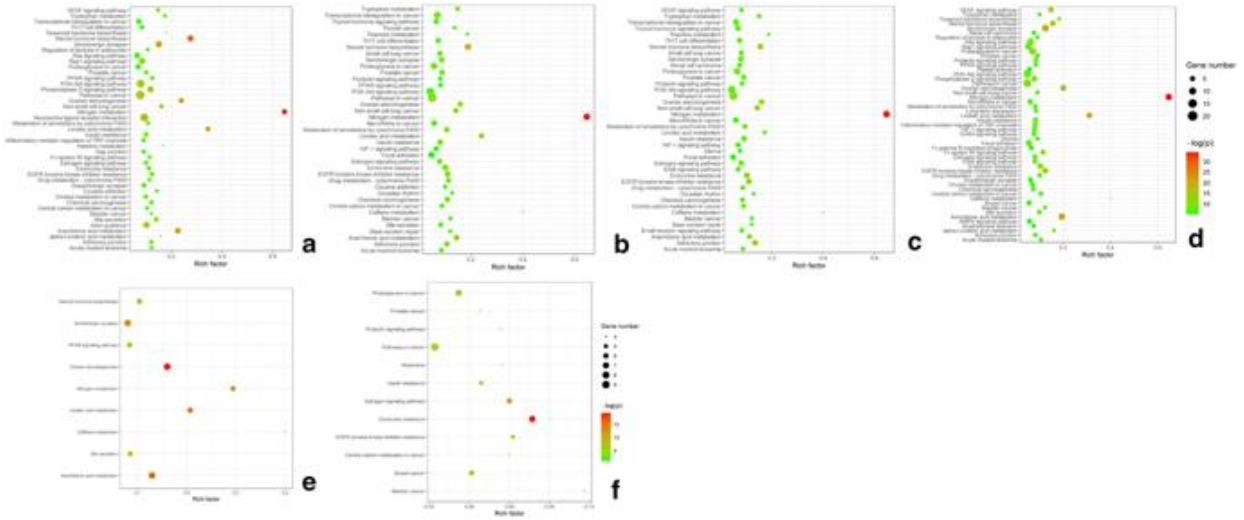


Active compounds (C)-putative targets (T) network of QFPDD
The hexagon on the left represents the compounds, and the different colours from top to bottom represent the different compounds in mild, moderate, severe, critical and recovery stages of the disease. The right circle represents the putative targets, the yellow represents the 58 common targets of the five disease stages, and the orange represents 197 non-common targets. The edges show the relationship between the compounds and putative targets obtained by SEA prediction. Size of the point is related to its degree value, and the larger the value, the larger the point.

The blue hexagon represents the compound, and the red circle points represent the 60 targets shared by QFPDD and the recommended compound of the first four disease stages. The rest is consistent with Fig. 2.

As shown in Fig. 4, according to the enrichment results of pathways corresponding to the five disease stages, figure B, C and D show a large number of enrichment pathways, while E and F are more concentrated. Nitrogen metabolism is presented in the first four diseases stages, with a high enrichment rate as well as a low *p* value, and the expression of arachidonic acid metabolism in the critical disease stages was the most obvious, indicating that formulas were more inclined to inhibit pathogens in the critical diseases stage. Ovarian steroidogenesis exists mainly in severe and critical disease stages. For QFPDD, nitrogen metabolism, linoic acid metabolism and steroid hormone biosynthesis have high enrichment rate (Figure A), which is roughly consistent with the results of mild and moderate stages.

Fig. 4



The bubble diagram of KEGG pathway enrichment analysis
Figure A represents QFPDD and figure B-F represent recommended formulas of mild, moderate, severe, critical and recovery stages of the disease, respectively. The x axis means enrichment ratio and the y axis means the name of KEGG pathways.

Efficacy of TCM treatment on NCP

Generally, the clinical results of our study showed that 54 NCP patients enhanced immune ability against COVID-19 by detecting blood samples after TCM treatment and category of ISRD appears to shorten patients' hospitalization days.

Firstly, the hemogram changes of patients in this study can reflect the efficacy of TCM treatment on NCP in a certain extent. As a common symptom among NCP patients, the lymphocytopenia was observed in this study, though it was not severe. According to recent studies on clinical characteristics of NCP, lymphocytopenia was one of the most common laboratory abnormalities observed in NCP patients, and occurred in 70.3–83.2% of patients [8, 9]. The abnormalities like lymphocytopenia suggested that COVID-19 infection may be associated with cellular immune deficiency [9], thus the increase of lymphocyte during the TCM treatment in this study could be regarded as a result of TCM improving patients' hemogram by adjusting immune system [10].

In this study, the average hospitalization time of 54 NCP patients in moderate type was 8.96 days under the treatment of TCM, and the length of hospital stay was related to the TCM in category of ISRD, suggesting that Chinese medicines could effectively shorten the pathological evolution of NCP. As the pathogenesis of NCP in Chinese medicine theory belongs to dampness and toxin, and dampness pathogen mainly influence the digestive function of spleen, stomach and intestine, the primary therapeutic strategy focuses on improving digestive function for dispelling dampness pathogen.

Additionally, our study also found that abnormal digestion was associated with the clinical symptoms of fever and cough by correlation analysis, which powerfully illustrated the relationship of the pathogenesis of NCP with the dysfunction of digestive system (e.g. spleen, stomach and intestine). Therefore, our study demonstrates that Chinese medicines with an effect of ISRD should be beneficial to attenuate the pathological evolution of NCP.

Interestingly, the category of removing toxin did not account for the predominant relation with the length of hospital stay from the result of multiple linear regression analysis, showing the therapeutic methods were primarily attributed to ISRD rather than removing toxin. The results indicated the function of Chinese medicines on NCP treatment focus on strengthening sufficient healthy Qi (enhancing body immune ability) instead of dispelling pathogen (targeting against COVID-19). One of supported evidences was that the number of leukocytes, neutrophil, lymphocyte and platelet significantly increased at the end of integrated treatment.

Pathological mechanism of anti-NCP TCM

During rescuing critical NCP patients in Wuhan, Academician Li Lanjuan's team mentioned that it was important to maintain patients' intestinal microenvironment balance, and TCM had advantages on reducing intestinal bacterial migration to lung and secondary infection. This view has been included into the Diagnosis and Treatment Guideline of NCP (Edition 7) [7]. As the development of intestine and lung originate from the same source in embryonic stage, studies indicate that bacterial and viral infections in lung can affect intestinal microenvironment through the "Gut-lung axis". And vice versa, in animal model of sepsis and acute respiratory distress syndrome, the intestinal specific bacteria (such as bacteroides spp) can directly transfer to lung through damaged intestinal barrier, revealing that these two critical and high mortality diseases have a common pathogenesis mechanism [11]. Sepsis and acute respiratory distress syndrome are also the pathological results of severe and critical NCP patients suffering from cytokine storm, which are the main death cause for patient [12, 13]. Previous studies have confirmed that intestinal microenvironmental disorders and imbalance of intestinal flora can cause the occurrence and development of respiratory

diseases through the Gut-lung axis. Therefore, lung diseases can be treated by adjusting intestinal microbiota [14, 15].

In the theoretical system of TCM, there is a doctrine of "lung and large intestine in pair (肺合大肠)". Some scholars have suggested that TCM pathogenesis of NCP is related to the "lung-spleen-large intestine" dysfunction [10, 16, 17]. It has been discovered that Houttuynia cordata polysaccharide (HCP), a Chinese herbal medicine extract from *Houttuynia cordata* Thunb. can keep the intestinal homeostasis in H1N1 virus-infected mice, and protect the intestinal wall barrier, inhibit IL-1β production mediated by Toll receptors, and improve the expression of IL-10, suggesting that the effect of anti-inflammatory damage is related to regulation of intestinal microbiota. As a main active ingredient of *Scutellaria baicalensis* Georgi and *Coptis chinensis* Franch., Berberine's bioavailability and in vivo metabolism are found to have close relationship with intestinal organic acids and microbiota [18, 19]. The doctrine of lung and large intestine in pair in TCM theory is similar to the Gut-lung axis in the theory of modern medicine. The most frequently used CMs for NCP patients in our study also suggests channel tropism (归经) of numerous CMs belongs to lung, spleen, stomach and intestine. Therefore, CMs can achieve the purpose of relieving and curing lung diseases by adjusting the intestinal microenvironment balance. Moreover, it is not only one of the mechanisms of Chinese medicine treatment for NCP, but also one important approach to screen novel Chinese medicines for treating NCP in the future.

The network pharmacology results in this study indicated that the recommended formulas in five disease stages have both generality and individuality in the compound level, in which compound quercetin, luteolin, kaempferol, acacetin etc., all are involved in the treatment of multi-stages of the disease. The target and KEGG pathway enrichment results also show the certain integrity and the different tendency of formulas. Meanwhile, by comparing the QFPDD with the recommended formula of the first four stages [20, 21], we discover that the QFPDD has a certain integrity on the compound level. However, on the target level, the QFPDD has covered most of the predicted target of the recommended formula of the first four disease stages, and the mutual target from both two sources have a higher network parameter. The KEGG pathways enrichment results about QFPDD are consist with the effects of recommended formulas of mild and moderate stages as well. All these results confirmed the consistency of QFPDD with the recommended formulas of the first four disease stages from the perspective of network pharmacology.

In summary, this study suggests TCM has a systemic theoretical understanding on the pathological evolution of NCP (COVID-19), and the symptom positive correlation between fever and abnormal digestion indicates that researchers should pay more attention on intestinal functional recovery and microenvironmental balance in the treatment of NCP. Additionally, the network pharmacology results demonstrate the appropriateness of theatrical formulas for QFPDD and other recommended formulas in guideline, for the compounds corresponding to targets can cover various stages of the disease.

Advantages and implications of TCM utilization

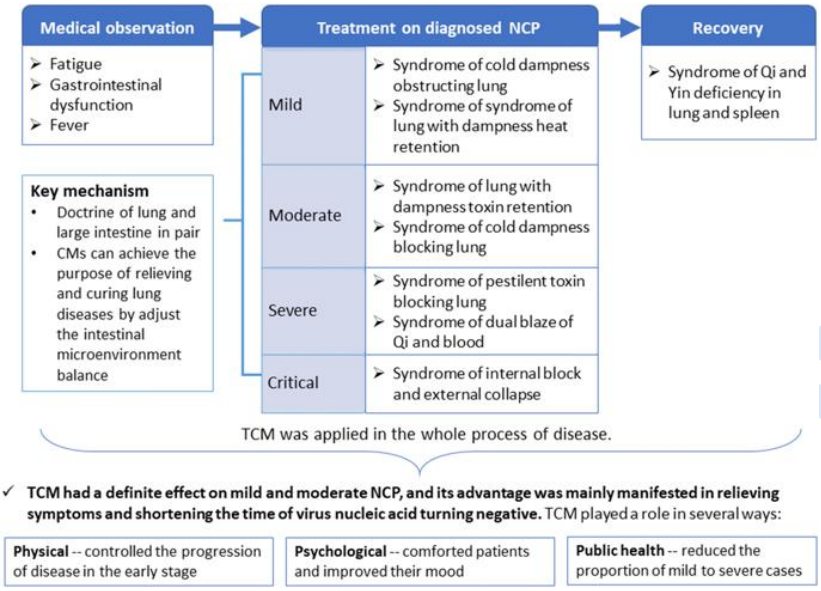
As there has been no effective targeted drugs for NCP up to now, the early intervention to NCP is important in controlling the spread of COVID-19. According to the Protocol for Prevention and Control of COVID-19 (Edition 6) by Chinese Center for Disease Control and Prevention, the use of TCM in the prevention and treatment of infectious diseases was encouraged and supported, and TCM was applied in the whole process of disease [7, 22].

Numerous data have showed that the early intervention of TCM and modern medicine have positive effects on shortening time of hospitalization and ameliorating symptoms, reducing the development of mild and moderate case to severe case and the mortality rate, improving the cure rate and the rehabilitation of the recovery population of NCP patients [23]. A report from Hunan province suggested that due to the increasing the use of TCM for NCP, the patients' average hospitalization day was shortened for 2 days, and none of the patients turned to critical stage [15]. A study about 34 NCP patients (mostly moderate type)

from Wuhan found satisfied efficacy of integrated use of CM and western medicine on protecting heart, liver, kidney and other organ functions, as well as reducing inflammatory response and improving immune function, through the observation of patients treated with conventional western medicine, CM decoctions, traditional Chinese patent medicines and CM injections [24, 25]. In a study on 6 cases of severe and critical NCP, the results showed that Qufeidu No.1 Formulae (祛肺毒一号方) combined with conventional western medicine treatment, as well as integrating Xuebijing injection (血必净注射液) and/or Lianhua Qingwen capsule (连花清瘟胶囊) significantly improved pathological evolution of critical patients, consequently all of patients recovered [26, 27].

However, there were still deficiencies of the clinical research evidence on the integration of traditional Chinese and western medicine treatment for NCP, mainly embodying small sample size, the absence of control group, and the diversification of treatment programs. Therefore, constantly expanding the sample size and employing standardized randomized controlled trial (RCT) design will give more convincing scientific evidence to demonstrate the superiority of TCM for NCP treatment.

Fig. 5



The major advantages and key mechanisms of TCM on the treatment of NCP

At present, NCP has been nearly controlled in China. By the end of April 1st, 2020, up to 92.44% of NCP patients have recovered in China. On March 23, the state council information office of China reported that 74,187 confirmed COVID-19 patients (91.5% of total confirmed cases) accepted the combined treatment with TCM and modern medicine, and the clinical efficacy observations showed that the total effective rate reached more than 90% [28].

As Tong Xiaolin said, practice proved the importance of sticking to both TCM and modern approaches, and Chinese medicine had the unique advantage in establishing a collaboration mechanism between them [29]. In summary, TCM had a definite effect on mild and moderate NCP, mainly manifested in relieving symptoms and shortening the time of virus nucleic acid turning negative (Fig. 5). Moreover, TCM not only played a role in controlling the progression of disease in the early stage, but also comforted patients psychologically [30]. In addition, TCM reduced the proportion of mild to severe cases, which could contribute to save medical resources from the perspective of public health.

The utilization of TCM has implications for the development of medicines in the future. As the NCP virus has some mutations when compared to SARS or MERS coronavirus, the natural compounds effectiveness against the two previous coronaviruses might not be present in the new virus, which urges updated TCM formulas. In this study, the anti-NCP effects of the natural compounds have been mainly confirmed by the screening method, and the treatment effect of dampness pathogen results in the shortening of hospital stay for patients of mild type. Thus, TCM treatment was not only effective for the NCP patients, but also advantageous to the clinical development of medicines to dampness pathogen and lung infection diseases, which have high incidence rate of respiratory system syndrome. Therefore, Chinese medicine treatment has positive impact on infectious lung diseases, as well as the development of new medicines.

Conclusion

Traditional Chinese medicine has a systemic theoretical understanding on the pathological evolution of novel coronavirus pneumonia and plays a positive role in NCP treatment according to the clinical data from Wuhan in this study. Multiple linear regression analysis shows that the TCM category of invigorating spleen and removing dampness improved patients' recovery, suggesting the vitalness of regulating intestinal function and keeping microenvironmental balance in the TCM treatment of novel coronavirus pneumonia. The network pharmacology results demonstrate active compounds from QFPDD and recommended formulas by National Health Commission contribute to recovery of different disease progresses during TCM treating novel coronavirus pneumonia. Chinese medicine treatment is advantageous in treating NCP, as well as the development of new medicines for infectious lung diseases.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations

Bas: Alkaline granulocytes

CM: Chinese medicine

EOS: Eosinophils

HCT: Hematocrit

HGB: Hemoglobin concentration

HIS: Hospital information system

KEGG: Kyoto encyclopedia of genes and genomes

Lym: Lymphocytes

MCH: Average red blood cell hemoglobin concentration

MCV: Average red blood cell volume

Mon: Mononucleosis

MPV: Average platelet volume

NCP: Novel coronavirus pneumonia

Neu: Neutrophils

NHC: National Health Commission

ORA: Over-representation analysis

PCT: Platelet pressure

PDW: Platelet distribution width

PLT: Platelet number

QFPDD: Qingfei Paidu decoction

RBC: Red cell count

SARS: Severe acute respiratory syndrome

SEA: Similarity ensemble approach

TCM:Traditional Chinese medicine

WBC: White cell count

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Can Chinese Medicine Be Used for Prevention of Corona Virus Disease 2019 (COVID-19)? A Review of Historical Classics, Research Evidence and Current Prevention Programs LUO Hui, TANG Qiao-ling, SHANG Ya-xi, LIANG Shi-bing, YANG Ming, Nicola Robinson, and LIU Jian-ping

Results: The use of CM to prevent epidemics of: The use of CM to prevent epidemics of infectious diseases was traced back to ancient Chinese practice infectious diseases was traced back to ancient Chinese practice cited in Huangdi's Internal Classic (Huang Di Nei Jing) where preventive ef (Huang Di Nei Jing) where preventive effects were recorded. There were 3fects were recorded. There were 3 cited in Huangdi's Internal Classic studies using CM for prevent studies using CM for prevention of SARS and 4 studies for H1N1 inion of SARS and 4 studies for H1N1 infl uenza. None of uenza. None of the participants who took CM the participants who took CM contracted SARS in the 3 studies. The infection rate of H1N1 infl uenza in the CM group was signifi cant cantly lower thanly lower tan contracted SARS in the 3 studies. The infection rate of H1N1 in uenza in the CM group was sign in the non-CM group (relative risk 0.36, 95% con the non-CM group (relative risk 0.36, 95% confi dence interval 0.24–0.52; dence interval 0.24–0.52; n=4). For prevention of COVID-19, 23 =4). For prevention of COVID-19, 23 provinces in China issued CM programs. The main principles of CM use were to tonify qi to protect from external provinces in China issued CM programs. The main principles of CM use were to tonify qi to protect from external pathogens, disperse wind and discharge heat, and resolve dampness. The most frequently used herbs included pathogens, disperse wind and discharge heat, and resolve dampness. The most frequently used herbs included Radix astragali Radix astragali (Huangqi), (Huangqi), Radix glycyrrhizaeRadix glycyrrhizae (Gancao), (Gancao), Radix saposhnikoviae, Radix saposhnikoviae (Fangfeng), (Fangfeng), Rhizoma Atractylodis Rhizoma Atractylodis Macrocephalae Macrocephalae (Baizhu), (Baizhu), Lonicerae Japonicae FlosLonicerae Japonicae Flos (Jinyinhua), and (Jinyinhua), and Fructus forsythiaFructus forsythia (Lianqiao). (Lianqiao). ConclusionsConclusions: Based on historical records and human evidence of SARS and H1N1 influenza prevention, Chinese herbal Based on historical records and human evidence of SARS and H1N1 influenza prevention, Chinese herbal formula could be an alternative approach for prevention of COVID-19 in high-risk population. Prospective, rigorous formula could be an alternative approach for prevention of COVID-19 in high-risk population. Prospective, rigorous population studies are warranted to con population studies are warranted to confi rm the potential preventive effect of CM. rm the potential preventive effect of CM. KEYWORDSKEYWORDS Chinese medicine, corona virus disease 2019 (COVID-19), prevention program, clinical evidence, Chinese medicine, corona virus disease 2019 (COVID-19), prevention program, clinical evidence, review

In December 2019, a pneumonia associated with the corona virus disease 2019 (COVID-19) emerged in Wuhan, Hubei Province, China.(1)

It is highly contagious and has quickly spread to many other parts of China and some other countries within 1 month since the fi rst report emerged. As of February 11, 2020, 44,653 cases of confi rmed infections and 1,113 deaths have been reported in Chinese mainland.(2) Outside of China, there had been 395 confirmed cases and 1 death from 24 countries were reported as of February 11, 2020.(3) The outbreak of COVID-19 raised intense attention not only within China but internationally.(4) On 20 January 2020, the Chinese government added it to the Notifiable Communicable Disease List and gave the highest priority to its prevention and treatment.(5) On 30 January 2020, the World Health Organization (WHO) declared a public health emergency of international concern for China's COVID-19.

Although the WHO said: "To date, there is no specific medicine recommended to prevent or treat the novel coronavirus",(6) in China, historically, when the outbreak started, Chinese medicine (CM) approaches

including oral administration of preventive herbal formulae, wearing CM sachets, indoor herbal medicine fumigation, etc. were recommended for prevention and treatment.(7,8). For example, in 2003, CM approaches were used to prevent and treat severe acute respiratory syndrome (SARS),(9,10) which was the most serious infectious disease outbreak in China prior to the COVID-19. In 2009, during the pandemic of H1N1 infl uenza around the world, the National Administration of Traditional Chinese Medicine of China issued a CM prevention program, which included 4 Chinese herbal medicine (CHM) formulae for adults of different CM body constitutions and one for children.(11)

The current outbreak of COVID-19 resulted in many provinces in China issuing CM prevention and control programs, among which the prevention programs are mainly oral CHM formulae. This study has reviewed the historical and human research evidence on CM in preventing and control of infections in order to provide guidance for the prevention of COVID-19.

METHODS

Data Sources

Three types of data were searched, including historical classics records, human research evidence and current prevention programs. (Ⅰ) Historical classics records: records on the prevention of epidemic diseases in ancient CM books were searched, including history, treatment principles, medicines and application of CM to prevent epidemic disease. (Ⅱ) Human research studies: studies to evaluate the preventive effects of CM on contagious respiratory virus diseases were included. The inclusion criteria were as follows. (1) Study design: clinical trials, cohort studies, and other population studies without control. (2) Population: high-risk populations exposed to SARS or H1N1 infl uenza. (3) Intervention: oral CHM formulae, including decoction, granules, or patent medicine. (4) Control: placebo, blank or without control group. (5) Outcome: infection rate defi ned as laboratory-confi rmed incidence of disease. (Ⅲ) Current prevention programs: CM prevention programs for COVID-19 issued by the state or provincial health authorities in China. Considering that some provinces had regularly updated the programs according to the local prevalence and clinical practice, the most recent versions of the programs were included for analyses in this study.

Literature Search

Retrieval strategy differed among the above three types of data. The fi rst type of data was based on mainly manual retrieval of ancient books of CM on epidemic diseases, supplemented by electronic database retrieval. The list of literature retrieved was determined by discussion among all authors. Secondly, 6 databases were searched including PubMed, Google Scholar, the Cochrane Library, China National Knowledge Infrastructure (CNKI), Wanfang Data, and CQVIP database, with the key words of "severe acute respiratory syndrome" (or "SARS"), "infl uenza", "H1N1", "prevent*" and "Chinese medicine" (pinyin: zhongyi or zhongyao). Thirdly, government websites or offi cial media websites were searched for prevention programs on COVID-19. Two authors (Luo H and Tang QL) conducted the literature search independently. The search date was up to February 10, 2020.

Data Extraction and Analysis

The following data were extracted and analyzed: source of evidence, time of publication or release, author, setting, basis for formulation of CM prevention strategy, composition of CM prescription, target disease, course of prevention, effect, and adverse reaction. The data was qualitatively described and presented, and if possible, quantitative or descriptive statistics were conducted. When the data were available for pooling, meta-analysis would be conducted by RevMan 5.3 software (https:// community.cochrane.org/help/tools-and-software/ revman-5/revman-5-download).

RESULTS

CHM Formula for Preventing "Pestilence" in Ancient CM Classics

The theory of prevention and treatment of "pestilence" (refers to fatal epidemic disease, pinyin: wenyi) in CM originated from Huangdi's Internal Classic (Huang Di Nei Jing), which was written about 2000 years ago.(12)

It suggested two aspects which should be employed to prevent the spread of epidemics. One was to maintain and improve the healthy qi in the body by taking preventive medicine [Xiaojin Dan (小金丹) in Huangdi's Internal Classic, the first recommended formula of CM to prevent pestilence], healthy diet care, exercise and so on, so as to resist the invasion of external pathogen, and the other was to avoid the source of infection.(13)

These two principles of epidemic disease prevention have been followed by CM practitioners till now.(12,14)

Since Huangdi's Internal Classic, a large number of formulae for preventing epidemic diseases have been recorded in other ancient CM classics, such as, the Handbook of Prescriptions for Emergencies (Zhou Hou Bei Ji Fang), Essential Prescriptions Worth a Thousand Gold for Emergencies (Bei Ji Qian Jin Yao Fang), Medical Secrets of an Official (Wai Tai Mi Yao), Compendium of Materia Medica (Ben Cao Gang Mu), etc.(15)

The famous doctor SUN Si-miao (541–682 AD) expounded the basis of medicines to prevent "pestilence" in his book Essential Prescriptions Worth a Thousand Gold for Emergencies: "pestilence comes from nature, so to prevent it, we need to fi nd medicinal herbs that also come from nature. People would not be infected if they know and take preventive medicine."(16)

A literature study compared the characteristics of medicinal formulae for preventing pestilence in different periods of ancient China, found that during the Jin and Tang Dynasties (3rd–10th century AD), medicinal formulae were mainly used to eliminate the pathogenic factors, while Ming and Qing dynasties (14–20th century AD) focused on fortifying Spleen (Pi), resolving dampness, clearing heat, and detoxifying.(17)

Although many formulae for pestilence prevention were recorded in ancient CM books, the case description of prevention was relatively rare. Through limited literature searches, we found an interesting case report: SU Shi (1037-1101 AD), a famous poet in the Northern Song Dynasty, accidentally found a formula for preventing pestilence named Sheng San Zi (圣散子), a powder consisting of 22 herbs.(18)

Later, when he was demoted to Huangzhou, Hubei Province, the pestilence had been outbreak for several years. He disclosed the prescription to the local people. After taking this formula, the number of patients with the disease was significantly reduced, and many lives were saved. This story was recorded by SU Shi himself, when he wrote a preface to his doctor friend PANG An-shi's book General Treatise on Febrile Diseases (Shang Han Zong Bing Lun).(18)

Evidence of CHM Formula for Preventing SARS Three studies were identified including 1 controlled study (19) and 2 single cohort studies (20,21) conducted during the epidemic of SARS. Lau, et al (19) designed a controlled study to evaluate a herbal formula for prevention of SARS (no herbal intervention in the control group) and conducted it in Hong Kong SAR, China. The sample size was 16,437 (1,063 in the herbal group and 15,374 in the non-herbal group), and all participants were hospital care workers including doctors, nurses, and other staff. The result showed that none of the participants who took modifi ed formula of Yupingfeng Powder (玉屏风散) plus Sangju Decoction (桑菊饮) contracted SARS, while 64 out of 15,347 (0.4%) in the non-herbal group were infected with SARS (P=0.035). Nineteen cases (1.8%) appeared minor adverse effects after 14 days taking herbal medicine, including diarrhea, sore throat, dizziness, and nausea.

Both single cohort studies were conducted in Beijing, China with sample sizes of 3,561(21) and 163, respectively. All participants were medical staff from two hospitals, where SARS patients were recruited and treated during the study period. Among them, Xu, et al's study (20) only included fi rst-line medical staff in treating SARS. The courses of taking herbal formulae for prevention were 6 days (20) and 12–25 days,(21) respectively. The formulae used in these studies were both classical formula Yupingfeng Powder plus some heat-clearing and detoxifying herbs. The results showed that none of the participants who took the preventive

herbal medicine had contracted SARS in the two studies. Information on the safety of the herbal medicines was not reported.

The details of the preventive herbal formulae of the three studies are presented in Table 1.

Evidence of CHM Formula for Preventing H1N1 Influenza

Four studies were identified, including 3(22-24) randomized controlled trials (RCTs) and 1(25) non-randomized controlled clinical study. All the studies were conducted during the prevalence of H1N1 influenza in Chinese mainland and published in Chinese. In these studies, participants were exposed to high-risk environments, such as hospitals and schools where H1N1 influenza occurred. The total sample size was 25,636 with the largest one of 25,329.(25)

The testedherbal interventions included self-made herbal formulae and Chinese patent medicines [Qingjie Fanggan Granule (清解防感颗粒), Kangbingdu Oral Liquid (抗病毒口服液); Ganmao Qingre Granule (感冒清热胶囊)]; while in the control group, 1 study used placebo and 3 used blank control. The course of herbal formulae ranged from 3 to 7 days, while the follow-up ranged from 5 to 30 days. The outcome measure was infection rate of H1N1 infl uenza tested by laboratory serological diagnosis. One study reported that no adverse events occurred,(22) while the others did not report. The details of the characteristics of included trials are presented in Table 2.

The data on infection rate of H1N1 influenza from 4 studies were pooled in meta-analysis. The results showed that the infection rate in the herbal formulae group was signifi cantly lower than that in the control group [relative risk (RR) 0.36, 95% confi dence Interval (CI) 0.24–0.52, P<0.01]. A sensitivity analysis was conducted to exclude non-RCT and the results showed similar effect (RR 0.36, 95% CI 0.21–0.62, P<0.01, Figure 1).

Summary of Officially Issued CM Prevention Recommendations for COVID-19

Up to February 12, 2020, the National Health Commission of China has issued 5 versions of diagnosis and treatment programs for COVID-19, but none have included any content on CM prevention and control, but on treatment since the 3rd versions.(26)

Table 1. Ingredients of Herbal Formulae for Preventing SARS

Study	Latin name	Pinyin	Chinese name
Lau JT 2005[19]	*Folium mori*	Sangye	桑叶
	Flos chrysanthemi	Juhua	菊花
	Semen armeniacae amarum	Xingren	杏仁
	Fructus forsythia	Lianqiao	连翘
	Herba menthae	Bohe	薄荷
	Radix platycodonis	Jiegeng	桔梗
	Radix glycyrrhizae	Gancao	甘草
	Rhizoma phragmitis	Lugen	芦根
	Radix astragali	Huanggi	黄芪
	Radix saposhnikoviae	Fangfeng	防风
	Folium isatidis	Banlangen	板蓝根
	Radix scutellariae	Huangqin	黄芩
Xu JY 2006[20]	*Lonicerae Japonicae Flos*	Jinyinhua	金银花
	Radix astragali	Huanggi	黄芪
	Rhizoma Atractylodis Macrocephalae	Baizhu	白术
	Radix saposhnikoviae	Fangfeng	防风
	Glehniae Radix	Shashen	沙参
	Crystal sugar	Bingtang	冰糖
Zhang L 2005[21]	*Radix astragali*	Huanggi	黄芪
	Rhizoma Atractylodis Macrocephalae	Baizhu	白术
	Radix saposhnikoviae	Fangfeng	防风
	Cyrtomium fortune J. Sm.	Guanzhong	贯众
	Isatidis Folium	Daqingye	大青叶
	Radix Scutellariae	Huangqin	黄芩
	Talcum	Huashi	滑石
	Radix glycyrrhizae	Gancao	甘草

Table 2. Characteristics of Included Trials of Herbal Formulae for H1N1 Influenza

Study	Design type	Population	Average age (Year)	Sample size (Case, P/C)	Herbal intervention	Control	Course (d)	Follow up (d)	Outcome
Song YP 2019[22]	RCT	Population in close contact with H1N1 influenza patients; high-risk population	P: 25.6 ± 14.2 C: 27.1 ± 14.5	200 (100/100)	Qingjie Fanggan Granule	Placebo	3	30	Infection rate; adverse event
Liu L 2013[23]	RCT	Medical staff	P: 30.5 ± 5.3 C: 31.4 ± 4.7	3 (28/25)	Decoction of self-made formula[#1]	Blank	7	10	Infection rate
Xia BL 2010[24]	RCT	Population in close contact with H1N1 influenza patients	23.5 (18–26)	54 (27/27)	Kangbingdu Oral Liquid; Ganmao Qingre Granule	Blank	3	14	Infection rate
Liu BL 2010[25]	CCT	Student	Not report	25329 (23947/1382)	Decoction of self-made formula[#2]	Blank	5	5	Infection rate

Notes: RCT: randomized controlled trial; CCT: controlled clinical trial; C: control group; P: prevention group. Ingredients of formulae: [#1] *Amebiae Radix* (Zicao), *Herba Menthae* (Bohe), and *Radix Glycyrrhizae* (Gancao). [#2] *Cyrtomium Fortune* J. Sm (Guanzhong), *Lonicerae Japonicae Flos* (Jinyinhua), *Fructus Forsythiae* (Lianqiao), *Folium Isatidis* (Banlangen), *Fructus Arctii* (Niubangzi), *Herba Agastaches* (Huoxiang), *Lophatheri Herba* (Zhuye), *Radix Glycyrrhizae* (Gancao), and *Isatidis Folium* (Daqingye).

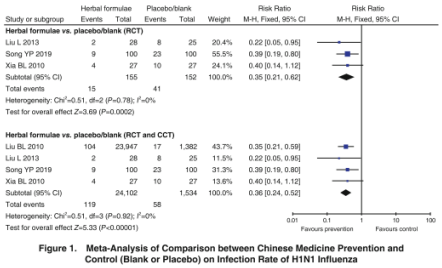

Figure 1. Meta-Analysis of Comparison between Chinese Medicine Prevention and Control (Blank or Placebo) on Infection Rate of H1N1 Influenza

Of the 31 provinces (including autonomous regions, and municipalities) in Chinese mainland, health authorities in 23 provinces had offi cially issued programs recommending herbal formulae to preventing COVID-19. These 23 provinces cover 7 regions of mainland: Northeast, North, Central (including Wuhan, Hubei Province, the original outbreak of COVID-19), South, East, Northwest, and Southwest China. All programs were formulated by clinical experts organized by local health authorities according to local geographic and climate characteristics and COVID-19 prevalent conditions. The earliest program recommending CM for prevention was issued by Sichuan Province on January 21, 2019. Ten provinces have updated their programs since the fi rst announcement, 7 of them have issued the 2nd edition and 3 issued the third edition. The applicable population of preventive programs included general and special population (such as the elderly, children, pregnant women, patients with chronic comorbidity diseases). Different groups of populations had specifi ed preventive CM formulae. The programs issued by the 23 provinces included CM formulae ranging from 1 up to 10, with an average of 3.4 per program. With regard to the course of CM formulae for prevention, 11 provinces recommended from 3 to 14 days, while 12 provinces did not mention. In addition, Tibet Autonomous Region recommended Tibetan medicine and Guizhou province recommended Miao medicine formulae (one of the minority folk medicines). The basic characteristics of 23 provincial programs are shown in Appendix 1.

We counted the frequency of the herbs used in CM formulae for prevention of general population issued by the 23 provinces. The results showed that these formulae contained 54 different herbs, of which 19 herbs with a frequency of use for 3 or more times in preventive formulae for general population (Figure 2). The top two were Radix astragali (Huangqi) and Glycyrrhizae Radix Et Rhizoma (Gancao).

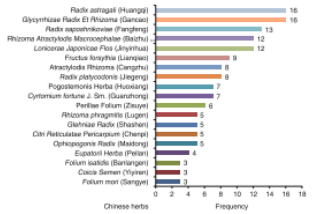

Figure 2. Frequency of Commonly Used Herbs in Preventive Formulae for COVID-19

DISCUSSION

As a new emerging acute respiratory infectious disease, COVID-19 lacks effective methods to control and treat the infection. It is urgent and reasonable to explore effective intervention strategies from traditional medicine for its prevention. This study examines the historical records for infection prevention in CM, as well as previous clinical evidence on CM prevention for similar public health emergencies such as SARS and H1N1 influenza. Recorded literature showed that the use of CM to prevent epidemics of infectious diseases can be traced back to ancient CM practice over thousands of years, and its successful effects were preliminarily substantiated by modern human clinical researches when applied to SARS and H1N1 influenza epidemics suggesting that historical CM experience is a worthwhile approach.

Based on the comprehensive analyses of the prevention programs issued by 23 provinces since the COVID-19 outbreak, we found that the main CM principles in preventing COVID-19 were to tonify qi to protect and provide defense from external pathogens, disperse wind and discharge heat, and resolve dampness with aroma. It was also similar to the characteristics of CHM formulae for preventing "pestilence" in ancient times and SARS in 2003.(17,19)

The 6 most commonly used herbs were Astragali Radix (Huangqi), Glycyrrhizae Radix Et Rhizoma (Gancao), Saposhnikoviae Radix (Fangfeng), Atractylodis Macrocephalae Rhizoma (Baizhu), Lonicerae Japonicae Flos (Jinyinhua), and Forsythiae Fructus (Lianqiao). Astragali Radix (Huangqi), Saposhnikoviae Radix (Fangfeng), and Atractylodis Macrocephalae Rhizoma (Baizhu) are all ingredients of a classical herbal formula Yupingfeng Powder, for tonifying qi to protect from external pathogens. In Lao, et al's controlled study (19) of CM formula

for preventing SARS, Yupingfeng Powder was also the main ingredients. Some studies have confirmed that Yupingfeng Powder has antiviral, anti-inflammatory and immunoregulatory effects.(27,28)

Japonicae Flos (Jinyinhua) and Forsythiae Fructus (Lianqiao) are the core components of Yinqiao Powder, which is a classical formula used to prevent and treat respiratory infectious diseases in ancient.(29)

An experimental study found that the effect of Yinqiao Powder (银翘 散) in preventing and treating upper respiratory tract infection could be explained by its antibacterial and antiviral properties and improvement of the function of upper respiratory mucosal immune system.(30)

A multicenter, large-scale, randomized trial found that Yinqiao Powder plus another heat-clearing formula could reduce time to fever resolution in patients with the infl uenza virus infection.(29)

At present, the National Health Commission of China has not issued a CM prevention program for COVID-19. The reasons may be, first, according to the CM theory of three-factors concerned treatment (Sanyin Zhiyi, 三因制宜), due to the differences of individual, regional, and seasonal factors in the occurrence and distribution of diseases, these factors should be considered in prevention and treatment,(31,32) and second, lack of solid evidence of CM formula for COVID-19. By comparing and analyzing the prevention programs issued by provincial levels, we also found that there was slight regional difference in the recommended herbal formulae and prescription principles. For example, due to the dry climate in northern China, there are additional one or two herbs for nourishing yin in the formula, like Glehniae Radix (Shashen) and Ophiopogonis Radix (Maidong), while in the south, due to the humid climate, aromatic herbs with the function of resolving dampness and turbidity are used in the formulae, like Pogostemonis Herba (Huoxiang) and Eupatorii Herba (Peilan).

Individual difference was also considered in the prevention programs in some provinces. There were two or more formulae recommended in 18 provinces' programs, which were applicable for different populations, such as the elderly, children, pregnant women, or patients with chronic comorbidity diseases, population in close contact with COVID-19 patients, etc. In addition, 7 provinces or province- level municipality (Beijing, Tianjin, Shanxi, Henan, Hunan, Shandong, Yunnan) recommended formulae according to the types of CM body constitutions of the population. This tailored prevention strategy might help to improve the preventive effect.

We suggest that the safety should also be paid attention to when taking CHM formula to prevent COVID-19, especially when they are used for long period. The public should choose the prescriptions under the guidance of CM doctors according to the program issued by provincial health authorities, and avoid taking the prescriptions or herbs with unknown origin and without offi cially approval. It should also be noted that the prevention advice for taking decoction were not reported in the 12 provinces' program. According to the programs of other provinces, it is appropriate to take the decoction for 1 week.

Based on the consideration of health economics and balance of risks and benefits, we do not recommend that all people should take CHM to prevent COVID-19. Due to the highly contagion,(33,34) high-risk populations exposed to COVID-19 patients, including medical personnel, family members, and other people who are in close contact with COVID-19 patients, as well as residents living in COVID-19 outbreak areas, would probably benefit from taking CHM formulae for prevention. These formulae recommended in the prevention programs are easily available in pharmacies and hospitals across the country.

As there is no direct clinical evidence for the prevention of the new emerging COVID-19, currently reported researches were from previous literature on the prevention of SARS and H1N1 influenza by CM which can only be considered as indirect evidence to refer to the current outbreak. Thirdly, the prevention programs for preventing COVID-19 were issued shortly after the outbreak, which were formulated by CM experts based on their previous experience in the prevention and treatment of similar diseases and their initial understanding of the disease; therefore, the actual effect of these programs needs to be verifi ed in clinical application, and updated and improved according to the evidence of new researches on COVID-19.

For future studies, we recommend prospective cohort studies, RCTs or registry studies to evaluate the effect of CHM formulae in prevention of COVID-19. At present, as the COVID-19 has not yet been controlled, we expect that a series of prospective population studies with rigorous design and large sample should commence with protocol registration, ethical approval, and implementation in a timely manner, to produce reliable evidence for CM prevention of COVID-19 or similar emerging respiratory infectious diseases in the future.

In conclusion, based on historical records and clinical evidence of SARS and H1N1 infl uenza prevention, CHM formula could be an alternative approach for the prevention of COVID-19 in high-risk population while waiting for the development of a successful vaccine. Prospective well design population studies are needed to evaluate the preventive effect of CM.

Conflicts of Interest

The authors declare that they have no competing interest.

Author Contributions

Luo H, Tang QL, and Liu JP conceived of the design and carried out the study. Tang QL undertook the literature review of historical evidence and assisted in writing the manuscript. Shang YX and Liang SB translated and assisted in analyzing Chinese data. Yang M provided suggestions for the design of study. Luo H undertook the literature review of prevention programs and wrote the manuscript. Liu JP supervised the study and revised the manuscript. Robinson N revised the manuscript and provided important perspectives. All authors read and approved the final manuscript. Luo H and Tang QL contributed equally to this work.

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Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has spread worldwide through person-to-person contact, causing a public health emergency of international concern. At present, there is no specific antiviral treatment recommended for SARS-CoV-2 infection. Liu Shen capsule (LS), a traditional Chinese medicine, has been proven to have a wide spectrum of pharmacological properties, such as anti-inflammatory, antiviral and immunomodulatory activities. However, little is known about the antiviral effect of LS against SARS-CoV-2. Herein, the study was designed to investigate the antiviral activity of SARS-CoV-2 and its potential effect in regulating the host's immune response. The inhibitory effect of LS against SARS-CoV-2 replication in Vero E6 cells was evaluated by using the cytopathic effect (CPE) and plaque reduction assay. The number of virions of SARS-CoV-2 was observed under transmission electron microscope after treatment with LS. Proinflammatory cytokine expression levels upon SARS-CoV-2 infection in Huh-7 cells were measured by real-time quantitative PCR assays. The results showed that LS could significantly inhibit SARS-CoV-2 replication in Vero E6 cells, and reduce the number of virus particles and it could markedly reduce pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, IL-8, CCL-2/MCP-1 and CXCL-10/IP-10) production at the mRNA levels. Moreover, the expression of the key proteins in the NF-κB/MAPK signaling pathway was detected by western blot and it was found that LS could inhibit the expression of p-NF-κB p65, p-IκBα and p-p38 MAPK, while increasing the expression of IκBα. These findings indicate that LS could inhibit SARS-CoV-2 virus infection via downregulating the expression of inflammatory cytokines induced virus and regulating the activity of NF-κB/MAPK signaling pathway in vitro, making its promising candidate treatment for controlling COVID-19 disease.

1. Introduction

Coronaviruses (CoVs) are a group of enveloped viruses named for their coronary appearance with positive single-stranded RNA genomes that infect animal hosts [1]. Many of the coronaviruses were recognized as viruses typically causing pneumonia and colds until the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 from zoonotic sources [2]. In addition, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing severe acute respiratory disease emerged recently in Wuhan, China in December 2019 [3,4]. Like the other two highly pathogenic coronaviruses SARS-CoV and MERS-CoV, SARS-CoV-2 can also cause severe respiratory illness and even death. Moreover, the population's susceptibility to these highly pathogenic coronaviruses has contributed to large outbreaks and evolved into the public health events, highlighting the necessity to prepare for the future emerging viruses [5].

Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 spreads rapidly among humans and likely originates in bats [3,6]. The initial patient cluster with confirmed SARS-CoV-2 infection was reported as pneumonia with unknown aetiology, which bore some resemblance to both SARS-CoV and MERS-CoV infections and was associated with ICU admission and high mortality. Moreover, patients requiring ICU admission had higher concentrations of G-CSF, IP-10, MCP-1, MIP-1A, and TNF-α than did those not requiring ICU admission, suggesting that the cytokine storm was associated with disease severity [7]. To date the severity of SARS-CoV-2 has tended to be mild, the risk of fatality among hospitalized cases was 4.3 % in a single-center case series of 138 hospitalized patients [8], and the infection fatality risk could be below 1% or even below 0.1 % in a large number of undetected relatively mild infections [9]. However, the basic reproduction number (R_0) of person-to-person spread was about 2.6, which meant that the cases of infection would grow at an exponential rate. As of 7 February, 2020, 57,620 cases of SARS-CoV-2 have been reported in China, including 26,359 suspected cases, and a sustained increase is predicted. It is challenging to judge the severity and predict the consequences of the information available to date. Since no specific antiviral treatment for SARS-CoV-2 infection is currently available, supportive cares, including symptomatic controls and prevention of complications remain the most important management strategy, especially in preventing acute respiratory distress syndrome [4,10]. Currently, there is no special antiviral treatment or vaccine against the new virus. Therefore, it is of great importance to research and develop effective and safe antiviral drugs.

It has been reported that many traditional Chinese medicine (TCM) prescriptions could not only inhibit the replication of virus directly, but also attenuate excessive pro-inflammatory responses and tissue damage by viruses [[11], [12], [13]]. Therefore, it is significantly to study the traditional Chinese medicines that have obvious advantages in the treatment of the new virus. Liu Shen capsule (LS), a traditional Chinese medicine, has been used to treat influenza, tonsillitis, pharyngitis, and mumps for more than a century [14]. It consists of Bezoar (the gall-stone of Bos taurus domesticus Gmelin), Musk (the excretion of Moschus), cinobufagin venom toad (the excretion of Venenum Bufonis), pearl (the shell of Pernulo), realgar, and borneol. In previous studies, it was shown that the LS exhibited a wide spectrum of pharmacological properties, such as anti-inflammatory, anticancer, antiviral, analgesic, antibacterial, and immunomodulatory activities [15,16]. In addition, LS could inhibit viral propagation and regulate immune function and achieved similar therapeutic effectiveness with oseltamivir in reducing the course of H1N1 virus infection. Notably, the anti-influenza activity of LS in infected mice might depend on the regulation of cytokines, particularly in cytokine storm associated cytokines, such as IFN-γ, IL-1β, IL-6, and TNF-α [17]. However, there is no exact evidence that LS is effective in the treatment of SARS-CoV-2 and its mechanisms of action remain obscure. Therefore, to study the antiviral activity of LS on SARS-CoV-2 and its potential effect in regulating the host's immune response, we evaluated the antiviral and anti-inflammatory efficiency of LS against a clinical isolate of SARS-CoV-2 from Guangzhou *in vitro*.

In this study, a comprehensive evaluation of the antiviral and anti-inflammatory activity of LS was performed *in vitro* with SARS-CoV-2 infection. It demonstrated that LS inhibited the replication of the virus in a dose-dependent manner and found that LS could reduce the number of viral particles. Moreover, LS could markedly decrease pro-inflammatory cytokine expression in infected human hepatocellular carcinoma cell lines (Huh-7), which may result in gaining a comprehensive understanding of the inhibition of LS against SARS-CoV-2 infection.

2. Methods

2.1. Reagents

LS (lot: SA01004C) used in this study was produced and provided by Leiyunshang Pharmaceutical Group Co., Ltd. (Suzhou, China). The LS was triturated, 50 mg was prepared in 10 mL dimethyl sulfoxide (DMSO). The mixture was ultrasonicated for 4 h and then centrifuged at 4000 *g* for 15 min. The supernatant was filtered through a 0.22 μm syringe filter before use. In the previous study, the index components in LS were detected by high-performance liquid chromatography (HPLC). HPLC revealed that LS contained 0.12 % gamabufotalin, 0.10 % arenobufagin, 0.26 % telocinobufagin, 0.21 % desacetylcinobufotalin, 0.25 % bufotalin, 0.41 % cinobufotalin, 0.27 % bufalin, 0.70 % resibufogenin, 0.68 % cinobufagin, 1.81 % cholic acid, 0.27 % anserine deoxycholic acid, and 0.23 % deoxycholic acid [18]. Remdesivir was kindly provided by Prof. Jiancun Zhang from Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences and was dissolved in DMSO to 100 μg/mL and stored at −20 °C before using. IκBα rabbit monoclonal (lot: 4812), p-IκBα rabbit monoclonal (lot: 2859), NF-κB p65 rabbit monoclonal (lot: 8242), p-NF-κB p65 rabbit monoclonal (lot:3033), p38 MAPK rabbit monoclonal (lot: 8690) and p-p38 MAPK rabbit monoclonal (lot: 4631) antibodies were provided by Cell Signaling Technology, Inc. (Danvers, MA, USA).

2.2. Cell lines and the virus

The African green monkey kidney epithelial (Vero E6) cells and human hepatocellular carcinoma cell lines (Huh-7) were purchased from ATCC. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) with 10 % fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. SARS-CoV-2 (Genebank accession no. MT123290.1) was clinical isolates from the First Affiliated Hospital of Guangzhou Medical University. The virus was propagated and adapted as previously described [19]. The 50 % tissue culture infective dose ($TCID_{50}$) of the virus was determined using the Reed Muench method ($TCID_{50}$ = 10^{-6}/100 μL). Virus stocks were collected and stored at −80 °C. All the infection experiments were performed in a biosafety level-3 (BLS-3) laboratory.

2.3. Cytotoxicity assay

The cytotoxic effects of LS or Remdesivir on Vero E6 and Huh-7 cells were evaluated by MTT assay [20]. Briefly, Vero E6 (5×10^4 cells/well) and Huh-7 (5×10^4 cells/well) cells grown in a monolayer in 96-well plates were rinsed with PBS followed by incubation with indicated concentrations of LS. After 72 h, the cells were stained with MTT solution at 0.5 mg/mL for 4 h. The supernatants were then removed, and the formed formazan crystals were dissolved in 200 μL dimethyl sulfoxide (DMSO). The absorbance at 570 nm was determined using a Multiskan Spectrum reader (Thermo Fisher, USA).

2.4. Cytopathic effect (CPE) inhibition assay

To investigate the antiviral effects of LS against SARS-CoV-2, the CPE inhibition assay under the nontoxic concentration of LS was employed. Briefly, the Vero E6 cell monolayers were grown in 96-well plates and inoculated with 100 $TCID_{50}$ of coronavirus strains at 37° C for 2 h. The inoculum was removed, and the cells were subsequently incubated with indicated concentrations of LS and the positive control Remdesivir. Following 72 h of incubation, the infected cells showed 100 % CPE under the microscope. The percentage of CPE in LS-treated cells was recorded. The 50 % inhibition concentration (IC_{50}) of the virus-induced CPE by LS was calculated as described and the selectivity index (SI) was determined from the CC_{50} to EC_{50} ratio [21].

2.5. Plaque reduction assay

The plaque reduction assay was performed as previously described [21]. Briefly, Vero E6 cells monolayers in 6-well plates were rinsed with PBS and incubated with 100 plaque-forming unit (PFU) of SARS-CoV-2. Following 2 h of incubation, the inoculum was removed, and the cells were covered with agar/basic medium mixture, which contained 0.8 % agar and indicated concentrations of LS or Remdesivir. The plates were then incubated at 37 ℃ for 48 h, followed by fixation in 4 % formalin for 30 min. The overlays were then removed and stained with 0.1 % crystal violet for 3 min. The plaques were visualized and counted. The IC_{50} of the virus-induced plaques by LS was calculated as described [21].

2.6. RNA isolation and reverse transcriptase-quantitative PCR analysis (RT-qPCR)

To further identify the possible underlying mechanisms of LS, we used several drug concentrations, with high antiviral efficiency for subsequent experiments. The primers of TNF-α, IL-6, CCL-2/MCP-1, IL-1β, IL-8, CXCL-10/IP-10 and GAPDH genes (Table 1) were designed by using Primer 5.0.

Table 1
Primer sequence for RT-qPCR.

Target Gene	Direction	Sequence (5′-3′)
IL-1β	Forward	GCACGATGCACCTGTACGAT
	Reverse	AGACATCACCAAGCTTTTTTGCT
	Probe	FAM-ACTGAACTGCACGCTCCGGGACTC-TAM
TNF-α	Forward	AACATCCAACCTTCCCAAACG
	Reverse	GACCCTAAGCCCCCAATTCTC
	Probe	FAM-CCCCCTCCTTCAGACACCCTCAACC-TAM
IL-6	Forward	CGGGAACGAAAGAGAAGCTCTA
	Reverse	CGCTTGTGGAGAAGGAGTTCA
	Probe	FAM-TCCCCTCCAGGAGCCCAGCT-TAM
MCP-1	Forward	CAAGCAGAAGTGGGTTCAGGAT
	Reverse	AGTGAGTGTTCAAGTCTTCGGAGTT
	Probe	FAM-CATGGACCACCTGGACAAGCAAACC-TAM
IP-10	Forward	GAAATTATTCCTGCAAGCCAATTT
	Reverse	TCACCCTTCTTTTTCAT-TGTAGCA
	Probe	FAM-TCCACGTGTTGAGATCA-TAM
IL-8	Forward	CTTGGTTTCTCCTTTATTTCTA
	Reverse	GCACAAATATTTGATGCTTAA
	Probe	FAM-TTAGCCACCATCTTACCTCACAGT-TAM
GAPDH	Forward	GAAGGTGAAGGTCGGAGTC
	Reverse	GAAGATGGTGATGGGATTTC
	Probe	FAM-CAAGCTTCCGTTCTCAGCC-TAM

Table 2
Inhibitory effect of LS and Remdesivir on coronavirus-infected Vero E6 cells.

Virus	LS (μg/mL)			Remdesivir (μM)		
	[a]TC_{50}	[b]IC_{50}	SI	[a]TC_{50}	[b]IC_{50}	SI
SARS-CoV-2	4.930	0.6024	8.18	105.60	0.6505	162.34

[a] The concentration of LS and Remdesivir required to reduce cell viability by 50 %.
[b] The concentrations of LS and Remdesivir required to inhibit virus proliferation by 50 %; [c]Selectivity index calculated as ratio of TC_{50} to IC_{50}.

Briefly, Huh-7 cell monolayer in 12-well plates were rinsed with PBS and then exposed to coronavirus at the multiplicity of infection (MOI) of 1 for 2 h. The inoculums were removed after infection, and the cells were divided into six groups: normal control group (NC), virus-infected group (virus), Remdesivir and three concentrations of LS. The cells were harvested at 48 h. Total RNA from the different groups was extracted according to the specification of the RNA reagent (Invitrogen, MA, USA), and reverse transcription of RNAs was quantified by using the PrimeScript™ RT Master Mix Kit (Takara Bio, Japan). Then, RT-PCR was performed on cDNA samples via the SYBR Premix Ex Tap™ II (Takara Bio, Japan). The PCR data were analysed using the detection system (ABI PRISM® 7500 Real-time PCR system, Applied Biosystems Co., USA). The relative amount of PCR products was calculated using the 2−ΔΔCt method as previously described [22].

2.7. Transmission electron microscope (TEM) observation of the surface of infected cells

Confluent monolayer culture of Vero E6 cells prepared in 6-well plates was inoculated with the virus and incubated at 37 °C for 2 h. At 2 h p.i., the culture was washed twice with DMEM to remove free viruses and incubated at 37 °C with or without 1 μg LS and Remdesivir. After 24 h, the cells were fixed with 2.5 % glutaraldehyde in 0.15 M phosphate buffer (pH 7.4) and post-fixed with 1% osmium tetroxide. The fixed cells were dehydrated through a series of ethanol and butanol mixtures [23], and observed under the JSM-6340 SEM (JEOL, Japan).

2.8. Western blot assay

The total proteins of the samples were extracted from the cells with radioimmunoprecipitation assay (RIPA) buffer (DGCS Biotechnology, China). The protein concentrations of the samples were detected by using the BCA kit (Beyotime, China). Then, 20 mg of the cell extract was separated by 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then they were transferred to a polyvinylidene fluoride (PVDF) membranes (Millipore, USA). The membranes were blocked with 5% BSA and incubated with different primary antibodies over night at 4 °C. And then, the membranes with different primary antibodies were incubated with different secondary antibodies for 1 h. The immune complexes were immunoblotted and the immunodetection was performed by using the enhanced chemiluminescence reagents (Fdbio, China).

2.9. Data analysis

All data in this study were analysed by using analysis of variance (ANOVA) with SPSS ver. 19.0 (Armonk, NY, USA). Data were presented as the mean ± standard deviation (SD). Differences in multiple groups were determined by one-way ANOVA with Tukey's honest significant difference (HSD) test. A P-value＜0.05 was considered statistically significant.

3. Result

3.1. Antiviral activity of LS on SARS-CoV-2 *in vitro*

The cytotoxicity of LS in Vero E6 cells was first evaluated by non-radioactive cell proliferation assay (MTT). The TC50 values, corresponding to a 50 % cytotoxic effect after 72 h of inhibitor treatment, were determined. The TC50 of LS and Remdesivir towards Vero E6 cells was 4.930 μg/mL and 105.60 μM, respectively, and the TC50 of LS and Remdesivir towards Huh-7 cells was 3.382 μg/mL and 138.40 μM, respectively (Table 2). As shown in Fig. 1, LS showed unapparent cytotoxicity for the cell lines at concentrations up to 2.0 μg/mL, and Remdesivir was chosen as the positive control and showed no cytotoxicity to cell lines at a concentration of 50 μM. The antiviral activities of LS and Remdesivir against SARS-CoV-2 were evaluated using cytopathic effect (CPE) inhibition assay (Fig. 1) and plaque reduction assay (Fig. 2). SARS-CoV-2 caused a severe CPE in Vero E6-infected cells, including cell rounding, detachment and death. A reduction in SARS-CoV-2-induced CPE after 72 h incubation indicated the antiviral activity of LS and Remdesivir. It was found that LS (2 μg/mL, 1 μg/mL and 0.5 μg/mL) and Remdesivir (5 μM and 2.5 μM) significantly reduced the CPE caused by infection in Vero E6 cells, and the IC50 values of LS and Remdesivir were 0.6024 μg/mL and 0.6505 μM, respectively, and the selectivity index (SI) of LS and Remdesivir was 8.18 and 203.84, respectively. The results showed that LS was able to protect cells from virus-induced cell death in a dose-dependent manner.

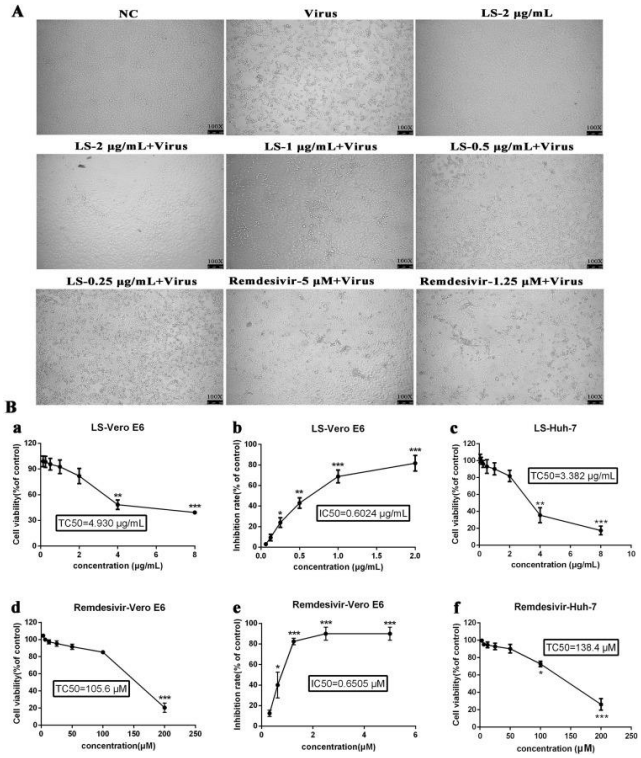

Fig. 1. Reduction of SARS-CoV-2-induced cytopathic effect by LS. A. Vero E6 cells were not-infected (NC) or infected with SARS-CoV-2 and the inhibitory effect of LS and Remdesivir on virus proliferation was evaluated. Images under a NiKon Eclipse TE300 microscope (NiKon Corporation, Tokyo, Japan) at 100 magnification. B. Inhibiting the activity of SARS-CoV-2 when given different concentrations in vitro. (a) The cytotoxicity effects of LS in Vero E6 cells were detected using MTT assay. (b) The inhibitory effects of LS on SARS-CoV-2 in Vero E6 cells. (c) The cytotoxic effects of LS in Huh-7 cells were detected using MTT assay. (d) The cytotoxic effects of Remdesivir in Vero E6 cells were detected using MTT assay. (e) The inhibitory effects of Remdesivir on SARS-CoV-2 in Vero E6 cells. (f) The cytotoxic effects of Remdesivir in Huh-7 cells were detected using MTT assay. Error bars indicate the range of values obtained from counting in triplicate are represented as the mean ± SD of three individual experiments.

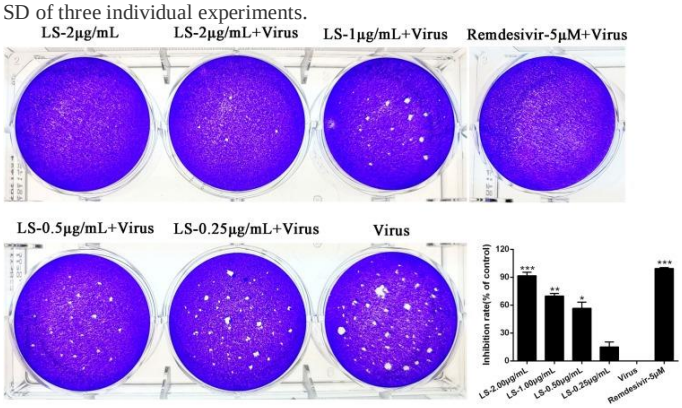

Fig. 2. Dose-dependent reduction of SARS-CoV-2 plaque formation after treatment with LS. (A) Inhibitory effect of LS or Remdesivir on plaque formation of SARS-CoV-2. (B) The quantitative analysis of the plaque formation in different groups was analysed by SPSS ver. 19.0. Data are presented as the mean ± SD obtained from three separate experiments. *p < 0.05; **p < 0.01; ***p < 0.001, compared with SARS-CoV-2-infected cells.

The plaque reduction assay was carried out to confirm the efficacy of LS on SARS-CoV-2 propagation. Vero E6 cells were infected with SARS-CoV-2 (MOI = 0.1) and incubated with overlay medium containing various concentrations of LS. After three days, the overlays were then removed and stained with 0.1 % crystal violet. The plaques were visualized and counted. Remdesivir was used as a positive control at 5 μM. The results

showed the average size and plaque number in LS-treated cells were markedly reduced in a dose-dependent manner and LS (2.00, 1.00, 0.50 μg/mL) and Remdesivir (5 μM) could significantly inhibit the viral plaque formation ($p < 0.05$) (Fig. 2). The above assays showed that LS might be a potential antiviral drug for SARS-CoV-2.

3.2. LS strongly inhibits the virion in Vero E6 cells by TEM

To verify the antiviral efficacy on SARS-CoV-2, TEM assay was performed in the infected-cells which were treated with LS (2.00 μg/mL) or Remdesivir (5 μM) (Fig. 3). The results showed that no virus particles were found in the cell control group (NC) (Fig. 3A and E) and many virions were found in the cytoplasm, intracellular vesicles, and cell membrane and presented typical coronavirus morphology in the virus group under electron microscopy after 48 h p.i. (Fig. 3B and F). Treatment with LS (2.00 μg/mL) (Fig. 3C and G) and Remdesivir (5 μM) (Fig. 3D and H) resulted in a reduction of the number of virions and inhibited the entrance into the intracellular vesicles of the virus compared with the virus group.

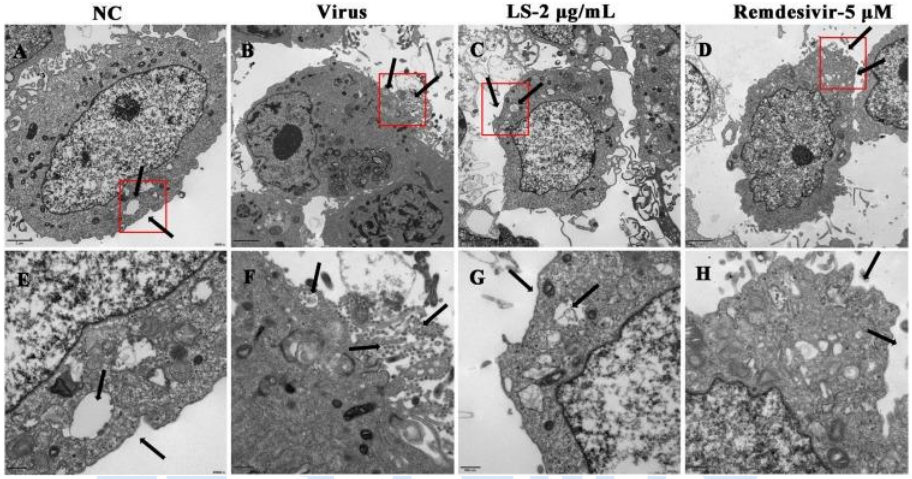

Fig. 3. Effect of LS on virus morphology in Vero E6 cells. (A, E) uninfected cells (NC), (B, F) SARS-CoV-2 infected cells (Virus), (C, G) LS-treated infected cells and Remdesivir-treated infected cells. The red boxes and black arrows indicated changes in the number of virus particles after treatment with or without LS and Remdesivir. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.3. LS strongly inhibits the expression of proinflammatory cytokines *in vitro*

SARS-CoV-2 infection is known to be able to induce a strong inflammatory reaction, hallmarked by the production of cytokines and chemokines. Therefore, the expression of cytokines on the infected cells was measured. To determine the influence of LS or Remdesivir (5 μM) on the expression of pro-inflammatory cytokines induced by SARS-CoV-2, the mRNA and protein expression of IL-6, TNF-α, IL-1β, CXCL10/IP-10, CCL2/MCP-1 and IL-8 in Huh-7 cells were detected by RT-qPCR and ELISA. As shown in Fig. 4, Fig. 5, the mRNA and protein expression of IL-6, TNF-α, IL-1β, CXCL10/IP-10, CCL2/MCP-1 and IL-8 in the virus group were significantly up-regulated 48 h after infection in the Huh-7 cells infected by SARS-CoV-2 ($p < 0.01$) compared with that in the NC group. Compared with the virus group, LS and Remdesivir significantly reduced the mRNA and protein expression of IL-6, TNF-α, IL-1β, CXCL10/IP-10, CCL2/MCP-1 and IL-8 in a dose-dependent manner in Huh-7 cells 48 h after infection ($p < 0.01$ or $p < 0.05$), respectively. This indicated that LS might be an effective anti-inflammatory agent.

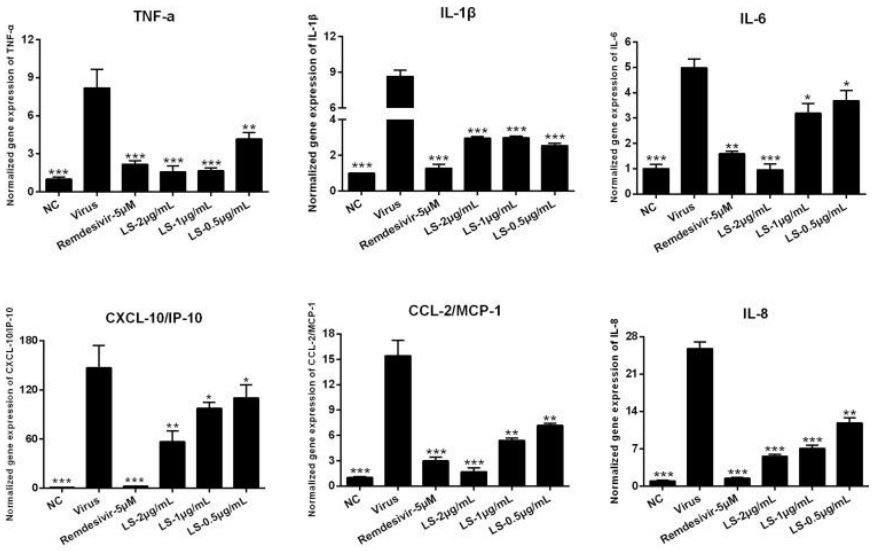

Fig. 4. Effects of treatment with LS or Remdesivir on the mRNA expression levels of inflammatory mediators in SARS-CoV-2-infected Huh-7 cells. TNF-α, IL-1β, IL-6, CXCL-10/IP-10, CCL-2/MCP-1 and IL-8. Data are presented as the mean ± SD obtained from three separate experiments. *p < 0.05; **p < 0.01; ***p < 0.001, compared with SARS-CoV-2-infected cells.

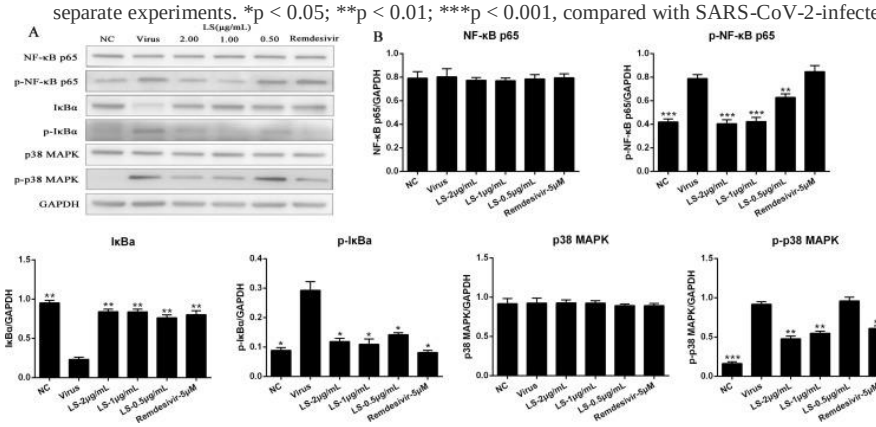

Fig. 5. LS inhibited the inflammation induced by the virus through regulating the NF-κB/MAPK signaling pathway *in vitro*. (A) The protein expressions of NF-κB p65, p-NF-κB p65, p-IκBα, IκBα, p-p38 MAPK and p38 MAPK in the cells was detected by western blot analysis; (B) The quantitative analysis of the NF-κB p65, p-NF-κB p65, p-IκBα, IκBα, p-p38 MAPK and p38 MAPK proteins was analysed by Image J. The values are presented as the means ± SD of three individual experiments. *p < 0.05; **p < 0.01; ***p < 0.001, when compared to the viral control.

3.4. LS strongly inhibits the expression of the key proteins related to the NF-κB/MAPK signaling pathway *in vitro*

To further study whether the antiviral and anti-inflammation mechanisms induced by virus of LS was related to the inhibitory of NF-κB/MAPK signaling pathway activation, the expression of the key proteins related to the NF-κB/MAPK signaling pathway was examined. As shown in Fig. 5, the protein expressions of p-NF-κB p65, p-IκBα, and p-p38 MAPK of the virus group were significantly increased compared with the NC group ($p < 0.05$), and the expression of IκBα of the virus group were significantly decreased compared with the NC group ($p < 0.05$). Compared with the virus group, the protein expression of the p-NF-κB p65, p-p38 MAPK and p-IκBα were significantly reduced in Huh-7 cells with LS (2.0, 1.0, and 0.5 μg/mL) and Remdesivir, and IκBα was significantly upregulated, while the expression of NF-κB p65 and p38 MAPK was no significant difference.

4. Discussion

The number of infections caused by SARS-CoV-2 continues to rise sharply in the world [24,25]. The viral infection has widely and rapidly spread worldwide [26]. It is a major coronavirus infection, which threatens human life after SARS and MERS [27]. For this sudden and lethal disease, no specific antiviral drugs or vaccines have been developed, and supportive care and non-specific treatment of patients are currently the

only options to ameliorate the symptoms [[28], [29], [30]]. Therefore, effective and safe antiviral agents are urgently needed. Currently, the use of TCM is a popular and acceptable therapy, and many TCM prescriptions have been proven to have obvious therapeutic effect on the virus by inhibiting the replication of the virus directly and improving the immune functions of the host organism. It may be a potential effective drug source for new drug discovery. LS is a TCM used to treat tonsillitis, influenza, pharyngitis, and mumps. However, the antiviral and anti-inflammatory effects induced by SARS-CoV-2 and the potential mechanisms of LS in treating viral pneumonia are less understood. Therefore, the potential antiviral and anti-inflammatory activities of LS induced by SARS-CoV-2 were investigated. In the present study, it was the first time to clarify that LS could not only inhibit SARS-CoV-2 infection, but also significantly suppress the inflammation caused by SARS-CoV-2 through a mechanism that involved the downregulation of inflammatory cytokines.

First, the effects of LS against SARS-CoV-2 *in vitro* by CPE assay and plaque reduction assay (Fig. 1, Fig. 2) were measured. The results showed that LS was able to protect cells from virus-induced cell death and inhibit the average size and plaque number in LS-treated cells in a dose-dependent manner. The SI index of LS reached 8.18 (Table 1). All these results confirmed that LS might be a potential antiviral drug for SARS-CoV-2. TEM has been a potent tool to observe virus entry, virus particle assembly, viral ultrastructure, and budding from the plasma membrane [31]. To understand the antiviral details of LS, TEM images were taken from each group (Fig. 3). Abundant virus particles assembled at the surface of the membrane, cytoplasm, and plasma vesicles in cells infected with SARS-CoV-2, decreased with the treatment of LS at 2.00 μg/mL.

It is reported that highly pathogenic coronaviruses such as SARS-CoV and MERS-CoV cause fatal pneumonia, which is mainly associated with rapid virus replication, massive inflammatory cell infiltration and elevated proinflammatory cytokine/chemokine responses. Although the pathophysiology of fatal pneumonia caused by highly pathogenic coronaviruses has not been completely understood, recent studies suggest a crucial role of cytokine storm in causing fatal pneumonia [32]. Early studies have shown that increased amounts of proinflammatory cytokines (e.g., IL-1β, IL-6, CXCL-10/IP-10, and CCL-2/MCP-1) in the serum of SARS patients [33], which was similar to the serum patients with MERS with increased concentrations of proinflammatory cytokines (i.e., IFN-γ, TNF-α, IL-15, and IL-17) [34]. It also reported that the expression of the cytokine storm in the NCIP patients in ICU than those in non-ICU patients [7]. Therefore, the mRNA and protein production of IL-1β, TNF-α, CCL-2/MCP-1, CXCL-10/IP-10, IL-6 and IL-8 induced by SARS-CoV-2 was detected. The results showed that LS inhibited the release of IL-1β, TNF-α, CCL-2/MCP-1, CXCL-10/IP-10, IL-6 and IL-8 induced by SARS-CoV-2 in Huh-7 cells (Fig. 4, Fig. 5) in a dose-dependent manner. The change of cytokine profiles suggested that LS might have a potential effect on the inhibition of the cytokine storm induced by SARS-CoV-2.

Since the SARS syndrome is characterized by an uncontrolled inflammatory response and NF-κB is the major transcription factor activated in acute respiratory distress syndrome (ARDS) [35]. Similar to SARS-CoV, SARS-CoV-2 can also cause severe respiratory illness and even death. NF-κB plays an important role in mediating inflammation, immune responses, and other cellular activities [36]. Activation of NF-κB can induce cytokines production, and these cytokines can react upon in turn and they produce a positive autoregulatory loop and exacerbate the inflammatory response [37]. Therefore, to understand the molecular mechanism of LS against virus infection, the regulation of the NF-κB/MAPK signaling pathway contributes to the alleviation of inflammation. The results showed that SARS-CoV-2 activated the NF-κB /MAPK signaling pathway and LS could significantly decrease SARS-CoV-2-induced activation of p-NF-κB p65, p-IκBα, and p-p38 MAPK and increase the expression of the IκBα (Fig. 5). We inferred that the underlying mechanism of LS is to impair the upregulated proinflammatory cytokines induced by SARS-CoV-2 via inhibiting the activity of NF-κB/MAPK signaling pathway. The change of cytokine profiles suggested that LS might have a potential effect on the inhibition of cytokine storm induced by SARS-CoV-2.

5. Conclusions

In conclusion, our results revealed that LS could significantly protect cells from virus-induced cell death and inhibit the average size and plaque number *in vitro*. The anti-SARS-CoV-2 effect was attributed to the blocking of the proliferation of virus, inhibiting the formation of virus particles, and inhibiting the upregulated expression of pro-inflammatory cytokines induced by SARS-CoV-2 via regulating the activity of NF-κB/MAPK signaling pathway. These findings warrant further evaluation of LS as a potential agent for

SARS-CoV-2 treatment and provide information to further reveal the mechanisms. LS may be an effective anti-inflammatory agent and can be used to treat the inflammation induced by SARS-CoV-2.

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Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)

(Released by National Health Commission & State Administration of Traditional Chinese Medicine on March 3, 2020)

Since December 2019, multiple cases of novel coronavirus pneumonia (NCP) have been identified in Wuhan, Hubei. With the spread of the epidemic, such cases have also been found in other parts of China and other countries. As an acute respiratory infectious disease, NCP has been included in Class B infectious diseases prescribed in the Law of the People's Republic of China on Prevention and Treatment of Infectious Diseases, and managed as an infectious disease of Class A. By taking a series of preventive control and medical treatment measures, the rise of the epidemic situation in China has been contained to a certain extent, and the epidemic situation has eased in most provinces, but the incidence abroad is on the rise. With increased understanding of the clinical manifestations and pathology of the disease, and the accumulation of experience in diagnosis and treatment, in order to further strengthen the early diagnosis and early treatment of the disease, improve the cure rate, reduce the mortality rate, avoid nosocomial infection as much as possible and pay attention to the spread caused by the imported cases from overseas, we revised the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 6) to Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7).

I. Etiological Characteristics

The novel coronaviruses belong to the β genus. They have envelopes, and the particles are round or oval, often polymorphic, with diameter being 60 to 140 nm. Their genetic characteristics are significantly different from SARS-CoV and MERS-CoV. Current research shows that they share more than 85% homology with bat SARS-like coronaviruses (bat-SL-CoVZC45). When isolated and cultured in vitro, the 2019-nCoV can be found in human respiratory epithelial cells in about 96 hours, however it takes about 6 days for the virus to be found if isolated and cultured in Vero E6 and Huh-7 cell lines. Most of the know-how about the physical and chemical properties of coronavirus comes from the research on SARS-CoV and MERS-CoV. The virus is sensitive to ultraviolet and heat. Exposure to 56°C for 30 minutes and lipid solvents such as ether, 75% ethanol, chlorine-

containing disinfectant, peracetic acid, and chloroform can effectively inactivate the virus. Chlorhexidine has not been effective in inactivating the virus.

II. Epidemiological Characteristics

1. Source of infection Now, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. 2. Route of transmission Transmission of the virus happens mainly through respiratory droplets and close contact. There is the possibility of aerosol transmission in a relatively closed environment for a long-time exposure to high concentrations of aerosol. As the novel coronavirus can be isolated in feces and urine, attention should be paid to feces or urine contaminated environmental that leads to aerosol or contact transmission. 3. Susceptible groups People are generally susceptible.

III. Pathological changes

Pathological findings from limited autopsies and biopsy studies are summarized below: 1. Lungs Solid changes of varying degrees are present in the lungs. Alveolar damage involves fibromyxoid exudation and hyaline membrane formation. The exudates are composed of monocytes and macrophages, with plenty of multinucleated syncytial cells. Type II alveolar epithelial cells are markedly hyperplastic, some of which are desquamated. Viral inclusions are observed in type II alveolar epithelial cells and macrophages. Alveolar interstitium is marked with vascular congestion and edema, infiltration of monocytes and lymphocytes, and vascular hyaline thrombi. The lungs are laden with hemorrhagic and necrotic foci, along with evidence of hemorrhagic infarction. Organization of alveolar exudates and interstitial fibrosis are partly present. The bronchi are filled with desquamated epithelial cells, mucus and mucus plugs. Hyperventilated alveoli, interrupted alveolar interstitium and cystic formation are occasionally seen. On electron microscopy, cytoplasmic NCP virions are observed in the bronchial epithelium and type II alveolar epithelium. NCP virus antigen positivity in some alveolar epithelia and macrophages are revealed through immunohistochemistry staining, which are positive for NCP virus nucleic acid via RT-PCR. 2. Spleen, hilar lymph nodes and bone marrow The spleen is evidently shrunk. Lymphocytopenia and focal hemorrhage and necrosis are present. Macrophagocyte proliferation and phagocytosis are noted in the spleen. Lymph nodes are found with sparse lymphocytes and occasional necrosis. CD4+ and CD8+ T cells are present in reduced quantity in the spleen and lymph nodes, revealed by immunohistochemistry staining. Pancytopenia is identified in bone marrow. 3. Heart and blood vessels Degenerated or necrosed myocardial cells are present, along with mild infiltration of monocytes, lymphocytes and/or neutrophils in the cardiac interstitium. Endothelial desquamation, endovasculitis and thrombi are seen in some blood vessels. 4. Liver and gall bladder Appearing enlarged and dark-red, the liver is found degenerated with focal necrosis infiltrated with neutrophils. The liver sinusoids are found hyperemic. The portal areas are infiltrated with lymphocytes and monocytes and dotted with microthrombi. The gall bladder is prominently filled. 5. Kidneys The kidneys are noted with protein exudation in the Bowman’s capsule around glomeruli, degeneration and desquamation of the epithelial cells of renal tubules, and hyaline casts. Microthrombi and fibrotic foci are found in the kidney interstitium. 6. Other organs Cerebral hyperemia and edema are present, with degeneration of some neurons. Necrosis foci are noted in the adrenal glands. Degeneration, necrosis and desquamation of epithelium mucosae at varying degrees are present in the esophageal, stomach and intestine.

IV. Clinical Characteristics 1. Clinical manifestations Based on the current epidemiological investigation, the incubation period is one to 14 days, mostly three to seven days. Main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases. Severe cases mostly developed dyspnea and/or hypoxemia after one week. In severe cases, patients progress rapidly to acute respiratory distress syndrome, septic shock, metabolic acidosis that is difficult to correct, coagulopathy, multiple organ failure and others. It is worth noting that for severe and critically ill patients, their fever could be moderate to low, or even barely noticeable. Some children and neonatal cases may have atypical symptoms, manifested as gastrointestinal symptoms such as vomiting and diarrhea, or only

manifested as low spirits and shortness of breath. The patients with mild symptoms did not develop pneumonia but only low fever and mild fatigue. From current situations, most patients have good prognosis and a small number of patients are critically ill. The prognosis for the elderly and patients with chronic underlying diseases is poor. The clinical course of pregnant women with NCP is similar to that of patients of the same age. Symptoms in children are relatively mild.

2. Laboratory tests General findings In the early stages of the disease, peripheral WBC count is normal or decreased and the lymphocyte count decreases. Some patients see an increase in liver enzymes, lactate dehydrogenase (LDH), muscle enzymes and myoglobin. Elevated troponin is seen in some critically ill patients while most patients have elevated C-reactive protein and erythrocyte sedimentation rate and normal procalcitonin. In severe cases, D-dimer increases and peripheral blood lymphocytes progressively decrease. Severe and critically ill patients often have elevated inflammatory factors. Pathogenic and serological findings (1) Pathogenic findings: Novel coronavirus nucleic acid can be detected in nasopharyngeal swabs, sputum, lower respiratory tract secretions, blood, feces and other specimens using RT-PCR and/or NGS methods. It is more accurate if specimens from lower respiratory tract (sputum or air tract extraction) are tested. The specimens should be submitted for testing as soon as possible after collection. (2) Serological findings: NCP virus specific IgM becomes detectable around 3-5 days after onset; IgG reaches a titration of at least 4-fold increase during convalescence compared with the acute phase.

3. Chest imaging In the early stage, imaging shows multiple small patchy shadows and interstitial changes, apparent in the outer lateral zone of lungs. As the disease progresses, imaging then shows multiple ground glass opacities and infiltration in both lungs. In severe cases, pulmonary consolidation may occur while pleural effusion is rare.

V. Case Definitions 1. Suspect cases Considering both the following epidemiological history and clinical manifestations: 1.1 Epidemiological history 1.1.1 History of travel to or residence in Wuhan and its surrounding areas, or in other communities where cases have been reported within 14 days prior to the onset of the disease; 1.1.2 In contact with novel coronavirus infected people (with positive results for the nucleic acid test) within 14 days prior to the onset of the disease; 1.1.3 In contact with patients who have fever or respiratory symptoms from Wuhan and its surrounding area, or from communities where confirmed cases have been reported within 14 days before the onset of the disease; or 1.1.4 Clustered cases (2 or more cases with fever and/or respiratory symptoms in a small area such families, offices, schools etc within 2 weeks).

1.2 Clinical manifestations 1.2.1 Fever and/or respiratory symptoms; 1.2.2 The aforementioned imaging characteristics of NCP; 1.2.3 Normal or decreased WBC count, normal or decreased lymphocyte count in the early stage of onset. A suspect case has any of the epidemiological history plus any two clinical manifestations or or all three clinical manifestations if there is no clear epidemiological history.

2. Confirmed cases Suspect cases with one of the following etiological or serological evidences: 2.1 Real-time fluorescent RT-PCR indicates positive for new coronavirus nucleic acid; 2.2 Viral gene sequence is highly homologous to known new coronaviruses. 2.3 NCP virus specific Ig M and IgG are detectable in serum; NCP virus specific IgG is detectable or reaches a titration of at least 4-fold increase during convalescence compared with the acute phase.

VI. Clinical Classification

1. Mild cases The clinical symptoms were mild, and there was no sign of pneumonia on imaging.
2. 2. Moderate cases Showing fever and respiratory symptoms with radiological findings of pneumonia.
3. 3. Severe cases

<u>Adult cases meeting any of the following criteria</u>

(1) Respiratory distress (≧30 breaths/ min);

(2) Oxygen saturation≤93% at rest;

(3) Arterial partial pressure of oxygen (PaO2)/ fraction of inspired oxygen (FiO2)≦ 300mmHg (l mmHg=0.133kPa).

In high-altitude areas (at an altitude of over 1,000 meters above the sea level), PaO2/ FiO2 shall be corrected by the following formula: PaO2/ FiO2 x[Atmospheric pressure (mmHg)/760] Cases with chest imaging that showed obvious lesion progression within 24-48 hours >50% shall be managed as severe cases.

Child cases meeting any of the following criteria:

(1) Tachypnea (RR ≥ 60 breaths/min for infants aged below 2 months; RR ≥ 50 BPM for infants aged 2-12 months; RR ≥ 40 BPM for children aged 1-5 years, and RR ≥ 30 BPM for children above 5 years old) independent of fever and crying;

(2) Oxygen saturation ≤ 92% on finger pulse oximeter taken at rest;

(3) Labored breathing (moaning, nasal fluttering, and infrasternal, supraclavicular and intercostal retraction), cyanosis, and intermittent apnea;

(4) Lethargy and convulsion;

(5) Difficulty feeding and signs of dehydration.

4. Critical cases

Cases meeting any of the following criteria: 4.1 Respiratory failure and requiring mechanical ventilation; 4.2 Shock; 4.3 With other organ failure that requires ICU care.

VII. Clinical early warning indicators of severe and critical cases

1. Adults

1.1 The peripheral blood lymphocytes decrease progressively; 1.2 Peripheral blood inflammatory factors, such as IL-6 and C-reactive proteins, increase progressively; 1.3 Lactate increases progressively; 1.4 Lung lesions develop rapidly in a short period of time.

2. Children.

2.1 Respiratory rate increased; 2.2 Poor mental reaction and drowsiness; 2.3 Lactate increases progressively; 2.4 Imaging shows infiltration on both sides or multiple lobes, pleural effusion or rapid progress of lesions in a short period of time; 2.5 Infants under the age of 3 months who have either underlying diseases (congenital heart disease, bronchopulmonary dysplasia, respiratory tract deformity, abnormal hemoglobin, and severe malnutrition, etc.) or immune deficiency or hypofunction (long-term use of immunosuppressants).

VIII. Differential Diagnosis

1. The mild manifestations of NCP need to be distinguished from upper respiratory tract infections caused by other viruses. 2. NCP is mainly distinguished from other known viral pneumonia and mycoplasma pneumoniae infections such as influenza virus, adenovirus and respiratory syncytial virus. Especially for suspect cases, methods such as rapid antigen detection and multiplex PCR nucleic acid detection should be adopted as much as possible for detection of common respiratory pathogens. 3. It should also be distinguished from non-infectious diseases such as vasculitis, dermatomyositis and organizing pneumonia.

IX. Case Finding and Reporting Health professionals in medical institutions of all types and at all levels, upon discovering suspect cases that meet the definition, should immediately put them in single room for isolation and treatment. If the cases are still considered as suspected after consultation made by hospital experts or attending physicians, it should be reported directly online within 2 hours; samples should be collected for new

coronavirus nucleic acid testing and suspect cases should be safely transferred to the designated hospitals immediately. People who have been in close contact with patients who have been confirmed of new coronavirus infection are advised to perform new coronavirus pathogenic testing in a timely manner, even though common respiratory pathogens are tested positive. If two nucleic acid tests, taken at least 24-hour apart, of a NCP suspect case are negative, and the NCP virus specific IgM and IgG are negative after 7 days from onset, the suspect diagnosis can be ruled out.

X. Treatment 1. Treatment venue determined by the severity of the disease 1.1 Suspected and confirmed cases should be isolated and treated at designated hospitals with effective isolation, protection and prevention conditions in place. A suspect case should be treated in isolation in a single room. Confirmed cases can be treated in the same room. 1.2 Critical cases should be admitted to ICU as soon as possible.

2. General treatment 2.1 Letting patients rest in bed and strengthening support therapy; ensuring sufficient caloric intake for patients; monitoring their water and electrolyte balance to maintain internal environment stability; closely monitoring vital signs and oxygen saturation. 2.2 According to patients' conditions, monitoring blood routine result, urine routine result, c-reactive protein (CRP), biochemical indicators (liver enzyme, myocardial enzyme, renal function etc.), coagulation function, arterial blood gas analysis, chest imaging and cytokines detection if necessary. 2.3 Timely providing effective oxygen therapy, including nasal catheter and mask oxygenation and nasal high-flow oxygen therapy. If possible, inhalation of mixed hydrogen and oxygen (H_2/O_2: 66.6%/33.3%) can be applied. 2.4 Antiviral therapy: Hospitals can try Alpha-interferon (5 million U or equivalent dose each time for adults, adding 2ml of sterilized water, atomization inhalation twice daily), lopinavir/ritonavir (200 mg/50mg per pill for adults, two pills each time, twice daily, no longer than 10 days), Ribavirin (suggested to be used jointly with interferon or lopinavir/ritonavir, 500 mg each time for adults, twice or three times of intravenous injection daily, no longer than 10 days), chloroquine phosphate (500 mg bid for 7 days for adults aged 18-65 with body weight over 50 kg; 500 mg bid for Days 1&2 and 500 mg qd for Days 3-7 for adults with body weight below 50 kg), Arbidol (200 mg tid for adults, no longer than 10 days). Be aware of the adverse reactions, contraindications (for example, chloroquine cannot be used for patients with heart diseases) and interactions of the above- mentioned drugs. Further evaluate the efficacy of those drugs currently being used. Using three or more antiviral drugs at the same time is not recommend; if an intolerable toxic side effect occurs, the respective drug should be discontinued. For the treatment of pregnant women, issues such as the number of gestational weeks, choice of drugs having the least impact on the fetus, as well as whether pregnancy being terminated before treatment should be considered with patients being informed of these considerations. 2.5 Antibiotic drug treatment: Blind or inappropriate use of antibiotic drugs should be avoided, especially in combination with broad-spectrum antibiotics.

3. Treatment of severe and critical cases

3.1 Treatment principle: On the basis of symptomatic treatment, complications should be proactively prevented, underlying diseases should be treated, secondary infections also be prevented, and organ function support should be provided timely. 3.2 Respiratory support: 3.2.1 Oxygen therapy: Patients with severe symptoms should receive nasal cannulas or masks for oxygen inhalation and timely assessment of respiratory distress and/or hypoxemia should be performed. 3.2.2 High-flow nasal-catheter oxygenation or noninvasive mechanical ventilation: When respiratory distress and/or hypoxemia of the patient cannot be alleviated after receiving standard oxygen therapy, high-flow nasal cannula oxygen therapy or non-invasive ventilation can be considered. If conditions do not improve or even get worse within a short time (1-2 hours), tracheal intubation and invasive mechanical ventilation should be used in a timely manner. 3.2.3 Invasive mechanical ventilation: Lung protective ventilation strategy, namely low tidal volume (6-8ml/kg of ideal body weight) and low level of airway platform pressure (<30cmH_2O) should be used to perform mechanical ventilation to reduce ventilator-related lung injury. While the airway platform pressure maintained ≤30cmH_2O, high PEEP can be used to keep the airway warm and moist; avoid long sedation and wake the patient early for lung rehabilitation. There are many cases of human-machine asynchronization, therefore sedation and muscle relaxants should be used in

a timely manner. Use closed sputum suction according to the airway secretion, if necessary, administer appropriate treatment based on bronchoscopy findings. 3.2.4 Rescue therapy: Pulmonary re-tensioning is recommended for patients with severe ARDS. With sufficient human resources, prone position ventilation should be performed for more than 12 hours per day. If the outcome of prone position ventilation is poor, extracorporeal membrane oxygenation (ECMO) should be considered as soon as possible. Indications include: ①When Fi02＞90%, the oxygenation index is less than 80mmHg for more than 3-4 hours; ②For patients with only respiratory failure when the airway platform pressure ≥ 35cmH2O, VV-ECMO mode is preferred; if circulatory support is needed, VA- ECMO mode should be used. When underlying diseases are under control and the cardiopulmonary function shows signs of recovery, withdrawal of ECMO can be tried. 3.3 Circulatory support: On the basis of adequate fluid resuscitation, efforts should be made to improve microcirculation, use vasoactive drugs, closely monitor changes in blood pressure, heart rate and urine volume as well as lactate and base excess in arterial blood gas analysis. If necessary, use non-invasive or invasive hemodynamic monitor such as Doppler ultrasound, echocardiography, invasive blood pressure or continuous cardiac output (PiCCO) monitoring. In the process of treatment, pay attention to the liquid balance strategy to avoid excessive or insufficient fluid intake. If the heart rate suddenly increases more than 20% of the basic value or the decrease of blood pressure is more than 20% of the basic value with manifestations of poor skin perfusion and decreased urine volume, make sure to closely observe whether the patient has septic shock, gastrointestinal hemorrhage or heart failure.

3.4 Renal failure and renal replacement therapy: Active efforts should be made to look for causes for renal function damage in critical cases such as low perfusion and drugs. For the treatment of patients with renal failure, focus should be on the balance of body fluid, acid and base and electrolyte balance, as well as on nutrition support including nitrogen balance and the supplementation of energies and trace elements. For critical cases, continuous renal replacement therapy (CRRT) can be used. The indications include: ① hyperkalemia; ② acidosis; ③ pulmonary edema or water overload; ④ fluid management in multiple organ dysfunction. 3.5 Convalescent plasma treatment: It is suitable for patients with rapid disease progression, severe and critically ill patients. Usage and dosage should refer to Protocol of Clinical Treatment with Convalescent Plasma for NCP Patients (2nd trial version). 3.6 Blood purification treatment: Blood purification system including plasma exchange, absorption, perfusion and blood/plasma filtration can remove inflammatory factors and block "cytokine storm", so as to reduce the damage of inflammatory reactions to the body. It can be used for the treatment of severe and critical cases in the early and middle stages of cytokine storm. 3.7 Immunotherapy: For patients with extensive lung lesions and severe cases who also show an increased level of IL-6 in laboratory testing, Tocilizumab can be used for treatment. The initial dose is 4-8mg/kg with the recommended dose of 400mg diluted with 0.9% normal saline to 100ml. The infusion time should be more than 1 hour. If the initial medication is not effective, one extra administration can be given after 12 hours (same dose as before). No more than two administrations should be given with the maximum single dose no more than 800mg. Watch out for allergic reactions. Administration is forbidden for people with active infections such as tuberculosis.

3.8 Other therapeutic measures For patients with progressive deterioration of oxygenation indicators, rapid progress in imaging and excessive activation of the body's inflammatory response, glucocorticoids can be used in a short period of time (three to five days). It is recommended that dose should not exceed the equivalent of methylprednisolone 1-2 mg/kg/day. Note that a larger dose of glucocorticoid will delay the removal of coronavirus due to immunosuppressive effects. Xuebijing 100ml/time can be administered intravenously twice a day. Intestinal microecological regulators can be used to maintain intestinal microecological balance and prevent secondary bacterial infections. Child severe and critical cases can be given intravenous infusion of γ-globulin. For pregnant severe and critical cases, pregnancy should be terminated preferably with c-section. Patients often suffer from anxiety and fear and they should be supported by psychological counseling.

4. Traditional Chinese Medicine treatment

This disease belongs to the category of plague in traditional Chinese medicine (TCM), caused by the epidemic pathogenic factors. According to the different local climate characteristic and individual state of illness and physical conditions, the following treatment Protocol may vary. The use of over-pharmacopoeia doses should be directed by a physician. 4.1 During medical observation

Clinical manifestation 1: fatigue and gastrointestinal discomfort Recommended Chinese patent medicine: Huoxiang Zhengqi capsules (pills, liquid, or oral solution) Clinical manifestation 2: fatigue and fever Recommended Chinese patent medicine: Jinhua Qinggan granules, Lianhua Qingwen capsules (granules), Shufeng Jiedu capsules (granules), Fangfeng Tongsheng pills (granules)

4.2 During clinical treatment (confirmed cases) 4.2.1 Lung cleansing & detoxifying decoction Scope of application: It is suitable for light, moderate and severe patients, and can be used reasonably in combination with the actual situation of patients in the treatment of critically ill patients. Recommended prescription: Ephedra 9g, Zhigancao 6g, Almond 9g, Gypsum 15-30g (fried first), Guizhi 9g, Zixie 9g, Zhuling 9g, Baizhu 9g, Zhiling 15g, Bupleurum 16g, Scutellaria baicalensis 6g, and Pinellia 9g , Ginger 9g, aster 9g, winter flower 9g, shoot dry 9g, asarum 6g, yam 12g, coriander fruit 6g, tangerine peel 6g, aquilegia 9g. Suggested use: Traditional Chinese medicine decoction pieces for decocting in water. One dose per day, twice in the morning and evening (forty minutes after a meal), take with warm water, and three doses a course.

If conditions permit, the patient can take half a bowl of rice soup each time after taking the medicine, and can take up to one bowl if the patient has a dry tongue and is deficient in bodily fluids. (Note: If the patient does not have a fever, the amount of gypsum should be little. If having a fever or strong heat, the amount of gypsum can be increased). If the symptoms improve but do not fully recover, then take the second course of treatment. If the patient has special conditions or other underlying diseases, the prescription of the second course of treatment can be modified based on the actual situation and the medicine should be discontinued when the symptoms disappear. Source of prescription: Notice on Recommending the Use of 'Lung cleansing & detoxifying decoction' in Treatment of NCP by Integrated Traditional Chinese and Western Medicine by the Office of the State Administration of Traditional Chinese Medicine & the General Office of the National Health Commission. (2022 No.22)

4.2.2 Mild cases

4.2.2.1 Cold dampness and stagnation lung syndrome Clinical manifestations: fever, fatigue, sore body, cough, expectoration, chest tightness, suffocation, loss of appetite, nausea, vomiting, sticky stools. Tongue has thin fat tooth mark or is faint red, and the coating is white thick rot or white greasy and the pulse is moisten or slippery. Recommended prescription: Raw ephedra 6g, raw gypsum 15g, almond 9g, loquat 15g, gardenia 15g, Guanzhong 9g, Dilong 15g, Xu Changqing 15g, Huoxiang 15g, Peilan 9g, Cangzhu 15g, Yunling 45g, Atractylodes 30g, Jiao Sanxian 9g each , Magnolia officinalis 15g, betel coconut 9g, yarrow fruit 9g, ginger 15g. Suggested use: one dose daily, boiled with 600ml water, take it three times at morning, noon and evening before meal.

4.2.2.2 Dampness and heat-accumulation lung syndrome Clinical manifestations: low or no fever, slight chills, fatigue, heavy head and body, muscle soreness, dry cough, low phlegm, sore throat, dry mouth, do not want to drink more, or accompanied by chest tightness, no sweat or sweating, Or vomiting and loss of appetite, diarrhea or sticky stool. The tongue is reddish, and the coating is white, thick and greasy or thin yellow, and the pulse is slippery or sloppy. Recommended prescription: Betel nut 10g, apple 10g, Magnolia 10g, Zhimu 10g, scutellaria baicalensis 10g, Bupleurum 10g, red peony 10g, forsythia 15g, artemisia annua 10g (decocted later), 10g of green leaves, 10g of green leaves, 5g of raw licorice. Suggested use: one dose daily, boiled with 400ml water, take it twice at morning and evening.

4.2.3 Moderate cases 4.2.3.1 Dampness and stagnation lung syndrome Clinical manifestations: fever, low cough and sputum, or yellow sputum, suffocation, shortness of breath, bloating, and constipation. The tongue is dark red and fat; the coating is greasy or yellow and the pulse is slippery or stringy. Recommended prescription: raw ephedra 6g, bitter almond 15g, raw gypsum 30g, raw coix seed 30g, grass root 10g, patchouli

15g, artemisia annua 12g, Polygonum cuspidatum 20g, verbena 30g, dried reed root 30g, gardenia 15g 15g of orange red, 10g of raw licorice. Suggested use: one dose daily, boiled with 400ml water, take it twice at morning and evening.

4.2.3.2 Cold dampness lung syndrome

Clinical manifestations: low fever, low body temperature, or no heat, dry cough, low sputum, fatigue, chest tightness, nausea, or nausea. The tongue is pale or red, and the coating is white or greasy, and the veins are pulsating. Recommended prescription: Atractylodes lancea 15g, Chenpi 10g, Magnolia 10g, Aquilegia 10g, grass fruit 6g, raw ephedra 6g, Zhihuo 10g, ginger 10g, betel nut 10g. Suggested use: one dose daily, boiled with 400ml water, take it twice at morning and evening.

4.2.4 Severe cases 4.2.4.1 Plague poison and lung-closing syndrome Clinical manifestations: fever, flushing, cough, yellowish phlegm, or blood in sputum, wheezing, shortness of breath, tiredness, fatigue, dryness and stickiness, nausea, food loss, poor stool, and short urination. Red tongue, yellow greasy coating, slippery pulses. Recommended prescription: Raw ephedra 6g, almond 9g, raw gypsum 15g, licorice 3g, fragrant fragrant 10g (back), Magnolia 10g, atractylodes 15g, grass fruit 10g, pinellia 9g, Poria 15g, raw rhubarb 5g (back) 10g, gardenia 10g, red peony 10g. Suggested use: one or two doses daily, boiled with 100-200ml water, take it 2-4 times, oral or nasal feeding.

4.2.4.2 Syndrome of flaring heat in qifen and yingfen Clinical manifestations: Hot fever, thirst, shortness of breath, shortness of breath, blurred vision, or spotted rash, or vomiting blood, bleeding, or convulsions in the limbs. Tongue ridges have few or no moss, and the pulse sinks finely, or floats large and counts. Recommended prescription: 30-60g gypsum (fried first), 30g of Zhimu, 30-60g of raw land, 30g of buffalo horn (fried first), 30g of red sage, 30g of black ginseng, 15g of forsythia, 15g of paeonia, 6g of peony 12g, gardenia 15g, raw licorice 6g. Suggested use: 1 dose per day, decoction, first decoct gypsum and buffalo horn, then apply other pieces, 100ml-200ml each time, 2-4 times a day, orally or nasally. Recommended Chinese patent medicines: Xiyanping injection, Xuebijing injection, Reduning injection, Tanreqing injection, Xingnaojing injection. Drugs with similar efficacy can be selected according to individual conditions, or can be used in combination according to clinical symptoms. Traditional Chinese medicine injection can be used in combination with traditional Chinese medicine decoction.

4.2.5 Critical cases (syndrome of inner blocking causing collapse) Clinical manifestations: dyspnea, dyspnea, asthma or need mechanical ventilation, fainting, irritability, cold sweating, dark purple tongue, thick or dry moss, large floating roots. Recommended prescription: 15g of ginseng, 10g of Heishun tablets (decoct first), 15g of dogwood, delivered with Suhexiang Pill or Angong Niuhuang Pill. For patients on mechanical ventilation with abdominal distention or constipation: 5-10g of Dahuang. For patients with human-machine asynchronization: 5-10g of Dahuang and 5- 10g of Mangxiao while administering sedatives and muscle relaxants. Recommended Chinese patent medicines: Xuebijing injection, Reduning injection, Tanreqing injection, Xingnaojing injection, Shenfu injection, Shengmai injection, Shenmai injection. Drugs with similar efficacy can be selected according to individual conditions, or can be used in combination according to clinical symptoms. Traditional Chinese medicine injection can be used in combination with traditional Chinese medicine decoction

Note: Recommended usage of Chinese medicine injections for severe and critical cases The use of traditional Chinese medicine injections follows the principle of starting from a small dose and gradually adjusting the dosage according to the instructions of the drug. The recommended usage is as follows: Viral infection or combined mild bacterial infection: 0.9% sodium chloride injection 250ml plus Xiyanping injection 100mg bid, or 0.9% sodium chloride injection 250ml heated Duning injection 20ml, or 0.9% sodium chloride injection 250ml plus Tanreqing injection 40ml bid. High fever with disturbance of consciousness: 250ml of 0.9% sodium chloride injection and 20ml bid of Xingnaojing injection. Systemic inflammatory response syndrome or/and multiple organ failure: 250ml of 0.9% sodium chloride injection and 100ml of Xuebijing injection.

Immunosuppression: 250ml of 0.9% sodium chloride injection and 100ml bid of Shenmai injection. Shock: 250ml of 0.9% sodium chloride injection plus 100ml bid of Shenfu injection.

4.2.6 Convalescent period 4.2.6.1 Lung and spleen qi deficiency síndrome

Clinical manifestations: shortness of breath, fatigue, fatigue, anorexia, nausea, fullness, weak stool, and uneasiness. The tongue is pale and greasy. Recommended prescription: French Pinellia 9g, Chenpi 10g, Codonopsis 15g, Sunburn Astragalus 30g, Stir-fried Atractylodes 10g, Poria 15g, Huoxiang 10g, Amomum villosum 6g (later), and Licorice 6g Suggested use: 1 dose per day, boiled with 400ml of water, twice a day at morning and evening.

4.2.6.2 Qi and Yin deficiency syndrome Clinical manifestations: Fatigue, shortness of breath, dry mouth, thirst, palpitations, sweating, poor appetite, low or no lever, dry cough and little sputum; dry tongue, fine or weak pulses. Recommended prescription: North and south radix salviae 10g, 15g ophiopogonis, 6g American ginseng, 6g schisandra, 6g gypsum l5g, 10g light bamboo leaves, 10g mulberry leaves, 15g reed root, 15g salviae miltiorrhiza, 6g raw liquorice. Suggested use: 1 dose per day, boiled with 400ml of water, twice a day at morning and evening.

XI. Discharge criteria and after-discharge considerations 1.Discharge criteria 1) Body temperature is back to normal for more than three days; 2) Respiratory symptoms improve obviously; 3) Pulmonary imaging shows obvious absorption of inflammation, 4) Nuclei acid tests negative twice consecutively on respiratory tract samples such as sputum and nasopharyngeal swabs (sampling interval being at least 24 hours). Those who meet the above criteria can be discharged.

2. After-discharge considerations 2.1 The designated hospitals should contact the primary healthcare facilities where the patients live and share patients' medical record, to send the information of the discharged patients to the community committee and primary healthcare facility where the patients reside. 2.2. After discharge, it is recommended for patients to monitor their own health status in isolation for 14 days, wear a mask, live in well-ventilated single room if possible, reduce close contact with family members, separate dinning, practice hand hygiene and avoid going out. 2.3 It is recommended for the patients to return to the hospitals for follow-up and re-visit in two and four weeks after discharge.

XII. Patients Transportation Principles Patients should be transported in accordance with the Work Protocol for Transfer of the Novel Coronavirus Pneumonia Patients (Trial Version) issued by the National Health Commission.

XIII. Nosocomial Infection Prevention and Control Measures to prevent and control nosocomial infection should be implemented in accordance with the requirements of the Technical Guidelines for the Prevention and Control of Infection by the Novel Coronavirus in Medical Institutions (First Edition) and the Guidelines on the Usage of Common Medical Protective Equipment against Novel Coronavirus Infection (Trial Version) formulated by the National Health Commission.

The General Office of National Health Commission Office of State TCM Administration

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Combination of western medicine and Chinese traditional patent medicine in treating a family case of COVID-19 in Wuhan

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Abstract In December 2019, an outbreak of novel coronavirus (2019-nCoV) occurred in Wuhan, Hubei Province, China. By February 14, 2020, it has led to 66 492 confirmed patients in China and high mortality up to ~2.96% (1123/37 914) in Wuhan. Here we report the first family case of coronavirus disease 2019 (COVID-19) confirmed in Wuhan and treated using the combination of western medicine and Chinese traditional patent medicine Shuanghuanglian oral liquid (SHL). This report describes the identification, diagnosis, clinical course, and management of three cases from a family, suggests the expected therapeutic effects ofSHL on COVID-19, and warrants further clinical trials.

Keywords novel coronavirus (2019-nCoV); COVID-19; Chinese traditional patent medicine; Shuanghuanglian oral liquid

Introduction

Since December 31, 2019, a cluster of patients with pneumonia ofunknown cause has been reported in Wuhan, China. This special pneumonia was associated with a novel coronavirus, 2019-nCoV, named initially by the World Health Organization (WHO) in January 2020 [1]. An outbreak of 2019-nCoV pneumonia (officially and inter- nationally named as COVID-19 on February 11, 2020 by the WHO) occurred and spread to the entire China and multiple countries worldwide [2]. By February 14, the number of patients confirmed with COVID-19 reached 66 492, 8969 suspected patients, and 1523 deaths were identified in China [3]. By February 14, the number of confirmed patients and deaths were 37 914 and 1123, respectively, and the mortality ofCOVID-19 was 2.96% in Wuhan City, Hubei Province, which is considered as the origin of first cluster of patients [3]. The confusing and difficult thing for physicians is that they do not have specific drugs to either treat or prevent the aggravation and serious complications of COVID-19 for such patients [4]. Thus, Chinese herbs attract our attention, and investiga- tions for different clinical trials are in progress. The present report described a family case, including three cases who received western medicine and Chinese traditional patent medicine Shuanghuanglian oral liquid (SHL) treatments and achieved rapid recovery.

Case report

The family case includes parents and a daughter. The family lives in an apartment 3 km from the Huanan Seafood Wholesale Market in Wuhan, Hubei Province. These cases are a typical familial cluster where all ofthem had COVID-19 [5]. All the patients were informed about the clinical trial (ChiCTR2000029605) and signed informed consent. Case 1 is a 51-year-old female. On January 17, 2020, the patient presented a feeling ofgeneral malaise and coldness. On January 19, she had fever with body temperature of 37.3 °C and experienced diarrhea and vomiting. She started to receive intravenous injection of cefotaxime in community clinic and took oral Jinyebaidu granules (another Chinese traditional patent medicine) and oselta- mivir (75 mg, twice a day) for 4 days. She had persistent severe fever with body temperature from 37.6 °C to 38.3 °C for four days. On January 23, her chest computed tomography (CT) scan showed multiple patchy ground glass

opacity and consolidation shadow in bilateral lung and subpleural regions (Fig. 1A). She was highly suspected with COVID-19. Oral moxifloxacin and arbidol were prescribed, and she continued to take Jinyebaidu granules and oseltamivir. On January 24, her body temperature reached 39.9 °C and experienced severe fatigue, diarrhea, and breathlessness despite continuous treatments of the above drugs. She felt better after intravenous injection of immunoglobin (IVIG, 5 g per day) and dexamethasone (5 mg, once to twice a day). However, the patient experienced recurrent fever (body temperature of 39 °C) and breathlessness, and her blood oxygen saturation fluctuated from 90% to 95% at the night of January 27. On January 28, the second chest CT scan indicated that her pneumonia aggravated for the past 5 days (Fig. 1B). A nasopharyngeal swab specimen was obtained and sent for detection of 2019-nCoV. Although the patient's 2019-nCoV test was negative (Table 1), she was diagnosed of COVID-19 in accordance with her symptoms and chest CT display. On the same day, she was confined into an isolation ward and started to take oral SHL (twice a day, 20 mL once). On the next day, SHL administration increased to three times a day (20 mL once) without using any other drugs. From January 29 to 31, the patient's symptoms resolved with body temperature decreasing from 37.3 °C to 36.5 °C and without vomiting and diarrhea. The patient gradually felt strong except for slight cough. After February 6, the patient's symptoms disappeared, and her third chest CT scan (Fig. 1C) showed significant absorption of bilateral ground glass opacity compared with the previous ones. The association of her symptoms with treatments is shown in Fig. 2, and the clinical laboratory results are shown in Table 2. Cases 2 and 3 became simultaneously ill 9 days after they had close contacts with case 1 [5]

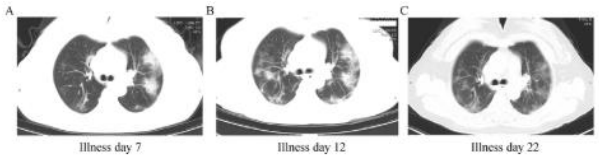

Fig. 1 Chest CT images of case 1. (A) CT imaging on January 23, 2020 shows ground glass opacity in both lungs on illness day 7. (B) Image taken on January 28, 2020, shows aggravation of pneumonia on illness day 12. (C) Image taken on February 6, 2020 shows the absorption of bilateral ground glass opacity after SHL treatment from January 28.

Table 1 Testing results of 2019-nCoV

Specimen	Case 1			Case 2			Case 3	
	Illness day 12	Illness day 20	Illness day 23	Illness day 3	Illness day 11	Illness day 14	Illness day 3	Illness day 11
Nasopharyngeal swab	Negative	Negative	NT	Negative	Negative	NT	Positive	Negative
Anal swab	NT	NT	Negative	NT	NT	Negative	NT	NT

NT, not tested.

Fig. 2 Symptoms, maximum body temperatures, and treatment timeline in accordance with day of illness and day of hospitalization (January 17 to February 10).

Table 2 Clinical laboratory results of case 1

Measure	Reference range	Illness day 13 Hospital day 2	Illness day 24 Hospital day 13
White cell count ($\times 10^9$/L)	3.50–9.50	5.93	5.46
Red cell count ($\times 10^9$/L)	3.80–5.10	4.13	4.06
Neutrophil count ($\times 10^9$/L)	1.90–6.30	3.04	3.27
Lymphocyte count ($\times 10^9$/L)	1.10–3.20	2.11	1.5
Eosinophil count ($\times 10^9$/L)	0.02–0.52	0.12	0.15
Platelet count ($\times 10^9$/L)	125.0–350.0	361	323
Hemoglobin (g/L)	115.0–150.0	113	111
Hematocrit (%)	35.0–45.0	35	35
Sodium (mmol/L)	136–214	140	140.4
Potassium (mmol/L)	3.50–5.10	4.22	4.8
Chloride (mmol/L)	99–110	104.6	102.4
Calcium (mmol/L)	2.15–2.50	2.06 ↓	2.19
Carbon dioxide (mmol/L)	22.0–29.0	21 ↓	25.1
Glucose (mmol/L)	4.11–6.05	–	5.01
Blood urea nitrogen (μmol/L)	2.6–7.5	3.1	3.6
Creatinine (μmol/L)	45–84	53	60
Total protein (g/L)	64–83	68.4	68.6
Albumin (g/L)	35–52	29.6 ↓	33.2 ↓
Total bilirubin (μmol/L)	⩽21	6.4	4.5
Procalcitonin (ng/mL)	⩽0.05	0.03	0.03
Alanine aminotransferase (U/L)	⩽33	17	20
Aspartate aminotransferase (U/L)	⩽32	16	19
Alkaline phosphatase (U/L)	35–105	59	83
Fibrinogen (g/L)	2.00–4.00	5.31 ↑	–
Lactate dehydrogenase (U/L)	135–214	248 ↑	–
Prothrombin time (s)	11.5–14.5	13.6	–
International normalized ratio	0.80–1.20	1.03	–
Creatine kinase (U/L)	⩽170	35	27
C-reactive protein (mg/L)	⩽3	57.8 ↑	–

Case 2 is a 27-year-old female nurse at the clinical trial center of Division of Cardiology of Tongji Hospital, Wuhan, daughter of case 1, who took care of her mother. On January 26, the patient presented mild weakness, diarrhea, and low fever, and she started to take oral Jinyebaidu granules, oseltamivir, moxifloxacin, and arbidol. On the next day, all her symptoms aggravated with body temperature reaching to 38.3 °C, frequent vomiting, and diarrhea for 5 times a day. At the night of January 27, her body temperature reached 39.5 °C with chest tightness and shortness of breath. Although she took nonsteroidal anti-inflammatory drug (loxoprofen), her fever remained high (39 °C). On January 28, her chest CT scan showed consolidation shadow in the left lung, and her 2019-nCoV test showed negative using nasopharyngeal swab speci- men. Considering her contact history with her mother, she was also confined into an isolation ward and started to take SHL 20 mL once for three times a day without taking other drugs. Her body temperature ranged from 37.5 °C to 38.5 °C during January 29 to 31 and decreased from 37.5 °C to 36.5 °C on February 1. All other symptoms resolved on February 2 with recovered appetite and spirit. Two repeated 2019-nCoV tests were negative (Table 1). On February 6, her second chest CT scan showed the absorption of the left lung shadow (Fig. 3). After her disease symptoms disappeared, the oral dose of SHL reduced to 10 mL once for three times a day. The patient's clinical laboratory results are shown in Table 3. Case 3 is a 53-year-old male, husband of case 1 and father of case 2, who presented mild diarrhea, vomiting, and fever on January 26. On January 28, the patient was diagnosed of COVID-19 with positive 2019-nCoV test using nasopharyngeal swab specimen (Table 1) and chest CT scan showing patchy ground glass opacity in the right lower lung subpleural fields (Fig. 4). He had no fever, cough, and breathlessness. Thus, the patient started to isolate himself at home and took SHL (20 mL once, three times a day), moxifloxacin, and arbidol on January 28.

Since February 2, all his symptoms resolved with exception of light nausea, and he continued taking SHL with other drugs. On February 4, he felt slightly weak. On February 7, the patient fully recovered, and the repeated 2019-nCoV RNA test was negative (Table 1).Niu, M., R. Wang, Z. Wang, P. Zhang, Z. Bai, J. Jing, Y. Guo, X. Zhao, X. Zhan, Z. Zhang, X. Song, E. Qin, J. Wang and X. Xiao. Rapid establishment of traditional Chinese medicine prevention and treatment for the novel coronavirus pneumonia based on clinical experience and molecular docking. China J. Chin. Mater. Med., 2020, doi:10.19540/j.cnki.cjcmm.20200206.501.

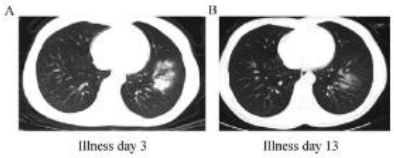

Fig. 3 Chest CT images of case 2. (A) CT imaging obtained from case 2 on January 28, 2020 shows consolidation shadow in the left lung on illness day 3. (B) Image taken on February 6, 2020 shows the absorption of consolidation shadow after SHL treatment from January 28.

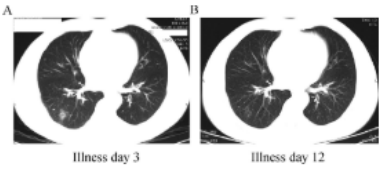

Fig. 4 Chest CT images of case 3. (A) CT imaging obtained from case 3 on January 28, 2020 shows ground glass opacity in the left lung on illness day 3. (B) Image taken on February 7, 2020 shows the absorption of ground glass opacity after SHL treatment from January 28.

Table 3 Clinical laboratory results of case 2

Measure	Reference range	Illness day 4 Hospital day 2	Illness day 15 Hospital day 13
White cell count ($\times 10^9$/L)	3.50–9.50	3.63	5.79
Red cell count ($\times 10^9$/L)	3.80–5.10	4.5	4.28
Neutrophil count ($\times 10^9$/L)	1.90–6.30	2.12	3.2
Lymphocyte count ($\times 10^9$/L)	1.10–3.20	1.25	1.98
Eosinophil count ($\times 10^9$/L)	0.02–0.52	0.01	0.17
Platelet count ($\times 10^9$/L)	125.0–350.0	139	308
Hemoglobin (g/L)	115.0–150.0	125	117
Hematocrit (%)	35.0–45.0	37.9	36.2
Sodium (mmol/L)	136–214	135.3	143.5
Potassium (mmol/L)	2.50–5.10	3.69	4.27
Chloride (mmol/ L)	99–110	97 ↓	106.3
Calcium (mmol/L)	2.15–2.50	2.25	2.27
Carbon dioxide (mmol/L)	22.0–29.0	22.3	25.2
Glucose (mmol/L)	4.11–6.05	4.31	4.82
Blood urea nitrogen (μmol/L)	2.6–7.5	2.7	3.4
Creatinine (μmol/L)	45–84	59	52
Total protein (g L)	64–83	77.2	65.6
Albumin (g/L)	35–52	41.5	37.5
Total bilirubin (μmol/L)	≤21	6.6	4
Procalcitonin (ng/mL)	≤0.05	0.04	–
Alanine aminotransferase (U/L)	≤33	8	50 ↑
Aspartate aminotransferase (U/L)	≤32	21	27
Alkaline phosphatase (U/L)	35–105	52	22 ↓
Fibrinogen (g/L)	2.00–4.00	3.42	–
Lactate dehydrogenase (U/L)	135–214	170	186
Prothrombin time (s)	11.5–14.5	13.6	–
International normalized ratio	0.80–1.20	1.03	–
Creatine kinase (U/L)	≤170	52	27
C-reactive protein (mg/L)	≤3	9.4 ↑	–

↓ The patient's value was below normal. ↑ The patient's value was above normal.

Discussion

This report described the first typical family case of COVID-19 treated using the Chinese traditional patent medicine SHL because of poor response to other treatments. The three patients were a close family, and the mother was the first victim with typical symptoms of severe viral pneumonia and confirmed as COVID-19 in accordance with the symptoms and chest CT scan. Cases 2 and 3 had close contact history with case 1, and 9 days later they became ill with positive diagnosis of COVID-19. All their symptoms resolved after using the Chinese traditional patent medicine SHL and rapidly recovered without obvious adverse effects when the patients showed no response and their symptoms continued to aggravate after other treatments, including IVIG (5 g per day) and dexamethasone, antibiotics, and antivirus compounds. SHL, a Chinese traditional patent medicine containing extracts of three Chinese herbs, namely, honeysuckle, forsythia, and Scutellaria baicalensis, is usually used to treat cold, sore throat, and cough with fever. SHL has been used in clinical practice for a long time because of its affordable cost and no serious adverse reaction. Recent news from preliminary study findings indicated that SHL can inhibit 2019-nCoV (http://www.cas.cn/yw/202001/t20200131_4733137.shtml, accessed on January 31, 2020). Considering that no specific drugs are recommended to treat COVID-19, we started our clinical trial (ChiCTR2000029605) to investigate whether SHL can treat this disease and the family case is a part ofthe clinical trial. These cases suggest that SHL might be effective for COVID-19 although subsequent clinical trials are needed. In this family case report, we described two patients who had poor response to other treatments but responded well to SHL therapy. Case 3 showed positive therapeutic effect although he simultaneously received arbidol. Early treat- ments may contribute to patients' outcome and several errors, including taking antibiotics, and combination of two antivirus drugs should be avoided. This report suggests that SHL treatment might be effective for COVID-19 and warrants subsequent clinical trials to obtain sufficient evidence for clinical recommendation.

Acknowledgements

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Li Ni, Ling Zhou, Min Zhou, Jianping Zhao, and Dao Wen Wang declared no potential conflicts ofinterest with respect to the research, authorship, and/or publication of this article. Informed consent was obtained from all

the patients in which their identifying information are included in this article. Other ethical board approval is not applicable in this case report.

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Abstract

We report a family cluster of coronavirus disease 2019 (COVID-19) caused by a presymptomatic case. There were 9 family members, including 8 laboratory-confirmed with COVID-19, and a 6-year-old child had no evidence of infection. Among the 8 patients, 1 adult and a 13-month-old infant were asymptomatic, and 1 adult was diagnosed as having severe pneumonia.

Since the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in early December 2019, the number of cases has been rapidly increasing [1]. Chan and colleagues reported SARS-CoV-2 in a family setting with person-to-person transmission [2]. We are here reporting a family cluster of

coronavirus disease 2019 (COVID-19) transmission and clinical features in Zhejiang, China, after a visit to a temple.

On 19 January, a couple participated in a temple activity to celebrate the Chinese Spring Festival. The 58-year-old woman (index 1) fell ill with fever, fatigue, and headache on 24 January. However, the 60-year-old man (index 2) was without any symptoms.

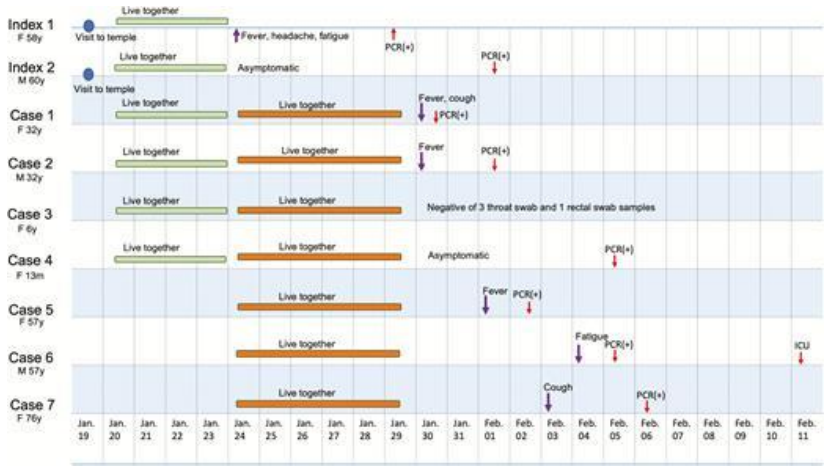
In between the temple visit and the onset of symptoms, the couple's daughter, son-in-law, and 2 grandchildren (cases 1–4) had visited and stayed with them from 20 to 23 January. Index 1 went to hospital on 26 January, and was diagnosed with COVID-19 on 29 January by reverse-transcription polymerase chain reaction (RT-PCR).

Index 2 and cases 1–4 were all admitted to hospital as persons under investigation on the day index 1's diagnosis was confirmed. The daughter (case 1) presented with fever and cough on 27 January, and was diagnosed with COVID-19 after being tested positive by RT-PCR using throat swab specimen.

On 1 February, index 2 tested positive via throat swab RT-PCR but stayed asymptomatic. Case 1's husband (case 2) started to suffer fever and was laboratory-diagnosed on the same day. The 6-year-old daughter (case 3) showed no symptoms and tested negative 3 times for throat swab and negative once for rectal swab by RT-PCR, and remained clear in chest computed tomographic scans. She was the only family member who was uninfected by SARS-CoV-2. The 13-month-old daughter (case 4) was asymptomatic but was laboratory-confirmed on 5 February.

On 23 January 23, before index 1 showed any symptoms, cases 1–4 had dinner with case 2's mother (case 5), father (case 6), and grandmother (case 7). They were admitted to hospital as persons under observation on 1 February, and were laboratory-confirmed to have contracted COVID-19 on 2, 5, and 6 February, respectively. Case 6 was a 57-year-old man who was transferred to the intensive care unit (ICU) as the symptoms got worse and the level lactic acid continued to increase (Figure 1).

Figure 1.



Timeline of exposure to index patients with coronavirus disease 2019 (COVID-19) in China. Abbreviations: F, female; ICU, intensive care unit; M, male; PCR, polymerase chain reaction.

This series of laboratory-confirmed cases of COVID-19 was diagnosed in the Zhejiang province, which lies outside Wuhan. First, although the family members were from 3 households, they all were infected directly or indirectly from the same 2 index patients. The clinical features were diverse across patients; in particular, there were 2 asymptomatic patients and 1 patient whose symptoms were so severe that he had to be transferred to ICU. Second, this cluster demonstrated that COVID-19 is transmittable during the incubation period, as the daughter and her family caught the disease during the incubation period of index 1 and index 2. Third, patients can stay asymptomatic, such as index 2 and case 4 in this cluster. Given that Zou et al [3] found that the viral load of symptomatic and asymptomatic patients were similar, asymptomatic patients can still infect others. These “silent patients” may remain undiagnosed and be able to spread the disease to large numbers of people. Last, it appears that children may not be as susceptible to this new virus as adults and elderly persons, and they may fare better when they have contracted the virus. As reported in this family cluster, the 6-year-old child was not infected and the 13-month-old was infected but stayed asymptomatic.

In summary, there are variations across individuals in the clinical manifestations of COVID-19, indicating that we should pay attention to how to prevent people from being infected by asymptomatic patients and patients who are in the incubation period.

Notes

Author contributions. L. W. and Xue. C. collected the data. Xiao C., G. Q., and N. Y. conceived the idea. G. Q. and N. Y. drafted the manuscript. A. H. Y. M. reviewed the manuscript. All authors read and approved the final manuscript.

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Abstract

Background: Development of a core outcome set (COS) for clinical trials for COVID-19 is urgent because of the pandemic wreaking havoc worldwide and the heterogeneity of outcomes in clinical trials.

Methods: A preliminary list of outcomes was developed after a systematic review of protocols of clinical trials for COVID-19. Then, two rounds of the Delphi survey were conducted. Stakeholders were traditional Chinese medicine (TCM) experts, Western medicine (WM) experts, nurses, and the public. Patients with confirmed COVID-19 were also invited to participate in a questionnaire written in understandable language. Then different stakeholders participated in a consensus meeting by video conference to vote.

Results: Ninety-seven eligible study protocols were identified from 160 clinical trials. Seventy-six outcomes were identified from TCM clinical trials and 126 outcomes were identified from WM clinical trials. Finally, 145 outcomes were included in the first round of the Delphi survey. Then, a COS for clinical trials of TCM and WM was developed. The COS included clinical outcomes (recovery/improvement/progression/death), etiology (SARS-CoV-2 nucleic-acid tests, viral load), inflammatory factor (C-reactive protein), vital signs (temperature, respiration), blood and lymphatic-system parameters (lymphocytes, virus antibody),

respiratory outcomes (pulmonary imaging, blood oxygen saturation, PaO2/FiO2 ratio, arterial blood gas analysis, mechanical ventilation, oxygen intake, pneumonia severity index), clinical efficacy (prevalence of preventing patients with mild-to-moderate disease progressing to severe disease), and symptoms (clinical symptom score). Outcomes were recommended according to different types of disease. Outcome measurement instruments/definitions were also recommended.

Conclusion: Though there are some limitations for the research, such as insufficient patients and the public involvement, and the unbalanced stakeholders' region, the COS for COVID-19 may improve consistency of outcome reporting in clinical trials. It also should be updated with research progression.

Introduction

Since December 2019, a novel coronavirus causing pneumonia has been spreading around the world. It was temporarily named “2019 novel coronavirus” on 2 January, 2020, but the World Health Organization (WHO) officially named it “coronavirus disease 2019” (COVID-19) on 11 February 2020. The coronavirus that causes COVID-19 was termed “severe acute respiratory syndrome coronavirus 2” (SARS-CoV-2) on 11 February 2020 by the WHO.

As of 10:00 CET on 2 April 2020, 896,450 cases of COVID-19 had been reported to the WHO, and 45,526 of these cases have died (WHO, 2020). COVID-19 is now a global threat, so its outbreak was declared to be a pandemic on 11 March 2020 by the WHO. There are significant knowledge gaps in the epidemiology, transmission dynamics, investigation tools, and management of COVID-19 (Khot and Nadkar, 2020). A specific drug or vaccine has not been approved to treat it. Hence, COVID-19 management is a major challenge for clinicians and researchers worldwide.

The first clinical trial of COVID-19 was registered on 23 January 2020 (Huang, 2020). Since then, an increasing number of clinical trials of COVID-19 have been registered using regimens based on traditional Chinese medicine (TCM) and Western medicine (WM). As of March 18, 2020, 585 protocols were searched from all the databases of International Committee of Medical Journal Editors (ICMJE)-accepted platforms of clinical-trial registries.

Previously, we found several problems regarding the protocols of clinical trials of COVID-19 [e.g., unclear study objectives, heterogeneity of outcome choices, and small sample size (Zhang et al., 2020)] that may reduce the value of clinical trials. In the meantime, clinicians' understanding of COVID-19 characteristics has been changing because they are treating many more patients than before. The diagnosis and management plan of COVID-19 also keeps changing. We believe that certain inappropriate outcomes may be chosen by researchers. To improve the consistency of outcomes and include more clinical trials in systematic reviews, development of a “core outcome set” (COS) for COVID-19 is crucial.

A COS is an agreed standardized set of outcomes that should be measured and reported, as a minimum, in all clinical trials in specific areas of health or healthcare (COMET). When researchers report outcomes in a COS, they can also report other outcomes.

This COS was based on: (i) a population with confirmed COVID-19 cases of "mild", "ordinary", "severe", or "critical" types; (ii) interventions that include TCM and WM; and (iii) the COS being applied in randomized controlled trials (RCTs) and observational studies.

Methods

Registry

This COS has been registered on the Core Outcome Measures in Effectiveness Trials (COMET) database (Shang and Qiu, 2020). This research was conducted and reported following COS-STAndards for Development (COS-STAD) (Kirkham et al., 2017) and COS-STAndards for Reporting (COS-STAR) (Kirkham et al., 2016).

Participants

Steering Group

A steering group was formed by a TCM expert, WM expert, methodologist, nurse, and statistician. They conducted the research protocol, made decisions if there was confusion, and attended the consensus meeting to facilitate COS development.

Stakeholders in the Delphi Survey

The stakeholders in the Delphi survey included TCM experts (clinicians and researchers), WM experts (clinicians and researchers), nurses, patients, and the public.

COVID-19 is a new infectious disease that is spreading rapidly. In China, many clinicians have been trained to face emergencies, irrespective of whether they are on the "frontline" of the "battle" against COVID-19. More than 40,000 clinicians and nurses from other areas of China moved to Hubei Province to support the local medical system. Not all of these clinicians and nurses were trained in respiratory medicine or critical care. To obtain perspectives on a larger scale, we used "snowball" sampling to extend the sample size. We invited members from the Clinical Research Information Association of China and the Information Association for Traditional Chinese Medicine and Pharmacy to participate in the Delphi survey. We asked them to send the questionnaire to their colleagues.

We believe that the perspectives of patients and the public are important. Hence, we sent the questionnaire *via* social media (WeChat, Tencent) to invite the public to participate.

To obtain patients' perspectives, frontline clinicians of Dongzhimen Hospital, Beijing University of Chinese Medicine (Beijing, China) invited and helped patients who consented to complete the questionnaire.

Stakeholders in the Consensus Meeting

The stakeholders in the consensus meeting were TCM clinicians, WM clinician, nurse, methodologist, evidence-based medicine researcher, and staff from the Chinese Clinical Trials Registry.

Information Sources

All the databases of ICMJE-accepted platforms of clinical-trial registries (ICMJE) were considered. Search terms for Chinese Clinical Trial Registry (ChiCTR) were: “COVID-19,” “2019-novel Corona Virus (2019-nCoV),” “Novel Coronavirus Pneumonia (NCP),” “Severe Acute Respiratory Infection (SARI),” and “Severe Acute Respiratory Syndrome - Corona Virus- 2 (SARS-CoV-2).” Search terms for the Netherlands National Trial Register were “nCoV,” “Coronavirus,” “SARS,” “SARI,” “NCP,” and “COVID.” Search terms for other databases were “2019-nCoV OR Novel Coronavirus OR New Coronavirus OR SARS-CoV-2 OR SARI OR NCP OR Novel Coronavirus Pneumonia OR COVID-19 OR Wuhan pneumonia.”

The search was conducted on 14 February 2020. The details of inclusion criteria, exclusion criteria, study identification, data extraction, and rejected/combined outcomes are described in the systematic review of protocols of clinical trials of COVID-19 (Qiu et al., 2020).

Consensus Process

Two rounds of the Delphi survey for professionals and the public, as well as one round of the Delphi survey for patients, were conducted. After the Delphi survey had been completed, a consensus meeting was conducted to determine the final COS.

Delphi Survey

The questionnaire for professionals and the public was sent by smartphone. It included individual outcomes in different outcome domains and scoring. At the end of the questionnaire of the first round of Delphi survey, there were two open-ended questions: (i) which outcomes do you think are important but were not included in the questionnaire? (ii) what is your opinion of this questionnaire?

The questionnaire for patients was sent by smartphone, too. It included outcomes/outcome domains that were understood readily by patients. Patients were asked to vote on which outcomes/outcome domains were important to them. There was one open-ended question: which outcomes do you think are important but were not included in the questionnaire?

Outcome Scoring

The questionnaire for professionals and the public employed a nine-point scoring system, which has been used in previous COS studies (Qiu et al., 2018; Qiu et al., 2019). A score of: “1–3” denoted that the outcome was not important for inclusion in the COS; “4–6” meant the outcome was important but not critical for inclusion in the COS; “7–9” denoted that the outcome was critical for inclusion in the COS. An outcome scored as ≥7 by ≤50% of participants for all stakeholders was removed from the next consensus process. The outcomes recommended by participants were added in the second round of the Delphi survey after discussion by the steering group.

Consensus Definitions

For the Delphi survey administered to professionals and the public, the consensus definitions were: (i) consensus in: ≥70% of participants in all stakeholders scored the outcome as 7–9, and <15% of participants in all stakeholders scored the outcome as 1–3; (ii) consensus out: ≤50% of participants in TCM experts and WM experts scored the outcome as 7–9; (iii). no consensus: anything else.

The voice of patients should be considered. Hence, for the patients' survey, the consensus definition was outcomes that were voted by >50% of patients.

For the consensus meeting, the consensus definitions were: (i) consensus in: outcomes that were voted by ≥70% of participants; (ii). consensus out: outcomes that were voted by <70% of participants.

Consensus Meeting

The consensus meeting was held by teleconference. The contents of the consensus meeting covered: (i) the reporting background and methods of the research; (ii) reporting the results of the Delphi survey of professionals and the public, and the results of the patients' questionnaire; (iii) discussing the candidate outcomes and their instruments/definitions; and (iv) voting on the outcomes and reaching a consensus.

Ethics and Consent

The entire project is part of a clinical trial of COVID-19, which was approved by the Ethics Committee of Dongzhimen Hospital (DZMEC-KY-2020-09). Because of the special circumstances of the COVID-19 pandemic, participants who completed the questionnaire were assumed to have provided consent for their data to be used.

Results

A total of 160 protocols from 19 platforms of clinical-trial registries were searched. After reading the titles and study details, 63 non-relevant or ineligible study protocols were excluded. Finally, 97 eligible study

protocols were included from ChiCTR and ClinicalTrials.gov. Thirty-four clinical trials were for TCM therapy and 63 clinical trials were for WM therapy. All clinical trials will be conducted in China. These clinical trials comprised 75 RCTs (53 for WM and 22 for TCM) and 22 non-RCTs (10 for WM and 12 for TCM). For 34 protocols of TCM clinical trials, there were 76 individual outcomes from 16 outcome domains after the merging and grouping of outcomes. For 63 protocols of WM clinical trials, there were 126 individual outcomes from 17 outcome domains after merging and grouping. The list of outcomes can be obtained from the systematic review (Qiu et al., 2020). There were >40 duplicated outcomes between the TCM and WM protocols for clinical trials.

After removing duplicated outcomes, we developed the questionnaire for first round of the Delphi survey. After review by the steering group, 145 outcomes were included in the questionnaire.

Round 1 of the Delphi Survey

We had incentive measures to improve the response of the Delphi survey (randomized rewards after completing and submitting the questionnaire). The time planned for round 1 of the Delphi survey was from 4 March to 12 March 2020. As of March 9, 2020, 176 participants had completed the questionnaire. After review, 51 questionnaires were found to be invalid. On March 8 and 9, 2020, ≤5 questionnaire/day were completed, and almost all of them were invalid. Most of the invalid questionnaires had been completed by the public within 5 min (it was not possible for people who were unfamiliar with COVID-19 to complete the questionnaire) or who had chosen the same score for all outcomes. After discussion with the steering group, we decided to stop the Delphi survey.

Finally, 125 valid questionnaires were evaluated. The characteristics of participants in the round 1 of the Delphi survey are shown in Table 1. The number of outcomes that achieved consensus and no consensus in different stakeholders are shown in Table 2. The list of outcomes is shown in Supplement 1.

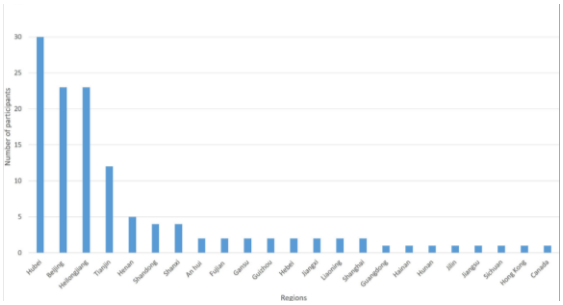
Characteristics	No. of population
Identification	
TCM experts	76 (76/125, 60.8%)
WM experts	16 (16/125, 12.8%)
Nurses	6 (6/125, 4.8%)
Public	27 (27/125, 21.6%)
Frontline working	
Yes	48 (48/125, 38.4%)
No	77 (77/125, 61.6%)
Designing or Participating in research of COVID-19	
TCM research	32 (32/125, 25.6%)
WM research	6 (6/125, 4.8%)
None	87 (87/125, 69.6%)

TCM, traditional Chinese medicine; WM, Western medicine.

Stakeholders	Consensus in	Consensus out	No consensus
TCM experts	34	50	61
WM experts	50	47	48
Nurses	126	2	17
Public	106	0	39

TCM, traditional Chinese medicine; WM, Western medicine.

Only 15 (15/125, 12%) participants were in Hubei Province. However, the Internet Protocol (IP) address that the electronic questionnaire obtained showed that 30 (30/125, 24%) participants were in Hubei Province. Only one person (1/125, 0.8%) was from outside of China (Canada). The regions of participants are shown in Figure 1.



More than 20 participants provided outcomes or significant proposals for round 1 of the Delphi survey. After discussion with the steering group, six of them were added to round 2 of the Delphi survey. There are some "consensus out" outcomes in different stakeholders, but no "consensus out" outcomes by all of the stakeholders were noted, so we did not delete any outcomes in the round 2 of Delphi survey.

Round 2 of the Delphi Survey

According to the significant proposals from participants in round 1 of the Delphi survey, the steering group decided to add more personal information. To reduce the risk of invalid questionnaires, participants would receive a random reward if the completed questionnaire was considered to be valid. Participants were also asked if they agreed to be mentioned in the "acknowledgements" section when the research was published. Computed tomography and magnetic resonance imaging of the hip were grouped as "hip imaging". There were 150 individual outcomes in round 2 of the Delphi survey.

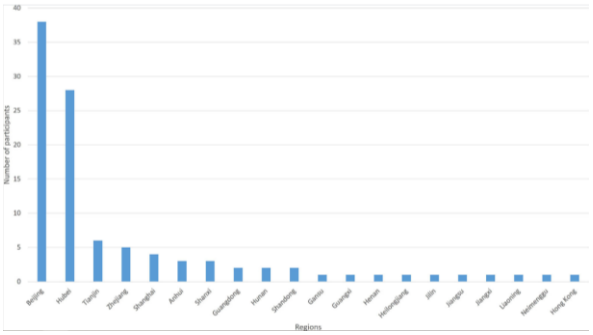
The feedback from participants in round 1 of the Delphi survey showed that scoring for some outcomes was difficult. Hence, in round 2 of the Delphi survey, participants had the opportunity to choose "unclear" for any outcome that was difficult to determine. The median score of each outcome from each stakeholder group was shown in round 2 of the Delphi survey. The steering group wanted more people to participate in the Delphi survey. Hence, the questionnaire was sent to potential participants (irrespective of whether they completed round 1 of the Delphi survey) and they were asked to invite colleagues who might be interested in this research. Round 2 of the Delphi survey was conducted from 11 to 13 March 2020.

A total of 110 questionnaires were completed, but seven of them were invalid, so 103 valid questionnaires were assessed. The characteristics of participants in round 2 of the Delphi survey are shown in Table 3.

Characteristics	No. of population	Characteristics	No. of population
dentification		Frontline working	
TCM experts	60 (60/103, 58.3%)	Yes	42 (42/103, 40.8%)
WM experts	22 (22/103, 21.4%)	No	61 (61/103, 59.2%)
Nurses	13 (13/103, 12.6%)	Designing or Participating in research of COVID-19	
Public	8 (8/103, 7.8%)	TCM research	25 (25/103, 24.3%)
Education background		Western medicine research	2 (2/103, 1.9%)
Doctor	35 (35/103, 34%)	None	76 (76/103, 73.8%)
Master	50 (50/103, 48.5%)	Participating in round 1 of Delphi survey	
Undergraduate	14 (14/103, 13.6%)	Yes	26 (26/103, 25.2%)
Others	4 (4/103, 3.9%)	No	77 (77/103, 74.8%)
Professional qualification			
Senior	30 (30/103, 29.1%)		
Intermediate	46 (46/103, 44.7%)		
Junior	17 (17/103, 16.5%)		
None	10 (10/103, 9.7%)		

TCM, traditional Chinese medicine; WM, Western medicine.

The IP address that the electronic questionnaire obtained showed that 28 (28/103, 27.2%) participants were in Hubei Province. The regions of participants are shown in Figure 2. The number of outcomes that achieved consensus and no consensus in different stakeholders are shown in Table 4. The list of outcomes is shown in Supplement 2.



Stakeholders	Consensus in	Consensus out	No consensus
TCM experts	91	35	24
WM experts	57	44	49
Nurses	141	0	9
Public	104	31	15

TCM, traditional Chinese medicine; WM, Western medicine.

After the results of round 2 of the Delphi survey had been reviewed by the steering group, outcomes that achieved "consensus out" by TCM experts and WM experts were excluded. Outcomes that achieved "consensus in" from stakeholders were grouped and presented according to the classification of disease and interventions. They were presented to consensus-meeting participants with "no consensus outcomes" before the consensus meeting was held.

Patients' Survey

Results suggested that nurses and the public may find it difficult to score outcomes because they may misunderstand the terminology. Hence, we developed a simple questionnaire with understandable language for patients. There were 43 outcomes/outcome domains in the questionnaire. The list of outcomes is in Supplement 3. Patients were recruited by frontline clinicians in our team on 12 and 13 March 2020. Finally, 10 cured patients agreed to participate in the survey. They were asked to choose which outcomes were important to them. The characteristics of patients are shown in Table 5. More than 50% of patients care about outcomes of pulmonary imaging, lung function, respiratory symptoms such as cough and dyspnea, fever, SARS-CoV-2 nucleic acid tests, recovery, and mental state.

Characteristics	No. of population	Characteristics	No. of population
Gender		Type of disease	
Male	6 (6/10, 60%)	Mild	4 (4/10, 40%)
Female	4 (4/10, 40%)	Ordinary	4 (4/10, 40%)
Age		Severe	0
≤18	0	Critical	2 (2/10, 20%)
18-29	3 (3/10, 30%)	Type of therapy	
30-39	5 (5/10, 50%)	TCM	0
40-49	1 (1/10, 10%)	Integrated TCM and WM	9 (9/10, 90%)
50-59	1 (1/10, 10%)	WM	1 (1/10, 10%)

TCM, traditional Chinese medicine; WM, Western medicine.

Consensus Meeting

The consensus meeting was held on 18 March 2020 and was a video conference. Six frontline clinicians (one from a WM hospital and five from TCM hospitals) as well as one frontline nurse, one methodologist, and one researcher who participated in the design of clinical trials of COVID-19 were invited to attend the consensus meeting. The participants were from Shanghai (one), Beijing (five), Tianjin (two), and Guizhou (one) and all were voting participants. All clinicians and nurses had worked in Hubei Province after the COVID-19 outbreak. Two additional participants (one coordinator and one staff member from the Chinese Clinical Trial Registry) attended the meeting but did not participate in the discussion or voting.

After reporting the results of the Delphi survey and patients' survey, participants discussed some outcomes they believed should/should not be measured in clinical trials. After discussion, voting participants were invited to vote on which outcomes should be included in the COS of COVID-19. The outcomes voted by ≥70% of participants were included in the COS. The voting results are shown in Supplement 4. The COS of COVID-19 is shown in Table 6.

Outcome domain	Outcome	Outcome measurement instruments/ definition	Type of disease				Interventions	
			Mild	Ordinary	Severe	Critical	TCM	WM
Clinical outcome	Recovery/improvement/progression/death	a. Recovery: recovery time or recovery prevalence b. Improvement: from severe type to ordinary type c. Progression: prevalence and time of progressing to severe or critical types d. Death: mortality	√	√	√	√	√	√
Etiology	SARS-CoV-2 nucleic acid tests	a. Proportion of patients negative for SARS-CoV-2 b. Time taken by SARS-CoV-2 RNA to become negative c. Declining speed of SARS-CoV-2	√	√	√	√	√	√
	Viral load		√	√	√	√	√	√
Inflammatory factor	CRP	CRP level and time for CRP recovery	√	√	√	√	√	√
Vital signs	Temperature	Prevalence of fever and clearance time of fever	√	√	√	√	√	√
	Respiration	a. Dyspnea prevalence b. Improvement in respiratory rate c. Time to achieve a normal respiration rate d. Prevalence of dyspnea clearance		√	√	√	√	√
Blood and lymphatic system outcomes	Lymphocyte	Lymphocyte count	√	√	√	√	√	√
	Virus antibody	Virus antibody level	√	√	√	√	√	√
Respiratory outcomes	Pulmonary imaging	Inflammation absorption or time to recovery	√	√	√	√	√	√
	Blood oxygen saturation	Blood oxygen saturation or prevalence of improvement	√	√	√	√	√	√
	PaO2/FiO2		√	√	√	√	√	√
	Arterial blood-gas analysis		√	√	√	√	√	√
	Mechanical ventilation	a. Duration of mechanical ventilation b. Frequency of requirement for mechanical ventilation c. Prevalence of mechanical ventilation				√	√	√
	Oxygen intake	a. Duration of supplemental oxygenation b. Frequency of requirement for supplemental oxygen c. Prevalence of supplemental-oxygen requirement d. Oxygen-intake method		√	√	√	√	√
	Pneumonia severity index			√	√	√	√	√
Clinical efficacy	Prevalence of preventing mild-to-moderate disease progressing to severe disease		√	√			√	√
Symptoms	Clinical symptom score		√	√	√	√	√	

CRP, c-reactive protein; PaO2/FiO2, partial pressure of oxygen/fraction of inspiration oxygen; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCM, traditional Chinese

Discussion

This COS was conducted rapidly and rigorously to report on an emergency in a specific environment. It can be used for any type of disease, intervention, and design. There is a specific outcome for TCM clinical trials: clinical symptom score. Researchers can measure the clinical symptom score according to different TCM syndromes. For some individuals, there are several measurements because there is no evidence to show which measurement is the best one. We hope researchers of clinical trials can use this COS to reduce heterogeneity in outcome reporting. Furthermore, our COS may help decision-makers to approve new agents for COVID-19 if researchers report important outcomes. However, researchers can report other outcomes according to the purpose of their research.

Our study had four main limitations. First, due to the highly infectious nature of SARS-CoV-2, patients and the public did not participate in the design or development of the preliminary list of outcomes, but they participated in the process of Delphi survey. Second, the preliminary list of outcomes was developed from protocols of clinical trials when there were knowledge gaps in the prevalence, therapy, prognosis, and clinical characteristics of COVID-19. With the research progression, new important outcomes may be reported. Hence, the COS must be updated in the future. Third, the number of patients was small and all of them were from Hubei Province, so their perspectives may not reflect those of other regions in China or overseas. Fourth, almost all stakeholders were from China. Though one participant in round 1 of the Delphi survey was from Canada, his/her opinion reflected a Chinese perspective because the questionnaire was written in Chinese.

Data Availability Statement

The data is from public databases and does not include identifiable patient data. All of the primary data was searched from different registry databases before it was analyzed, and the results can be found in another manuscript: https://medrxiv.org/cgi/content/short/2020.03.04.20031401v1. This manuscript was included in a priprint server https://www.medrxiv.org/content/10.1101/2020.03.23.20041533v2.

Ethics Statement

The studies involving human participants were reviewed and approved by Ethics Committee of Dongzhimen Hospital, Beijing University of Chinese Medicine. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author Contributions

RQ, JC, and HS designed the research. RQ drafted the manuscript. CZho and XW conducted the systematic review. TL, XH, and YH conducted the Delphi survey and patients' questionnaire. XZ, ZC, and MZ developed questionnaire. CZha, JH, ML, SH, TH, and YS revised the questionnaire. JC and HS revised the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00781/full#supplementary-material.

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The novel coronavirus disease (COVID-19) infection has been spreading for more than 2 months since it broke out early 2020.(1) The alarming levels of spread and severity have made this virus characterized as a pandemic which has been affecting at least 200 countries, areas or territories around the world. And 1051,635 cases and 56,985 deaths were confi rmed by 10:00 CET April 4, 2020.(2) So far, it is encouraging to note that the outbreak of COVID-19 in China has been basically contained and more than 77,200 patients in China have been cured after a series of treatments,(3) which suggests that patients can be cured through therapy even though there is an absence of specific medications and vaccines. Vaccines and targeted drugs are most effective in preventing and combating infectious diseases. However, developing such vaccines is particularly timeconsuming and arduous, and the long-term side effects of new medicine could also be a concern. Some recent studies and clinical data have demonstrated that among the limited therapies, Chinese medicine (CM), together with Western medicine (WM), plays an important role in halting the progress of the disease and promoting the recovery of patients in the absence of targeted drugs.(4,5) With discreet assessment, integrated CM and WM treatment with clear and constantly updated prescriptions has been approved for clinical use since the third version of the Handbook of Prevention and Treatment of the Pneumonia Caused by the Novel Coronavirus (2019-nCoV), which was issued by the Chinese authorities.(6) The latest handbook recommended different CM and WM prescriptions for diversified categories of COVID-19 cases including mild, moderate, severe, critical, convalescent, and suspected (Appendixes 1 and 2).(7) Recent studies have demonstrated that treatment with integrated CM and WM can signifi cantly alleviate fever, cough, and other clinical symptoms. A clinical observation showed that the patients' indexes such as

serum amyloid A, lymphocyte percentage, creatine kinase isoenzyme MB, alanine transaminase, aspartate transaminase, and blood urea nitrogen in the integrated therapy group recovered faster than those treated with WM only.(8) Combined administration of Toujie Quwen Granules (透解祛瘟颗粒, prepared from 16 CMs such as Fructus Forsythiae, Pseudobulbus Cremastrae, Flos Lonicerae, Radix Scutellariae, Folium Isatidis, etc.) and arbidol up-regulated the expressions of peripheral blood CD4+ /CD8+ and absolute value of lymphocyte in 37 mild COVID-19 patients while arbidol alone not, indicating the positive function of integrated therapy in regulating the balance of immune cells and promoting the recovery of immune function.(9) In another study, the integrated therapy showed a substantial increase in the rate of hospital discharge and reduction in the rate of deterioration.(10) In addition, inflammatory exudation and inflammatory storm associated with COVID-19 often result in acute lung injury, which subsequently causes severe respiratory failure and even death. Antibody drugs like tocilizumab can neutralize interleukin-6 directly, thus effectively alleviating cytokine storms, but they are too expensive to be widely adopted.(11) In comparison, the CM therapy which is relatively low-cost, can considerably relieve the inflammatory reaction as well through affecting the lymphatic system to modulate the patient's immune response.(12) Therefore, proper integration of the two therapies may make the treatment more economical for patients. This strategy is also suitable for regulating the activation of classic renin-angiotensin system regulatory pathway, which is activated when 2019-nCoV binds with angiotensin converting enzyme 2 receptor and is responsible for multiple organ injuries.(13) A comparative study on 710 cases jointly conducted by over 30 hospitals showed that combining regular treatment with Xuebijing Injection (血必净注射液, produced from 5 CMs including Flos Carthami, Radix Paeoniae rubra, Rhizoma chuanxiong, Radix Angelicae sinensis, and Radix Salviae Miltiorrhizae) reduced the mortality rate of severe pneumonic patients by 8.8% and shortened intensive care unit hospitalization by 4 days.(14) And a basic research performed on 156 patients at 32 hospitals recently also found that the injection has a certain antiviral effect in vitro that can inhibit inflammatory factors induced by 2019-nCoV infection.(15) Although the clinical reports are limited and required to be confirmed by further studies, such treatment is a reliable alternative option. While the therapeutic results are encouraging, the mechanisms and pathways through which CM works still need to be revealed. Side effects associated with the use of integrated CM and WM therapy should also be evaluated. In addition, the misuse of the integrated therapy by people who try to take precautions against COVID-19 infection should not be encouraged. Rational combination of CM and WM would benefi t the treatment of diverse emerging or serious diseases in the near future. Confl ict of Interest No confl ict is declared. Author Contributions Luo Y, Qing GC, and Zhang H conceived of the design and carried out the study. Qing GC and Zhang H undertook the literature review of historical evidence and wrote the manuscript. Luo Y and Bai Y provided important suggestions and revised the manuscript. Luo Y supervised the study. All authors read and approved the fi nal manuscript. Electronic Supplementary Material: Supplementary material (Appendixes 1 and 2) is available in the online version of this article at http://dx.doi.org/10.1007/s11655-020-3095-6. REFERENCES 1. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet 2020;395:470-473. 2. World Health Organization. Coronavirus disease 2019 (COVID-19) situation report–75. Accessed April 4, 2020. Available at https:// www.who.int/docs/default-source/coronaviruse/situationreports/20200404-sitrep-75-covid-19.pdf?sfvrsn=99251b2b_2 3. National Health Commission of the People's Republic of China. Update on the novel coronavirus pneumonia outbreak. Accessed April 4, 2020. Available at http://www.nhc.gov.cn/xcs/ yqtb/202004/4f4e36d54fc941d48f6ce6554514075e.shtml 4. Luo H, Tang QL, Shang YX, Liang SB, Yang M, Robinson N, et al. Can Chinese medicine be used for prevention of corona virus disease 2019 (COVID-19)? 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Despite advances in understanding the pathogenesis of 2019 novel coronavirus disease (COVID-19), there are yet no licensed vaccine or specific antiviral medicine to prevent or treat COVID-19 to date. Integrated multi-disciplinary treatment is recommended to improve therapeutic outcomes. To treat COVID-19 patients in China, Traditional Chinese Medicine (TCM) experts have recommended the use of herbal formulae based on their clinical expertise, and more than 90 % confirmed cases have received TCM therapy [1]. One of the TCM formulae was reported to be used in treating 701 confirmed cases across 10 provinces in China, of which most patients were relieved [2]. In addition, several ongoing randomized, clinical trials of TCM remedies have been registered in the international clinical trials registry platform (www.chictr.org.cn). However, these current data are insufficient to support the effectiveness and safety of TCM formulae in treating COVID-19 patients, the underlying mechanisms of TCM formula on blocking COVID-19 remain unclear. Computer-aided systematic approaches hold the promise of expanding understanding the rationale and mechanism for the clinical performance of TCM against COVID-19. In this study, we focused on the most widely used Chinese medicinal herbs contained in the prescribed formulae in treating COVID-19 and employed network pharmacology-based technologies to analyze and identify their underlying mechanisms from a holistic perspective.

To better interpret the scientific outlook on TCM, we collected all the clinically effective formulae that are included in the National Guidelines, Provincial Recommendations or Registered Clinical Trials (www.chictr.org.cn) up to February 23th. A total of 167 TCM formulae with 255 differentiated herbs, 22

mineral medicines and 22 animal parts were used in China (Supplementary table 1). Of note, 10 Chinese herbs, i.e. *Glycyrrhizae Radix Et Rhizoma, Armeniacae Semen Amarum, Gypsum Fibrosum, Scutellariae Radix, Forsythiae Fructus, Poria, Ephedrae Herba, Citri Reticulatae Pericarpium, Pogostemonis Herba, Lonicerae Japonicae Flos*, were applied in quite a high frequency, listed in at least 30 formulae. We have therefore defined these 10 herbs as a new formula called "Anti-COVID-19 Decoction" and analyzed their active compounds by adopting Traditional Chinese Medicine for Systems Pharmacology Database (TCMSP) (http://lsp.nwu.edu.cn/index.php), with absorption, distribution, metabolism and excretion (ADME) screening (oral bioavailability ≥30 % and drug-likeness value≥ 0.18), and Traditional Chinese Medicine Integrative Database (TCMID) (http://www.megabionet.org/tcmid/). As a result, a total of 258 compounds were identified as the bioactive ones (Supplementary table 2, 3), among which 20 compounds were contained in at least two herbs while the flavonoid quercetin was identified in 5 herbs of the Anti-COVID-19 Decoction, suggesting that functional coordination in some related biological processes may exist in the Decoction (Supplementary table 4).

Interestingly, we got 3215 compound-targets (with duplications) of the Anti-COVID-19 Decoction from TCMSP (Supplementary table 5), while 913 disease targets from GeneCards database (https://www.genecards.org/) and OMIM database (http://omim.org/) by adopting the keyword "new coronavirus" (Supplementary table 6). Of note, fifty-three overlapping genes were identified as the compound-targets of the Anti-COVID-19 Decoction by matching to the disease targets of COVID-19 (Supplementary Fig. 1), indicating that these 53 targets were the potential therapeutic targets of the Anti-COVID-19 Decoction against COVID-19. The Chinese herbal medicine of *Glycyrrhizae Radix Et Rhizoma* affected 46 COVID-19-associated disease targets, higher than other herbs in the Decoction (Supplementary Fig. 2). By conducting compound-target-disease network analysis, we found close correlations among the compounds in the Anti-COVID-19 Decoction and COVID-19-associated disease targets, indicating that multi-COmpounds contained in the Anti-COVID-19 Decoction might play their therapeutic roles through multiple COVID-19-associated targets (Supplementary Fig. 2). In particular, prostaglandin G/H synthase 2 (PTGS2), alpha-1D adrenergic receptor (ADRA1D), prostaglandin G/H synthase 1(PTGS1), nuclear receptor coactivator 2 (NCOA2) and peroxisome proliferator-activated receptor gamma (PPAR-γ) were targeted by 176, 151, 118, 112 and 102 compounds, respectively, indicating the potential synergistic effects in this herbal decoction.

We further performed the protein-protein interaction (PPI) analysis on those 53 potential therapeutic targets (https://string-db.org/), and found that interleukin 6 (IL-6) and mitogen-activated protein kinase3 (MAPK3) were the core targets of the Anti-COVID-19 Decoction (Fig. 1, Supplementary figure 4). The expression level of IL-6 was significantly increased in the severe COVID-19 patients compared to the level in mild cases [3]; while IL-6 receptor inhibitor tocilizumab has been applied in the patients to counteract the cytokine storm of COVID-19. Hence, inhibition of the expression level of IL-6 could be a promising therapeutic strategy for treating COVID-19. In this current study, we selected cytokine IL-6 as the major COVID-19-associated disease target for further investigation. We found that four compounds, i.e. quercetin,

wogonin, luteolin and oroxylin a, were closely correlated with IL-6. The action network of these four compounds on the COVID-19-associated disease targets have been simultaneously identified (Supplementary figure 5). MAPK signaling pathway involves a wide range of cellular functions like cell proliferation, differentiation and survival, which plays crucial roles in coronavirus propagation. Some compounds contained in “Anti COVID-19 Decoction”, such as naringenin, have been previously proven to significantly inhibit on activated MAPK3, supporting the results of the network analysis partly [3,4].

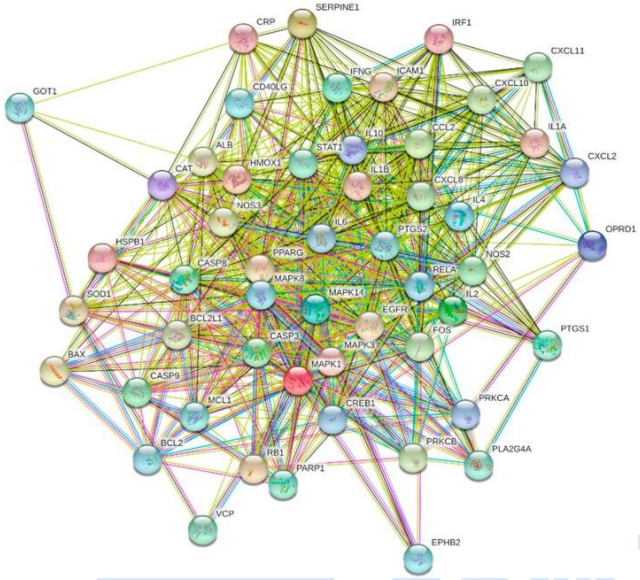

Fig. 1. PPI analysis on the potential therapeutic targets of the Anti-COVID-19 Decoction. Network nodes represent proteins. Edges represent protein-protein associations (Blue line: Known interactions from curated databases; fuchsia line: experimentally determined interactions; green line: gene neighborhood; red line: gene fusions; blue line: gene co-occurrence; yellow line: textmining; black line: co-expression; violet line: protein homology).

To uncover the underlying mechanisms of the Anti-COVID-19 Decoction, we conducted functional analysis on the 53 potential therapeutic targets by gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) analysis. The results of GO analysis mainly focused on cytokine receptor binding, cytokine activity and receptor-ligand activity (Supplementary figure 6), while the results of KEGG analysis revealed that the potential therapeutic mechanisms of the Anti-COVID-19 Decoction are most likely related to IL-17 signaling pathway and TNF signaling pathway (Supplementary figure 7&8, Supplementary table 7). Though the counts of peripheral T cells were substantially reduced, their status was hyperactivated and there was an increased concentration of the highly pro-inflammatory $CCR4^{+}CCR6^{+}$ Th17 in severe case [5]; moreover, the patients in ICU had a higher concentration of TNFα than those not requiring ICU admission [1], indicating that Th17 and TNFα account for the severe immune injury in the patients in part. Thus, modulation of IL-17 and TNFα signaling pathways by the Anti-COVID-19 Decoction may have significant potential to block the conversion of mild cases to severe cases, and even to save the critically ill patients.

Computed tomography imaging of COVID-19 patients is characterized by ground-glass opacity (56.4 %) and bilateral patchy shadowing (51.8 %) with partial consolidation which will be absorbed with the formation of fibrotic stripes [6,7]. Histological examination of the biopsy lung sample from the COVID-19 patient showed bilateral diffuse alveolar damage with cellular fibromyxoid exudates [5]. Therefore, the treatment for COVID-

19 needs to deliver one-two punch to block the progression of pneumonia as well as to prevent the formation of fibrosis. Previous reports showed that inhibition of transforming growth factor-β (TGF-β) has the capability of preventing lung fibrosis [8]. We further applied molecular docking to calculate the possible interactions between the key compounds (quercetin, wogonin, luteolin and oroxylin a) and TGF-β. Binding free energies of quercetin, wogonin, luteolin and oroxylin a with TGF-β were -10.97 kcal mol^{-1}, -7.878 kcal mol^{-1}, -10.319 kcal mol^{-1} and -8.408 kcal mol^{-1}, respectively (Fig. 2), indicating that these compounds might bind with TGF-β.

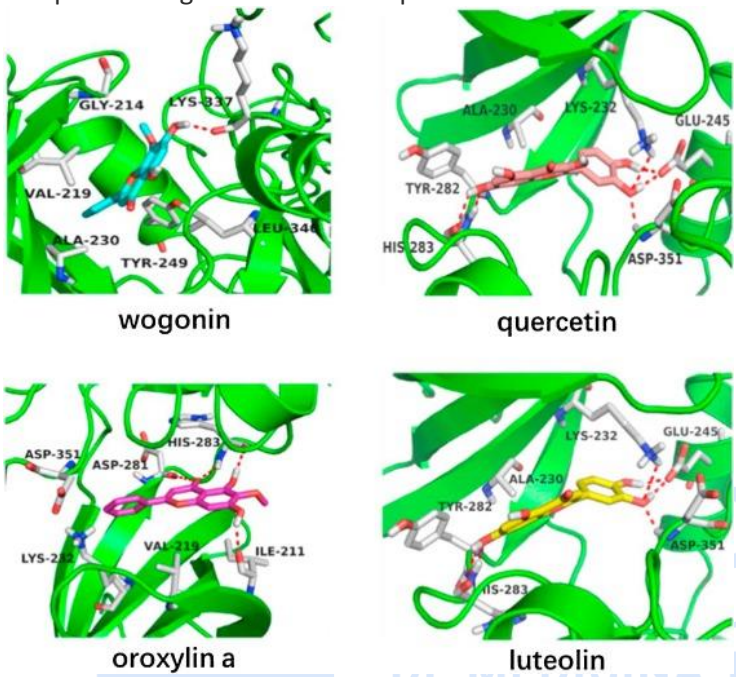

Fig. 2. The binding mode between the key compounds in the Anti-COVID-19 Decoction and TGF-β calculated by molecular docking.

In conclusion, it was the first report focusing on the most widely used herbs in treating COVID-19 patients, which encourages us to identify the most effective anti-COVID-19 decoction from Chinese herbal medicine. And, multi-COmpounds in the Anti-COVID-19 Decoction are proposed to play a synergistic and multiple effects on the multiple COVID-19-associated disease targets, especially on modulating the disordered virally driven hyperinflammation. These results indicate that this formula may play important roles on the different stages of the disease, rather than a single stage or TCM syndrome of the COVID-19 patients. The key compounds in the Anti-COVID-19 Decoction are quercetin, wogonin, luteolin and oroxylin a; and these four compounds might have potentials of further development as new anti-COVID-19 agents. We anticipate that comprehensive pharmacological experiments *in vivo* and *in vitro* are needed to verify the therapeutic mechanisms of the Anti-COVID-19 Decoction in TCM. At the same time, well-designed clinical trials are also required to develop world-wide acceptance of TCM in treating COVID-19 patients.

Authors' contributions

Study design, manuscript revision and funding acquisition, L Liu; Data analysis and writing, HD Pan; Molecular docking calculation, XJ Yao; Data collection, WY Wang; Manuscript revision, HY Lau.

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Declaration of Competing Interest

The authors have declared that there is no conflict of interest.

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http://www.ncbi.nlm.nih.gov/pubmed/32145402

Traditional Chinese medicine for COVID-19 treatment 1. Summary The current 2019-nCoV outbreak is moving rapidly [1], the cumulative number of confirmed cases in mainland China has reached 80151, with 47,204 (58.89 %) cured cases and 2943 (3.67 %) deaths as of 2-Mar-2020, and no specific drug has been discovered for Cor- onavirus Disease 2019 (COVID-19). However, a number of clinical practice results showed that traditional Chinese medicine (TCM) plays significant role in the treatment of COVID-19, bringing new hope for the prevention and control of COVID-19. TCM has a long history and played an indispensable role in the prevention and treatment of several epidemic diseases. During the SARS epidemic in 2003, the intervention of TCM has also achieved remark- able therapeutic effect. During the treatment period of COVID-19, more than 3100 medical staff of TCM were dispatched to Hubei province, and TCM scheme was included in the guideline on diagnosis and treatment of COVID-19 [2], and TCM experts fully participate in the whole rescue process. The decoction, Chinese patent medicine, acupuncture and other characteristic therapy of TCM was comprehensively employed, mainly treated based on syndrome differentiation. Specific TCM wards were set

up, and established the designated hospital, moreover, TCM team participates in treatment collectively. Currently, the total number of confirmed cases treated by TCM has reached 60,107 [3]. In 102 cases of mild symptoms treated with TCM, the clinical symptom dis- appearance time was shortened by 2 days, the recovery time of body temperature was shortened by 1.7 days, the average length of stay in hospital was shortened by 2.2 days, the improvement rate of CT image was increased by 22 %, the clinical cure rate was increased by 33 %, 27.4 % reduction in the rate of common to severe cases and 70 % in- crease in lymphocyte.3 In addition, in the treatment of severe patients with TCM, the average length of stay in hospital and the time of nucleic acid turning negative has been shortened by more than 2 days. From current treatment results, TCM based on an over-all symptoms of 2019-nCoV pneumonia patients, has suggested to prescribe pre- scription that are likely to be effective, such as qingfei paidu decoction (QPD), gancaoganjiang decoction, sheganmahuang decoction, qingfei touxie fuzheng recipe, etc. QPD which consisted of Ephedrae Herba, Glycyrrhizae Radix et Rhizoma Praeprata cum Melle, Armeniacae Semen Amarum, Gypsum Fibrosum, Cinnamomi Ramulus, Alismatis Rhizoma, Polyporus, Atractylodis Macrocephalae Rhizoma, Poria, Bupleuri Radix, Scutellariae Radix, Pinelliae Rhizoma Praepratum cum Zingibere et Alumine, Zingiberis Rhizoma Recens, Asteris Radix et Rhizoma, Farfarae Flos, Belamcandae Rhizoma, Asari Radix et Rhizoma, Dioscoreae Rhizoma, Aurantii Fructus Immaturus, Citri Reticulatae Pericarpium, and Pogostemonis Herba, has been promoted as a general prescription in the diagnosis and treatment plan of COVID-19 in China [2]. Among the 701 confirmed cases treated by QPD, 130 cases were cured and discharge clinical symptoms of 51 cases disappeared, 268 cases of symptoms improved, and 212 cases of stable symptoms without aggravation [3]. The effective cure rate of QPD against COVID-19 is over 90 %. Ac- cording to the theory of TCM, the target organ location of COVID-19 is the lung, and the etiology attribute is “damp and toxin plague”. The network pharmacology analysis showed that QPD has an overall reg- ulatory effect via multi-component and multi-target. The primary site of pharmacological action is the lung, as 16 herbs to lung meridian, which indicated that the decoction is mainly specific for lung diseases. In addition, it can play the role of dehumidification through the rise and fall of the spleen and stomach, and exhibited the protection for heart, kidney and other organs. Among the potential targets screen, most of them co-expressed with ACE-2, the receptor of COVID-19, indicating the potential improvement of COVID-19. It can inhibit the replication of COVID-19 by acting on multiple ribosomal proteins. COVID-19 can lead to strong immune response and inflammatory storm [4]. Functional enrichment analysis showed that QPD could inhibit and alleviate ex- cessive immune response and eliminate inflammation by regulating immune related pathway and cytokine action related pathway [5]. Furthermore, through the prediction of molecular docking, it was found that patchouli alcohol, ergosterol and shionone in the formula had better anti−COVID-19 effect, which provided new molecule structures for new drug development [6]. Here, we take one highly suspected COVID-19 patient treated with TCM as a case example to show its effectiveness [7]. The male patient was on a business trip in Wuhan for several days before the onset of the disease. During the admission period, fever and cough were repeated, and respiratory rales of both lungs were not obvious. Western medicine was used firstly, including orally take oseltamivir phosphate capsule, intravenous infusion of ganciclovir, aerosol inhalation of recombinant human interferon a1b, etc. Although the nucleic acid test was negative, the results of chest CT showed that the fusion of two lung ground glass shadows was enlarged and the density was increased, which was more advanced than that of admission (Fig. 1a-1c). As the serious illness, combined with the patient's performance of damp-heat syndrome, and the heat is more serious than damp, QPD was added for treatment. On the night of administration, the body temperature dropped to 36.2 ℃, and then tended to be normal. After 6 days of treatment, chest CT was better than before, tracheobronchial shadow was normal, and in- flammation was obviously absorbed (Fig. 1d). The patient had no fever or asthenia, coughing occasionally, and the rales of two lungs were weaker than before. After discharge, continue to take 7 doses of the prescription, occasionally cough, no special discomfort was found. The clinical symptoms and imaging examination of the patients improved significantly after the treatment, reflecting the advantages of TCM. TCM has own characteristics such as holistic concept, balance of Yin and Yang, syndrome differentiation and treatment, strengthening the body resistance to eliminate pathogenic factors. TCM has thousands of years of experience in regulating the body and enhancing the resistance to epidemic diseases, with unique insights and

prevention and control experience. For mild and common patients, the early intervention of TCM can effectively prevent the disease from transforming into severe and critical disease. In the severe cases, TCM has won time for rescuing them by improving symptoms (http://www.scio.gov.cn/xwfbh/ xwbfbh/wqfbh/42311/42560/index.htm).

Treatment practice of COVID-19 showed that early intervention of TCM is important way to improve cure rate, shorten the course of disease, delay disease pro- gression and reduce mortality rate. Furthermore, the reason why TCM works is not only to inhibit the virus, but might block the infection, regulate the immune response, cut off the inflammatory storm, and promote the repair of the body. Moreover, the prevention and control measures of COVID-19 fully reflect the ideology of “preventive treat- ment of disease”. Apart from the epidemic diseases recorded in the Han Dynasty should be isolated, the preventive measures of TCM also in- clude psychology, sports, diet, medication, etc. In the next prevention and control work of COVID-19, it should give full play to the advantages of TCM in syndrome differentiation and the whole therapeutic effect, reduce the complications as well as death rate. Besides, the scientific research should also be carried out on the TCM with definite curative effective of COVID-19, to comprehensively evaluating its action mechanism and in-depth understanding COVID- 19.

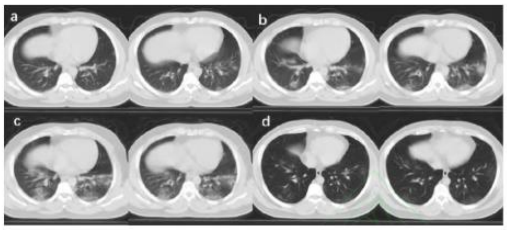

Fig. 1. Comparison of chest CT results of patients. (a), chest CT on January 24; (b), chest CT on January 28; (c), chest CT on January 30; (d), chest CT on February 4.

Declaration of Competing Interest There are no conflicts to declare.

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Appendix A. Supplementary data Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phrs.2020.104743.

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Abstract

Ethnopharmacological relevance

Traditional Chinese Medicine (TCM) has been widely used as an approach worldwide. Chinese Medicines (CMs) had been used to treat and prevent viral infection pneumonia diseases for thousands of years and had accumulated a large number of clinical experiences and effective prescriptions.

Aim of the study

This research aimed to systematically excavate the classical prescriptions of Chinese Medicine (CM), which have been used to prevent and treat Pestilence (Wenbing, Wenyi, Shiyi or Yibing) for long history in China, to obtain the potential prescriptions and ingredients to alternatively treat COVID-19.

Materials and methods

We developed the screening system based on data mining, molecular docking and network pharmacology. Data mining and association network were used to mine the high-frequency herbs and formulas from ancient prescriptions. Virtual screening for the effective components of high frequency CMs and compatibility Chinese Medicine was explored by a molecular docking approach. Furthermore, network pharmacology method was used to preliminarily uncover the molecule mechanism.

Results

574 prescriptions were obtained from 96,606 classical prescriptions with the key words to treat “Warm diseases (Wenbing)”, “Pestilence (Wenyi or Yibing)” or “Epidemic diseases (Shiyi)”. Meanwhile, 40 kinds of CMs, 36 CMs-pairs, 6 triple-CMs-groups existed with high frequency among the 574 prescriptions. Additionally, the key targets of SARS-COV-2, namely 3CL hydrolase (Mpro) and angiotensin-converting enzyme 2(ACE2), were used to dock the main ingredients from the 40 kinds by the LigandFitDock method. A total of 66 compounds components with higher frequency were docked with the COVID-19 targets, which were distributed in 26 kinds of CMs, among which *Gancao* (Glycyrrhizae Radix Et Rhizoma), *HuangQin* (Scutellariae Radix), *Dahuang* (Rhei Radix Et Rhizome) and *Chaihu* (Bupleuri Radix) contain more potential compounds. Network pharmacology results showed that *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) CMs-pairs could also interact with the targets involving in immune and inflammation diseases.

Conclusions

These results we obtained probably provided potential candidate CMs formulas or active ingredients to overcome COVID-19. Prospectively, animal experiment and rigorous clinic studies are needed to confirm the potential preventive and treat effect of these CMs and compounds.

1. Introduction

Corona Virus Disease 2019 (COVID-19), which is caused by a newly identified coronavirus SARS-COV-2, has been spread to more than 200 countries and regions around the world and posing significant threats to public health (Chen et al., 2020). Unfortunately, it is still raging with no effective drugs clinically approved. Given the severity of SARS-COV-2, it is critical to discovery and clinical application of specific drugs against SARS-COV-2 to alleviate the current epidemic situation. It is particularly important to screen possible

blockers for the potential target proteins of virus by computational chemical biology techniques such as molecular docking ("dry method"research) in special cases such as the outbreak of SARS-COV-2 (Li et al., 2020a). This approach conducive to large-scale screening in a short period of time. It has been recommended two main proteins, 3C-like protease (3CLpro) and angiotensin-converting enzyme 2 (ACE2), could be used as available targets for screening drugs that inhibiting the replication and proliferation of SARS-COV-2, benefit from rapid sequencing of SARS-COV-2 coupled with molecular modelling based on the genomes of related viral proteins (Chen et al., 2020; Chai et al., 2020).

Chinese Medicines (CMs), a long history system of medicine with distinct features of theories and practices, has been used for thousands of years (Qiu, 2015). CMs prescriptions embody the principles of system theory, and act on multiple cellular targets in multiple pathways to exert therapeutic effects (Hou et al., 2016; Liao et al., 2018). COVID-19 belongs to the category of pestilence or epidemic in CM (Li et al., 2020). CMs had been used to treat and prevent viral infection pneumonia diseases for thousands of years and had accumulated a large number of clinical experience and effective prescription (Luo et al., 2019). In the "Diagnosis and Treatment Program for Corona Virus Disease 2019 (COVID-19)" issued by the National Health Commission of China, it is recommended to treat with CMs and had achieved good clinical effects. Thus, it is very significant to explore and mine experiences of CMs in treating of pestilence or epidemic diseases based on the abundant historical classics of CMs combined with modern medical research method. In the present study, data mining and association network were used to mine the high-frequency CMs and formulas from ancient prescriptions. Furthermore, molecular docking approach were used to explore binding rates between the main ingredients in high frequency CMs and the key targets of SARS-COV-2. Then, we preliminarily uncover molecular mechanism by a network pharmacology process (Fig. 1). These results are expected to provide referenced candidate CMs formulas or active compounds to overcome COVID-19.

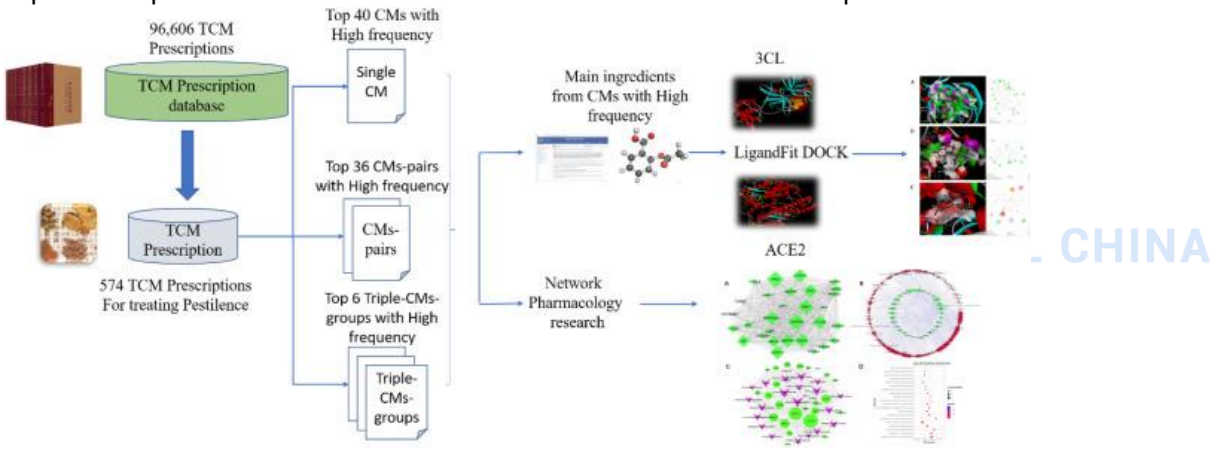

Fig. 1. The whole framework based on an integration strategy of screening system.

2. Material and method

2.1. Data mining

2.1.1. Data sources

In our study, the *Dictionary of Traditional Chinese Medicine Prescriptions* (Peng, 1996) and *Pharmacopoeia of the People's Republic of China* (Pharmacopoeia Commission China, 2015) were used to screened the prescriptions containing "Warm diseases (Wenbing)", "Pestilence (Wenyi or Yibing)" or "Epidemic diseases (Shiyi)".

The data processing process included the following three steps: Firstly, the relevant prescriptions retrieved were inputted into the word document to obtain the original literature file; Secondly, key words of prescription information contained number, name, source, formula, efficacy, therapy, dosage form and number of natural medicines and ancient literature classified information were inputted into the Excel file; Finally, prescription data were standardized on the basis of *Pharmacopoeia of the People's Republic of China* (Peng, 1996) and the *Chinese Materia Medica* (Zhu, 1998). Then standardized data was imported to database for following data mining and association network analysis.

2.1.2. Data analyze and association network analysis

In our present study, frequency analysis method, association rule mining method and association knowledge network construction method were used to analyze the collected prescriptions. The high frequency CMs were mined by frequency analysis method, and the compatibility rule of prescription was analyzed by association rules. The rules package was called into the formula basket data by R software platform, and the Apriori algorithm was used to mine the data for association rules with Confidence, Support and Promotion as the criteria (CSBTS, 1997). Support value was the percentage of preconditions that were true and used to measure universality; Confidence value was the percentage of preconditions for which records and the conclusions were both true, mainly used for measuring accuracy; Promotion value was used to evaluate the degree to which the appearance of one item set increased the appearance of another (Zhan and Fu, 2016). The mined related knowledge was screened and visualized by the arulesViz package to construct the associated knowledge network (Hahsler et al., 2011).

2.2. Molecular docking

2.2.1. Screening of active components in CMs

The chemical composition of high-frequency CMs were obtained from the TcmSP™ (Traditional Chinese Medicine System Pharmacology Database, http://tcmspw.com/tcmsp.php). Meanwhile, important pharmacology-related parameters of compounds were also obtained from TcmSP™, including drug-likeness (DL) and oral bioavailability (OB). The compounds with OB >30% and DL > 0.18 were selected as candidate compounds for further analysis (Wang et al., 2017). Besides, some compounds with low OB or DL values were also selected for candidate compounds because of their excellent pharmacological activities or high contents (S. J. Yue et al., 2017). The sdf format of the main active ingredients' structures were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) as candidates for molecular docking.

2.2.2. Preparation of the target proteins and the active site

The high-resolution crystal structure of COVID-19 3CL hydrolase (Mpro) was obtained from PDB (PDB_ID: 6LU7) (Jin et al., 2015) (Fig. 2). The active site of the protein is centered on the active amino acid site of the original ligand in the crystal structure. The corresponding "active pocket" was constructed. The system searched for the "active pocket" near the active site, and finally −8.669631, 12.384467, and 67.029640 with a point count of 8538 were defined as active pocket.

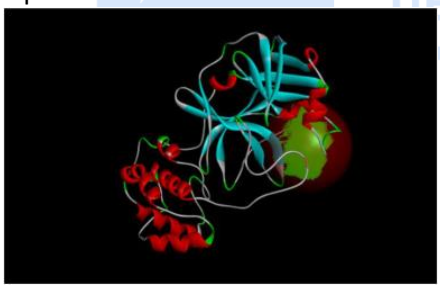

Fig. 2. High-resolution crystal structure of novel coronavirus target 3CL hydrolase (Mpro) (PDB 6LU7). The circle in the figure is the position of the active pocket.

The high-resolution crystal structure of angiotensin-converting enzyme 2 (ACE2) was obtained from PDB (PDB_ID:2AJF) (Fig. 2). The two active sites of the protein are centered on the active amino acid site of the original ligand in the crystal structure. The active site 1 of 15.262085, −17.780927, 57.786474 with the points count of 4198 (Fig. 3) and the active site 2 of 21.539614,7.755639,61.035558 with the points count of 4198 (Fig. 4) were constructed to dock.

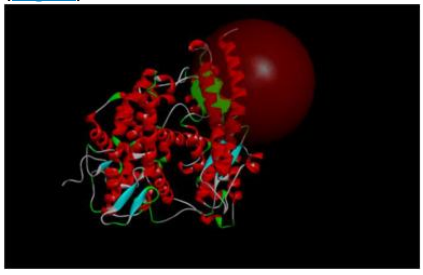

Fig. 3. High-resolution crystal structure of ACE2(PDB_ID:2AJF) site 1. The circle in the figure is the position of the active pocket.

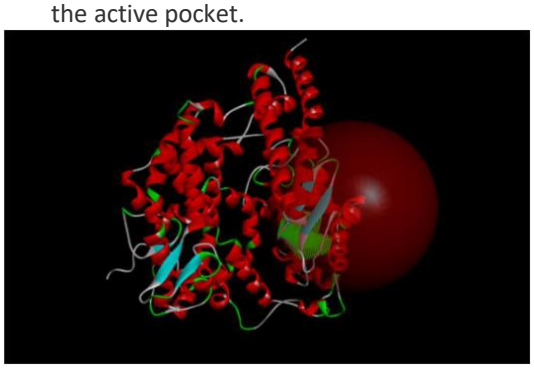

Fig. 4. High-resolution crystal structure of ACE2(PDB_ID:2AJF) site 2. The circle in the figure is the position of the active pocket.

2.2.3. Molecular docking method

Receptor-Ligand interaction module in Discovery Shannon, 2003) R2 were used to explore binding rates between the main ingredients in high frequency CMs and the key targets of SARS-COV-2. The LigandFit molecular docking parameter settings remained the default parameters.

In order to ensure the accuracy of the results, scoring was performed with seven scoring functions: DockScore, LigScore1, LigScore2, PLP1, PLP2, Jain and PMF. Then, consensus score was used to analysis of seven scoring functions selected for Ligandfit docking, which produces a single consensus score value for each ligand rather than for each posed to measure the result. The threshold was set at greater than four.

2.3. Potential action mechanisms of high-frequency CMs pairs

The Huangqin and Gancao CMs-pair were selected to explore their possible molecular mechanism. The chemical compositions were obtained according to "2.2.1 Screening of active components in CMs". We then predicted the potential targets using target prediction approach developed by Fu et al., (2017). Noteworthy, only the targets with reliability score greater than 0.9 of *Homo sapiens* were retained for further analysis. After, we established the Protein-Protein Interaction (PPI) network for the targets by using String Datasets (https://string-db.org/), and the PPI networks were further visualized by using Cytoscape (Version 3.5.0, available at http://www.cytoscape.org/) (Shannon, 2003).

DAVID, a database for annotation, visualization and integrated discovery to identify the functions, was used to perform GO enrichment analysis and KEGG pathway enrichment analyses for the potential targets (Huang et al., 2009). The *P*-value was calculated and further corrected by using the Benjamini-Hochberg method, and *P*-value <0.05 was selected as the cutoff criterion. Subsequently, the compound-target network, and target-pathway network were constructed and visualized by using Cytoscape (Version 3.5.0).

3. Results and discussion

3.1. CMs prescriptions screening results

Chinese medicine prescriptions (fang ji in Chinese) is the main form of CMs application in clinical practice (Ren et al., 2019). *Dictionary of Traditional Chinese Medicine Prescriptions* is the summary of research achievement in CMs prescriptions, which contains more than 1800 kinds of CMs and 90, 000 prescriptions in related literatures (Peng, 1996). In our study, the prescriptions for treatment of pestilence or epidemic diseases were mined from *Dictionary of Traditional Chinese Medicine Prescriptions*. 574 prescriptions were selected for the treatment of "Wenbing", "Wenyi", "Yibing" or "Shiyi". The age distribution of prescriptions showed that the use of CMs to prevent epidemics could be traced back to Jin dynasty (Fig. 5). The selected prescriptions were mainly distributed in the Song, Ming and Qing dynasties, especially the Qing dynasty with 325 kinds of prescriptions. It was estimated that it may be due to the academic development of Seasonal Febrile Disease after the Ming dynasty. Dr. Wu Youke from Ming dynasty proposed the etiological theory of "liqi" for the first time, which mean the evil epidemic pathogenic factors in *Wen Yi Lun* (Wu, 1991). Wu emphasized that Wen diseases (pestilence) was totally different from febrile disease and clearly pointed out that *"The wenyi was a disease, not feng(wind), han(cold), shu(heat) or shi(damp), but a strange feeling between heaven and earth"*. In addition, Wu established the thinking mode of syndrome differentiation and

created the effective prescription named “Dayuanyin” for treating pestilence diseases. While during the formation of Seasonal Febrile Disease school in the Qing Dynasty, febrile pathologists such as Ye Gui, Wu Jiao, Wang Shixiong and Xue Shengbai not only put forward the academic opinions of Wei Qi Ying Xue from *Wen Bing Lun* (Yue et al., 2017), Differentiation of Triple Energizer from *Wen Bing Tiao Bian* (Wu, 1972). Abundant publishes and excellent prescriptions of Wen diseases were also created in that times.

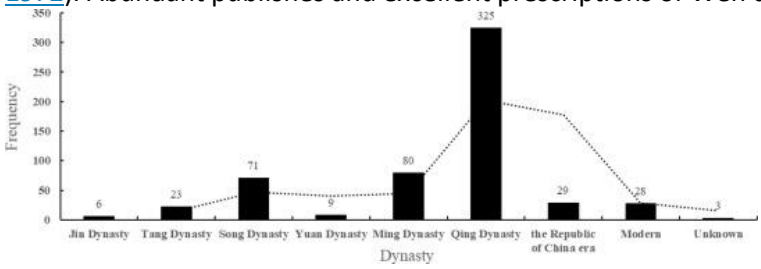

Fig. 5. Distribution times of 574 prescriptions for treatment of pestilence or epidemic diseases.

3.2. High frequency CMs from prescriptions

In order to screen high frequency CMs, we counted the frequency of the herbs used in screened CMs formulae by frequency analysis method. The results showed that 40 kinds of CMs with the highest frequency from above prescriptions (Table 1, Table 2). Glycyrrhizae Radix Et Rhizoma (Gancao; *Glycyrrhiza uralensis* Fisch, Rhizome) was the most frequency used in half of the CM prescriptions, followed by Scutellariae Radix (HuangQin; *Scutellaria baicalensis* Georgi, root) and Rhei Radix Et Rhizome (Dahuang; *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf., or *Rheum officinale* Baill., Rhizome), both of them were used more than 100 times. Most of these high frequency CMs were kinds of cold and heat-clearing CMs, which corresponding to the classic Warm disease's symptoms of syndrome differentiation of COVID-19. Among them, *HuangQin* (Scutellariae Radix) could relive lung heat corresponding to the high fever syndrome type of COVID-19, and *Dahuang* (Rhei Radix Et Rhizome) discharging damp-heat of large intestine, corresponding to diarrhea syndrome of some patients of COVID-19. At the same time, there were also some supplementing Qi and nourishing Yin CMs, such as Paeoniae Radix Alba (Baishao; *Paeonia lactiflora* Pall., root), Angelicae Sinensis Radix (Danggui; *Angelica sinensis* (Oliv.) Diels, root), Rehmanniae Radix (Shengdi; *Rehmannia glutinosa* (Gaertn.) DC., root) and Ginseng Radix Et Rhizoma (Renshen; *Panax ginseng* C.A.Mey., Rhizome), and expectorant CMs, such as Citri Reticulatae Pericarpium (Chenpi; *Citrus reticulata* Blanco, fruit) and Platycodonis Radix (Jiegeng; *Platycodon grandiflorus* (Jacq.) A.DC., root). The above results indicated the principle of Traditional Chinese Medicine with strengthening the body resistance to eliminate pathogenic factors and treating both symptom and root au.

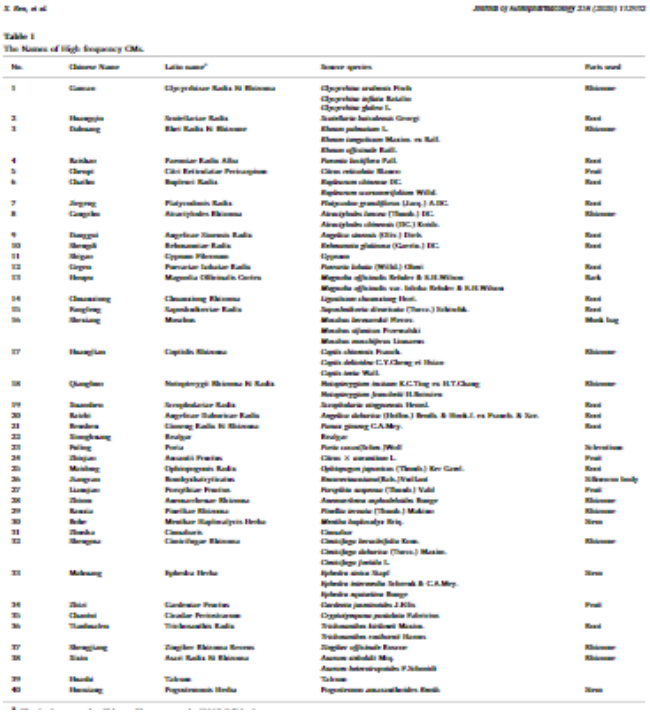

Table 2
High frequency CMs from Prescriptions (top 40).

No.	*Pinyin* Name	Latin name	Frequency	Percentage, %
1	Gancao	Glycyrrhizae Radix Et Rhizoma	296	51.57
2	Huangqin	Scutellariae Radix	123	21.43
3	Dahuang	Rhei Radix Et Rhizome	103	17.94
4	Baishao	Paeoniae Radix Alba	97	16.9
5	Chenpi	Citri Reticulatae Pericarpium	91	15.85
6	Chaihu	Bupleuri Radix	74	12.89
7	Jiegeng	Platycodonis Radix	74	12.89
8	Cangzhu	Atractylodes Rhizoma	69	12.02
9	Danggui	Angelicae Sinensis Radix	69	12.02
10	Shengdi	Rehmanniae Radix	69	12.02
11	Shigao	Gypsum Fibrosum	69	12.02
12	Gegen	Puerariae Lobatae Radix	66	11.5
13	Houpu	Magnolia Officinalis Cortex	65	11.32
14	Chuanxiong	Chuanxiong Rhizoma	63	10.98
15	Fangfeng	Saposhnikoviae Radix	62	10.8
16	Shexiang	Moschus	62	10.8
17	Huanglian	Coptidis Rhizoma	61	10.63
18	Qianghuo	Notopterygii Rhizoma Et Radix	60	10.45
19	Xuanshen	Scrophulariae Radix	58	10.1
20	Baizhi	Angelicae Dahuricae Radix	57	9.93
21	Renshen	Ginseng Radix Et Rhizoma	55	9.58
22	Xionghuang	Realgar	55	9.58
23	Puling	Poria	54	9.41
24	Zhiqiao	Aurantii Fructus	54	9.41
25	Maidong	Ophiopogonis Radix	53	9.23
26	Jiangcan	Bombyxbatryticatus	52	9.06
27	Lianqiao	Forsythiae Fructus	52	9.06
28	Zhimu	Anemarrhenae Rhizoma	52	9.06
29	Banxia	Pinelliae Rhizoma	51	8.89
30	Bohe	Menthae Haplocalycis Herba	51	8.89
31	Zhusha	Cinnabaris	51	8.89
32	Shengma	Cimicifugae Rhizoma	48	8.36
33	Mahuang	Ephedra Herba	46	8.01
34	Zhizi	Gardeniae Fructus	44	7.67
35	Chantui	Cicadae Periostracum	41	7.14
36	Tianhuafen	Trichosanthis Radix	41	7.14
37	Shengjiang	Zingiber Rhizoma Recens	40	6.97
38	Xixin	Asari Radix Et Rhizoma	40	6.97
39	Huashi	Talcum	38	6.62
40	Huoxiang	Pogostemonis Herba	38	6.62

3.3. High frequency CMs-pair and triple-CMs groups in prescriptions

CMs-pair and triple-CMs groups are the basic forms of compatibility of CMs. There were 36 CMs-pair with a frequency of more than 5% from above prescriptions (Table 3). *Gancao* (Glycyrrhizae Radix Et Rhizoma) was most commonly used in high frequency CMs-pair. Most frequently used CMs-pair was *Huangqin* (Scutellariae Radix) and *Gancao* (Glycyrrhizae Radix Et Rhizoma) pair, which was the sovereign drug of Gancao Huangqin Tang in the *Si Sheng Xin Yuan* (Huang, 2019). Huangqin is a frequently used CMs, and have the effects of clearing heat and depriving the evil wetness, heat-clearing and detoxicating, while *Gancao* (Glycyrrhizae Radix Et Rhizoma) have the effects of tonifying the middle body and supplementing Qi. *Huangqin* (Scutellariae Radix) and *Gancao* (Glycyrrhizae Radix Et Rhizoma) CMs-pair could be used for treating heat syndrome caused by SARS-COV-2.

Table 3
High frequency CMs-pair in prescriptions(percentage > 5%).

No.	CMs-pair	Frequency	Percentage, %
1	*Huangqin* (Scutellariae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	89	15.48
2	*Baishao* (Paeoniae Radix Alba) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	80	13.91
3	*Chenpi* (Citri Reticulatae Pericarpium) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	74	12.87
4	*Chaihu* (Bupleuri Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	59	10.26
5	*Gegen* (Puerariae Lobatae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	56	9.74
6	*Qianghuo* (Notopterygii Rhizoma Et Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	56	9.74
7	*Chuanxiong* (Chuanxiong Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	55	9.57
8	*Jiegeng* (Platycodonis Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	55	9.57
9	*Fangfeng* (Saposhnikoviae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	51	8.87
10	*Zhiqiao* (Aurantii Fructus) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	48	8.35
11	*Danggui* (Angelicae Sinensis Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	48	8.35
12	*Houpu* (Magnolia Officinalis Cortex) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	47	8.17
13	*Cangshu* (Atractylodes Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	44	7.65
14	*Baizhi* (Angelicae Dahuricae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	43	7.48
15	*Shigao* (Gypsum Fibrosum) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	42	7.3
16	*Puling* (Poria) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	42	7.3
17	*Huanglian* (Coptidis Rhizoma) & *Huangqin* (Scutellariae Radix)	41	7.13
18	*Renshen* (Ginseng Radix Et Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	40	6.96
19	*Baizhi* (Angelicae Dahuricae Radix) & *Chuanxiong* (Chuanxiong Rhizoma)	39	6.78
20	*Zhimu* (Anemarrhenae Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	38	6.61
21	*Xuanshen* (Scrophulariae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	38	6.61
22	*Bohe* (Menthae Haplocalycis Herba) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	38	6.61
23	*Chuanxiong* (Chuanxiong Rhizoma) & *Qianghuo* (Notopterygii Rhizoma Et Radix)	38	6.61
24	*Xionghuang* (Realgar) & *Shexiang* (Zingiber Rhizoma Recens)	37	6.43
25	*Zhizi* (Gardeniae Fructus) & *Huangqin* (Scutellariae Radix)	35	6.09
26	*Lianqiao*(Forsythiae Fructus) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	35	6.09
27	*Banxia* (Pinelliae Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	35	6.09
28	*Chantui* (Cicadae Periostracum) & *Jiangcan* (Bombyxbatryticatus)	33	5.74
29	*Xionghuang* (Realgar) & *Zhusha* (Cinnabaris)	33	5.74
30	*Zhusha* (Cinnabaris) & *Shexiang* (Moschus)	32	5.57
31	*Shengma* (Cimicifugae Rhizoma) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	32	5.57
32	*Mahuang* (Ephedra Herba) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	31	5.39
33	*Tianhuafen* (Trichosanthis Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	31	5.39
34	*Bohe* (Menthae Haplocalycis Herba) & *Jiegeng* (Platycodonis Radix)	30	5.22
35	*Bingpian* (Borneolum) & *Shexiang* (Moschus)	29	5.04
36	*Huoxiang* (Pogostemonis Herba) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	29	5.04

As for triple-CMs-groups, Chuanxiong Rhizoma (Chuanxiong; *Ligusticum chuanxiong* Hort.), Notopterygii Rhizoma Et Radix (Qianghuo; *Notopterygium incisum* K.C.Ting ex H.T.Chang, *Notopterygium franchetii* H.Boissieu, root) and *Gancao* (Glycyrrhizae Radix Et Rhizoma) group was used most commonly in the prescription, followed by Bupleuri Radix (Chaihu; *Bupleurum chinense* DC., *Bupleurum scorzonerifolium* Willd., root), *Huangqin* (Scutellariae Radix) and *Gancao* (Glycyrrhizae Radix Et Rhizoma) groups (Table 4). The CMs-pair of *Qianghuo* (Notopterygii Rhizoma Et Radix) and *Chuanxiong* (Chuanxiong Rhizoma) had properties of enhancing in compatibility, which was used in Jiuwei Qianghuo Decoction and Da Qianghuo Decoction recorded in *Difficult to Know* (Wang, 1956). *Qianghuo* (Notopterygii Rhizoma Et Radix) belongs to Taiyang meridian which performed guiding role in medicine applying; While Chuanxiong was belonged to Jueyin meridian played the role as guiding medicine. The combination of *Qianghuo* (Notopterygii Rhizoma Et Radix) and *Chuanxiong* (Chuanxiong Rhizoma) showed better pharmacological effects in headache therapy originated from the Taiyang and/or Jueyin, and could be helpful for headache syndrome caused by SARS-COV-2.

Table 4
High frequency Triple-CMs-group in prescriptions(percentage > 5%).

No.	Triple-CMs-group	Frequency	Percentage, %
1	*Chuanxiong* (Chuanxiong Rhizoma) & *Qianghuo* (Notopterygii Rhizoma Et Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	38	6.61
2	*Chaihu* (Bupleuri Radix) & *Huangqin* (Scutellariae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	37	6.43
3	*Baizhi* (Angelicae Dahuricae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma) & *Chuanxiong* (Chuanxiong Rhizoma)	32	5.57
4	*Fangfeng* (Saposhnikoviae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma) & *Qianghuo* (Notopterygii Rhizoma Et Radix)	32	5.57
5	*Chaihu* (Bupleuri Radix) & *Qianghuo* (Notopterygii Rhizoma Et Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	32	5.57
6	*Chuanxiong* (Chuanxiong Rhizoma) & *Fangfeng* (Saposhnikoviae Radix) & *Gancao* (Glycyrrhizae Radix Et Rhizoma)	29	5.04

3.4. Analysis of association rules and network of compatibility in CM prescriptions

Association rule mining is often used to find possible associations or connections between substance, and it has been applied to study the compatibility of CMs prescriptions (Zhang et al., 2020). We mined the association relationship (Supplementary Table S1) and matrix analysis (Fig. 6) for the compatibility relationship of CMs and built the association knowledge network of CMs (Fig. 6). From these results, *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) were key medicines of these prescriptions for treatment of pestilence or epidemic diseases. Most of the CM prescriptions were designed basing on these two CMs.

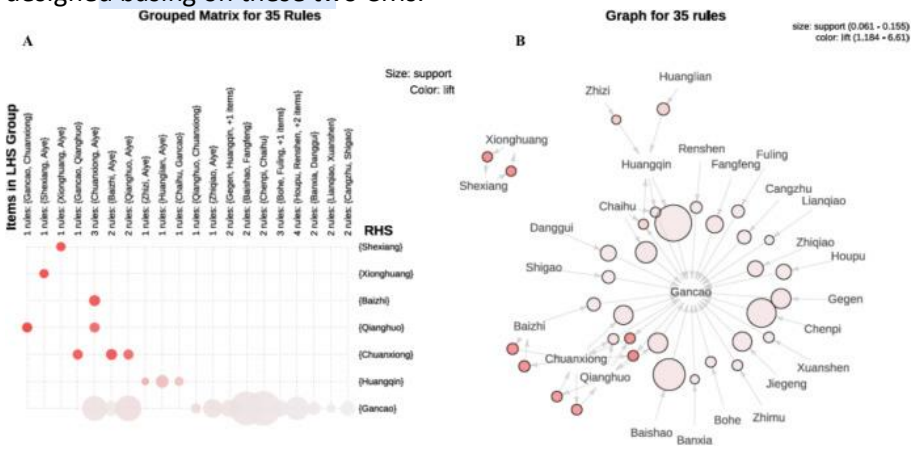

Fig. 6. CMs incidence matrix of prescription (A) and CMs association network of prescription (B).

3.5. LigandFit DOCK results of ingredients from high frequency CMs with SARS-COV-2 targets

The chemical composition of high frequency CMs were obtained from the Traditional Chinese Medicine System Pharmacology Database. There are 40 kinds of high-frequency CMs, five of which are not included in the database. Although a single CM usually contains a large number ingredient, only those with desirable pharmacodynamic and pharmacokinetic properties are key compounds for their therapeutic effects. In our current study, OB and DL were employed to screen candidate compounds from these high frequency CMs. In total, 35 high-frequency CMs includes 431 chemicals, which were molecularly docked with the SARS-COV-2 targets 3CL hydrolase and angiotensin converting enzyme 2 (ACE2) using LigandFit.

Consensus scoring, the combination of multiple scoring functions, is easier to find false positive than a single scoring function. The higher the Consensus scoring, the higher the binding rate of the molecule to the target. The score is greater than 4, indicating a better docking result. In our study, compounds with scoring values greater than 4 were screened for analysis. Therefore, 66 compounds were screened, of which 27 were docked with the 3CL hydrolase target and 48 were docked with the ACE2 target. The screened compounds were distributed in 27 kinds of CMs, among which *Gancao* (Glycyrrhizae Radix Et Rhizoma), *HuangQin* (Scutellariae Radix), *Dahuang* (Rhei Radix Et Rhizome) and *Chaihu* (Bupleuri Radix) contain more potential compounds (Fig. 7, Supplementary Table S2). The results of molecular docking were consistent with the frequency results of high frequency CMs.

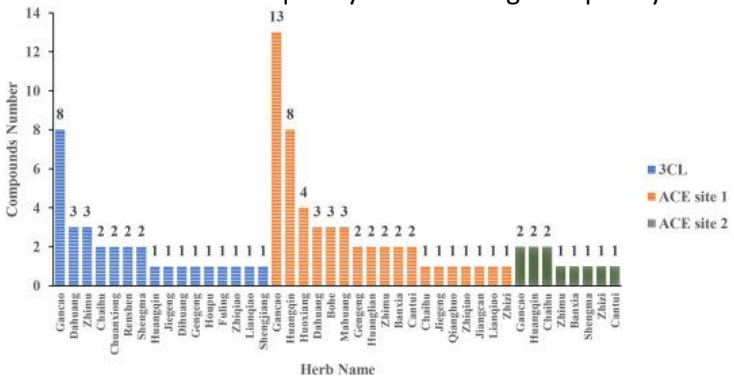

Fig. 7. High Frequency CMs with active components distribution.

3C-like protease ($3CL^{Pro}$) play an important role in the replication of the virus, which is considered to be an attractive target for drug development (Li et al., 2020). Acetoside (Consensus scoring = 7) has the strongest binding activity to 3CL hydrolase, which comes from *Shengdi* (Rehmanniae Radix). In Acetoside, hydrogen bonds were formed between phenolic hydroxyl groups and residues THR and PHE, and hydrophobic interaction was formed between benzene ring and target protein GLU (Fig. 8A). In addition, various components in high frequency CMs, such as *Gancao* (Glycyrrhizae Radix Et Rhizoma), *Dahuang* (Rhei Radix Et Rhizome) and *Chaihu* (Bupleuri Radix), also have potential anti-activity on 3CL protein (Supplementary Table S2).

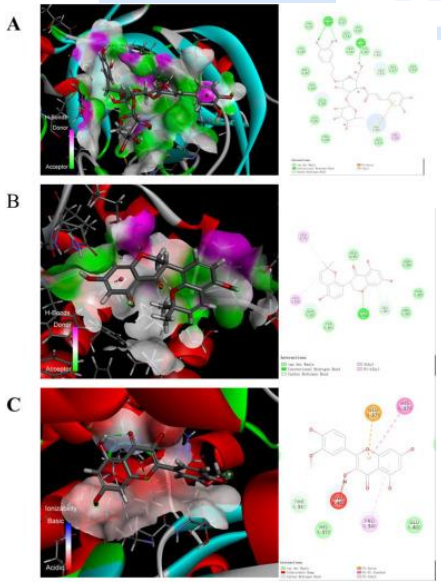

Fig. 8. Molecular docking patterns of candidate compounds with SARS-COV-2 targets. A: Acetoside with 3CL; B: Glyasperin F with ACE2 stie 1; C: Isorhamnetin with ACE2 site 2.

According to the two binding regions grid3 and grid4 between ACE2 and viral protein conformation (Niu et al., 2020), components which may block the binding of the two proteins were screened. Glyasperin F in *Gancao* (Glycyrrhizae Radix Et Rhizoma) had the strongest binding to site ACE 1(Consensus scoring = 6), and hydrogen bond and σ-p hyperconjugated system was formed between its phenolic hydroxyl group forms and target protein residue LEU (Fig. 8B). Isorhamnetin in *Gancao* (Glycyrrhizae Radix Et Rhizoma)

and *Chaihu* (Bupleuri Radix) has the strongest binding ability to ACE site 2 (Consensus scoring = 6), which mainly formed hydrophobic interaction between benzene ring and residue target protein residue PRO (Fig. 8C). Besides, various ingredient in CMs, such as *HuangQin* (Scutellariae Radix), *Chaihu* (Bupleuri Radix) and *Zhimu*(Anemarrhenae Rhizoma), could combined with ACE2 protein.

3.6. Systemic pharmacological analysis of high-frequency CMs pairs

According to the frequency analysis, association rule analysis and molecular docking results of high-frequency CMs, *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) were the key medicines pairs of these prescriptions for treatment of COVID-19. A systemic pharmacology model based on chemical, pharmacokinetic and pharmacological data was constructed to explore the molecular mechanisms. In the present work, the number of 85 and 34 kinds of active compounds were selected from *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix), respectively. The detailed information about those molecules were provided in Supplementary Table S3. An integrated *in silico* approach was introduced to identify the target proteins for the active compounds of CMs (Fu et al., 2017). We totally obtained 286 potential therapeutic targets for 119 kinds of candidate compounds from *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix). Then, we constructed a PPI (335 nodes and 4237edges) network for the putative targets of the compounds. Based on the average values for degree and distance of 21 and 2.3, respectively, we have identified 30 significant targets from *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) (Fig. 9A, Supplementary Table S4). In order to directly represent the interpretation of the complex relationships between active compounds and their targets, C-T network was constructed (Fig. 9B). Amongst them, those ones with high interconnection degrees were responsible for the high interconnectedness of the C-T network, especially Quercetin (degree = 10), 5,7,2′,6′-Tetrahydroxyflavone (degree = 12), Kaempferol (degree = 12), 4′-Hydroxywogonin (degree = 11), Ganhuangenin (degree = 11), Baicalein (degree = 9), Gancaonin O (degree = 8), and Norwogonin (degree = 8). As shown in the C-T network (Fig. 9B), a compound regulated multiple targets, while multiple compounds possibly regulate the same target.

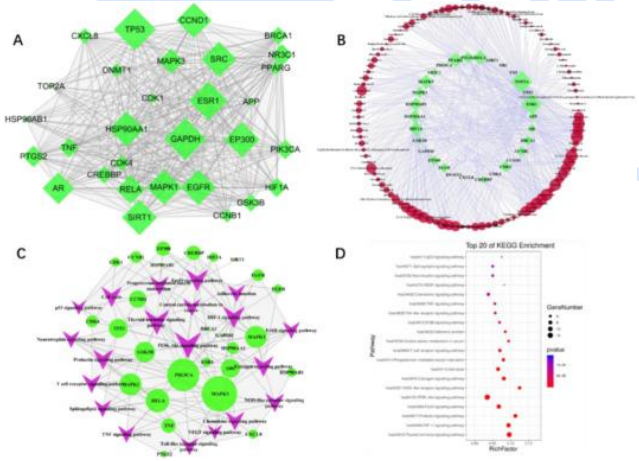

Fig. 9. Potential action mechanisms of *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) CMs-pair. A: PPI network of candidate Gancao-Huangqin CMs-pair targets; B: Construction of the Gancao-Huangqin CMs-pair compound-target network. The nodes representing candidate compounds are shown as red, and the targets are indicated as green; C:Construction of the Gancao-Huangqin CMs-pair target-pathway network; D: Pathway enrichment analysis of candidate targets. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The significant targets interacting with the active ingredients were mapped onto the KEGG pathways and the T-P network was generated as shown in Fig. 9C. Among the results of KEGG pathway enrichment, we selected the pathways in the basic biological processes of metabolism, genetic information processing, environmental information processing, cellular processes and organismal system. There are 20 target-enriched pathways (Table 5), which act on the immune system, inflammation, cellular processes, and endocrine system, respectively. Thus, we postulated that *Gancao* (Glycyrrhizae Radix Et Rhizoma)

and *HuangQin* (Scutellariae Radix) CM pair exerts therapeutic effects on multiple targets and pathways of the human body through hits complex active component.

Table 5
KEGG pathways regulated by *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) CMs-pair target.

Pathway classification	Pathway	Count	*P*-Value
Immune stystem	NOD-like receptor signaling pathway	7	7.26E-08
	T cell receptor signaling pathway	7	2.36E-06
	Toll-like receptor signaling pathway	6	5.86E-05
Inflammation	PI3K-Akt signaling pathway	12	4.29E-08
	TNF signaling pathway	6	6.13E-05
	Chemokine signaling pathway	7	8.27E-05
Angiogenesis	VEGF signaling pathway	5	9.77E-05
Nervous	Neurotrophin signaling pathway	6	0.000106
	ErbB signaling pathway	6	2.25E-05
Cellular processes	Adherens junction	6	8.3E-06
	Cell cycle	8	4.51E-07
	Sphingolipid signaling pathway	6	0.000106
	p53 signaling pathway	5	0.000141
	HIF-1 signaling pathway	9	2.65E-09
	FoxO signaling pathway	9	3.77E-08
	Central carbon metabolism in cancer	6	4.96E-06
Endocrine system	Thyroid hormone signaling pathway	11	1.17E-11
	Estrogen signaling pathway	8	9.55E-08
	Progesterone-mediated oocyte maturation	7	1.04E-06
	Prolactin signaling pathway	8	9.2E-09

Under the guidance of the theory of “treating non-disease” in TCM, CMs and CM prescriptions play a multi-effect synergistic effect, which makes them have more significant advantages in anti-COVID-19 (Luo et al., 2020). The classical CM prescriptions condense the experience in fighting against epidemics diseases for thousands of years. Its successful effects have been preliminarily confirmed in clinical studies when applied to SARS and H1N1 influenza epidemics (Lau et al., 2005,Liu et al., 2013). Meanwhile, Qing-Fei-Pai-Du decoction is a CM prescription recommended by the National Health Commission of China for the treatment of COVID-19, which was optimized by combination of a number of classical prescriptions from *Treatise on Febrile and Miscellaneous* (Han dynasty) (Zhong, 1963). Thus, the experiences and effective prescriptions of CM in treating and preventing viral infection diseases for thousands of years is worthwhile to explore and are of great significance and reasonable.

We excavated 574 CM prescriptions for treating epidemic diseases through 96, 606 prescription. Among them, 40 kinds of high frequency CMs, 36 high-frequency CMs-pairs and 6 kinds of high-frequency triple-CM-groups were mined by frequency analysis method were used to analyze the collected prescriptions. Among the commonly used CMs beneficial for antiviral, *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) were given high priority CM pairs for selection used in CM prescriptions against pestilence by mining the compatibility rules of prescriptions with association rules analyses. The molecular docking results implied that 66 compounds in 26 kinds of CMs probably show a potential anti-SARS-COV-2 activity by binding with the ACE2 and 3CL hydrolase. It's worth noting that *Gancao* (Glycyrrhizae Radix Et Rhizoma) contained the most potential compounds of 20, followed by *HuangQin* (Scutellariae Radix). Meanwhile, based on network-based computational methods, an integrated system pharmacology approach was used to predict targets, construct networks, and explore the molecular action of high-frequency *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) (GH) CMs-pair. In present study, 85 and 34 kinds of active ingredients with favorable bioactivities and contents were selected from *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) by ADME filtering, providing some foundational clues for thorough investigation on this CMs-pair. By analyzing the network topology of targets, 30 kinds of important targets were identified. By using network systematic analysis, GH CMs-pair could regulate the proteins related to immune system, inflammation, cellular processes, and endocrine system. COVID-19 leads to a strong immune response and inflammatory storm, in which a large number of cytokines are activated. GH CMs-pair may regulate the immune-related pathway Toll-like receptor signaling pathway, T-cell and B-cell receptor signaling pathway, as well as cytokine action related pathways such as TNF signaling pathway, NF-κB signaling pathway and PI3K-Akt signaling pathway signaling pathway to inhibit the activated cytokines, relieve the excessive immune response and eliminate inflammation. From

perspective of molecular network, GH CMs-pair exerted overall regulation through multi-ingredient and multi-target synergistic effect.

4. Conclusion

In conclusion, based on experience of ancient prescription and modern pharmacy research methods, 40 kinds of high frequency CMs, 36 high-frequency CMs-pair and 6 kinds of high-frequency triple-CMs-group were excavated. In addition, the molecular mechanism of the selected key CMs drug pair was preliminarily discussed. *Gancao* (Glycyrrhizae Radix Et Rhizoma) and *HuangQin* (Scutellariae Radix) CMs-pair with highest frequency show a potential anti-SARS-COV-2 activity by binding with the ACE2 and 3CL hydrolase and regulate the target related to immune system, inflammation, cellular processes, and endocrine system. Our results provide referenced candidate compatibility of CM and active ingredients against SARS-COV-2. The results fully reflected the synergistic mechanism of multi-components and multi-targets of CMs. In view of the limitations of virtual screening results, further experiments *in vivo* and *in vitro* are needed to verify the results of this study in the later stage, so as to provide experimental basis for the research and development of antiviral natural drugs.

Notes

The authors declare no competing financial interest.

Author contributions

Xian-Jun Fu, Zhen-Guo Wang, Xin-Hua Jia, Tao Song, Wu-Yi Zhou and Yan-Gang Zhao conceived and designed the experiments; Xia Ren, Xin-Xin Shao, Xiu-Xue Li, Yang Li, Xiao-Long Wang, Zhen-Yang Li,Yue Zhong performed the experiments; Xia Ren wrote the original draft; Peng Wang, Qing-Hua Cui; Pei-Ju Qin, Xue-Bo Li, Feng-Cong Zhang edited and reviewed the paper. All authors have read, revised and approved the final manuscript.

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Abstract

Background

At present, coronavirus disease 2019 (COVID-19), caused by infection with severe acute respiratory syndrome coronavirus 2, is spreading all over the world, with disastrous consequences for people of all countries. The traditional Chinese medicine prescription Dayuanyin (DYY), a classic prescription for the treatment of plague, has shown significant effects in the treatment of COVID-19. However, its specific mechanism of action has not yet been clarified. This study aims to explore the mechanism of action of DYY in the treatment of COVID-19 with the hope of providing a theoretical basis for its clinical application.

Methods

First, the TCMSP database was searched to screen the active ingredients and corresponding target genes of the DYY prescription and to further identify the core compounds in the active ingredient. Simultaneously, the Genecards database was searched to identify targets related to COVID-19. Then, the STRING database was applied to analyse protein–protein interaction, and Cytoscape software was used to draw a network diagram. The R language and DAVID database were used to analyse GO biological processes and KEGG pathway enrichment. Second, AutoDock Vina and other software were used for molecular docking of core targets and core compounds. Finally, before and after application of DYY, the core target gene IL6 of COVID-19 patients was detected by ELISA to validate the clinical effects.

Results

First, 174 compounds, 7053 target genes of DYY and 251 genes related to COVID-19 were selected, among which there were 45 target genes of DYY associated with treatment of COVID-19. This study demonstrated that the use of DYY in the treatment of COVID-19 involved a variety of biological processes, and DYY acted on key targets such as IL6, ILIB, and CCL2 through signaling pathways such as the IL-17 signaling pathway, AGE-RAGE signaling pathway in diabetic complications, and cytokine–cytokine receptor interaction. DYY might play a vital role in treating COVID-19 by suppressing the inflammatory storm and regulating immune function. Second, the molecular docking results showed that there was a certain affinity between the core compounds (kaempferol, quercetin, 7-Methoxy-2-methyl isoflavone, naringenin, formononetin) and core target genes (IL6, IL1B, CCL2). Finally, clinical studies showed that the level of IL6 was elevated in COVID-19 patients, and DYY can reduce its levels.

Conclusions

DYY may treat COVID-19 through multiple targets, multiple channels, and multiple pathways and is worthy of clinical application and promotion.

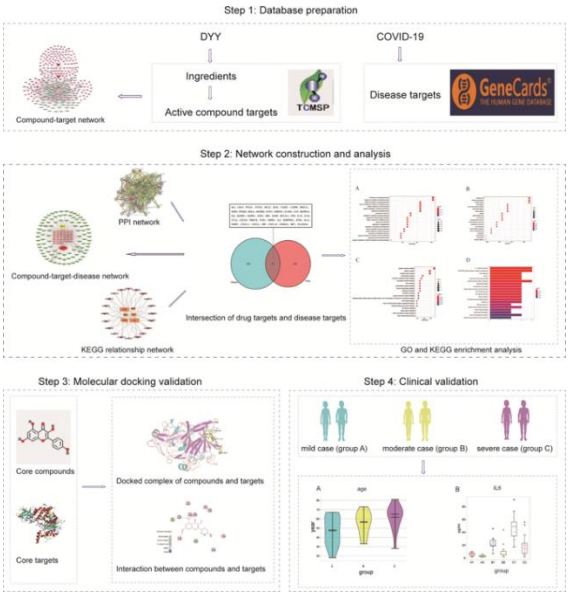
Background

Coronavirus disease 2019 (COVID-19) was first discovered in Wuhan, China, on December 12, 2019, but to date, no definitive conclusion has been drawn about its origin. According to the classification of syndromes in traditional Chinese medicine, COVID-19 is classified as an “epidemic disease” (damp-warm disease), and it is a highly contagious disease. In the early stages of damp-warm diseases, “damp-warm disease with syndrome of pathogen blocking pleuro-diaphragmatic interspace” is very common and is a specific stage and phenomenon in the pathological process of the disease. This symptom first appeared in the *Theory of Epidemic Febrile Disease* by Wu Youke during the Ming Dynasty, and he created Dayuanyin (DYY), described in the book. After 2019-nCoV invades the human body, it disturbs and damages the human immune system, further causing different degrees of damage to various organs throughout the body [1, 2]. DYY, a traditional Chinese medicine prescription, has played an important role in the prevention and treatment of epidemic diseases in documented history and literature. It has been used to treat influenza [3], atypical pneumonia [4], AIDS [5] and other diseases and has proven to be very effective in clinical applications. At the same time, through clinical observation of COVID-19 patients in the early stage of DYY treatment, it was found that this prescription can improve the clinical symptoms and signs of patients, improve the prognosis of patients, and shorten the course of disease [6, 7], making it worthy of clinical application and promotion. However, its mechanism of action in COVID-19 patients has not yet been clarified.

Network pharmacology, originally proposed by Andrew L Hopkins, includes systems biology, pharmacology, mathematics, computer network analysis, etc. As a useful tool for systematically evaluating and demonstrating the rationality of drugs, it has now been widely accepted [8, 9]. The application of network pharmacology in traditional Chinese medicine provides us with new possibilities for screening active ingredients of drugs and targets for disease treatment, which is helpful for explaining the mechanism of action of drugs for disease treatment at a system level [10]. Molecular docking is a theoretical simulation method that mainly studies intermolecular interactions and predicts their binding mode and affinity [11]. Not only can it be used for drug development, but it can also provide keen insights into protein function prediction and other important issues [12].

This study aimed to use network pharmacology and molecular docking to preliminarily explore the mechanism of action of this prescription in the treatment of COVID-19 patients, with the goal of widely using this prescription for COVID-19 patients with early damp-warm syndromes to improve the patients’ condition and to prevent the ongoing COVID-19 outbreak. A technological road-map of the experimental procedures of our study is shown in Fig. 1.

Fig. 1



Technological road-map

Methods

Acquisition of the chemical composition and target information of DYY

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) records 499 common traditional Chinese medicines (Chinese Pharmacopoeia 2010 edition) and elaborates their ingredients, the corresponding target information and common disease information related to traditional Chinese medicines [13]. The database provides pharmacokinetic information for each compound, such as drug-like (DL), oral bioavailability (OB), and blood-brain barrier (BBB). In this study, the TCMSP database (http://tcmspw.com/tcmsp.php) was used to search for and determine the active ingredients in the composition of the DYY decoction. At the same time, target genes were predicted for these active ingredients. OB and DL property are important reference standards for evaluating whether compounds can be used as drugs. In this study, OB ≥ 30% and DL ≥ 0.18 were used as screening thresholds [14]. According to the selected active ingredients of DYY, the target genes corresponding to the above active ingredients derived from DrugBank were further screened using Perl language in combination with the TCMSP database.

Gene name standardization

Perl language was used in combination with the UniProtKB search function in the UniProt database (http://www.uniprot.org/, update in 2018-04-10), the protein name was entered, the species was limited to humans, and the retrieved protein name was corrected to the official name of the protein.

Construction of network diagrams of compounds and corresponding targets

The compounds and predicted targets in the DYY formula obtained through the TCMSP database were imported into Cytoscape 3.6.1 software, and a compound-target network diagram was drawn to obtain the top five core compounds.

Acquisition of disease targets

The keyword “novel coronavirus pneumonia” was entered into the Genecards (https://www.genecards.org/, version 4.12) database to obtain target genes related to the COVID-19 disease.

Intersection of disease genes and drug genes

The target genes predicted from the active ingredients in DYY were intersected and mapped with the target genes predicted for the COVID-19 disease to obtain the target genes of DYY for the treatment of COVID-19. The Venn Diagram package in R was used to draw a Venn diagram.

Protein–protein interaction analysis and core target screening

The target genes for DYY treatment of COVID-19 were entered into the STRING database for protein–protein interaction (PPI) analysis, "Homo sapiens" was selected, the minimum required interaction score was set to > 0.9, the protein interaction network map was downloaded, and R3.5.0 was used to screen core genes.

Construction of network visualization

The active ingredients of DYY, the targets corresponding to the active ingredients, and the targets predicted for the COVID-19 disease were imported into Cytoscape 3.6.1 software, and a drug-target-disease network diagram was constructed for network visualization.

GO analysis and KEGG pathway enrichment analysis

The DAVID database (http://david.abcc.ncifcrf.gov/) can functionally annotate many genes and help us understand the biological process and meaning behind genes. The target genes selected above were combined with R language and DAVID database for Gene Ontology (GO) biological process enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment.

Construction of KEGG relationship network

The pathway ID numbers and the genes involved in the KEGG-enriched pathways were imported into Cytoscape software, the number of adjacent nodes in the network was calculated, and the size of the nodes in the network was determined according to the number of adjacent nodes to construct a KEGG relationship network.

Molecular docking verification of core compounds and core target genes

Firstly, the top five core compounds were selected, and the two-dimensional structure diagrams of the compounds were downloaded from the PubChem database, imported into Chem3D software to draw the three-dimensional structure diagrams of the core compounds and optimize the energy, and saved in mol2 format. Then, the files were imported into AutoDockTools-1.5.6 software to add charge and display rotatable keys and then saved in pdbqt format. Secondly, the protein crystal structures corresponding to the core target genes were downloaded from the PDB database, imported into Pymol software to remove water molecules and heteromolecules, imported into AutoDockTools-1.5.6 software to add hydrogen atoms and charge operations, saved to pdbqt format, and imported into Discovery Studio 3.5 Client software to search for active pockets. Finally, the above core compounds were used as ligands, and the proteins corresponding to the core target genes were used as receptors for molecular docking. The results were analysed and interpreted using PyMOL software and Discovery Studio 3.5 Client.

Clinical validation of the core target IL6

In this study, a total of 45 patients who were hospitalized in Third People's Hospital of Hubei Province and Lei Shen Shan Hospital during the period from January 25, 2019 to March 8, 2020 were selected. The TCM syndrome of the selected patients was "plague (syndrome of pathogen hidden in interpleuro-diaphramatic space)", and DYY was used for treatment. ELISA was used to detect changes in IL6 levels at the time of before and after treatment for 1 week. All statistical analyses were performed by GraphPad Prism version 7.00 software. T test was used for comparisons before and after treatment in each group. Our data are expressed as the mean ± standard deviation (SD). A value of $p < 0.05$ was considered significant.

Mechanism of action of DYY in the treatment of COVID-19

Adobe Illustrator CC software was used to draw the chart for the specific mechanism of DYY treatment of COVID-19.

Results

Acquisition of the active ingredient and target information of DYY

The composition of DYY is magnolia officinalis(MO), amomum(AM), arecae semen(AS), herbaceous peony(HP), scutellariae radix(SR), anemarrhenae rhizoma(AR) and licorice(LR), as shown in Additional file 1: Fig. S1. A total of 839 DYY active ingredients were obtained from TCMSP database. Among them, the number of active ingredients from magnolia officinalis, amomum, arecae semen, herbaceous peony, scutellariae radix, anemarrhenae rhizoma and licorice was 52, 139, 59, 85, 143, 81, 280, respectively. After screening by the ADME standard (OB ≥ 30%, DL ≥ 0.18), 174 compounds were obtained, among which the number of compound from magnolia officinalis, amomum, arecae semen, herbaceous peony, scutellariae radix, anemarrhenae rhizoma and licorice was 8, 2, 8, 13, 3, 15, 92, respectively. Among them, MOL000073 (ent-Epicatechin) was a common compound of amomum, scutellariae radix, and licorice; MOL004961 (quercetin) was a common compound of licorice and arecae semen; MOL000211 (mairin) was a common compound of herbaceous peony and licorice; MOL000358 (beta-sitosterol) was a common compound of scutellariae radix and herbaceous peony; MOL000359 (sitosterol) was a common compound of licorice, scutellariae radix and herbaceous peony; and MOL000449 (stigmasterol) was a common compound of anemarrhenae rhizoma and scutellariae radix.

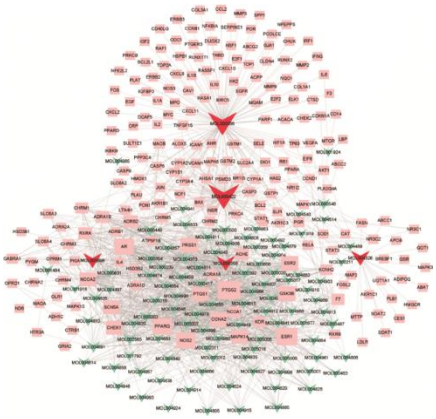
According to the results obtained by screening the active ingredients against the TCMSP database, there were a total of 7053 targets in the DrugBank. Among them, the number of targets of magnolia officinalis, amomum, arecae semen, herbaceous peony, scutellariae radix, anemarrhenae rhizoma and licorice was 379,1162,406, 990, 1203, 407 and 2506, respectively. After screening by the ADME standard (OB ≥ 30%, DL ≥ 0.18), 2766 targets related to the bioactive components were obtained, among which the number of targets from magnolia officinalis, amomum, arecae semen, herbaceous peony, scutellariae radix, anemarrhenae rhizoma and licorice was 32, 179, 41, 122, 436, 188, 1768, respectively. The distribution of candidate compounds and targets in each herb is shown in Table 1.

Table 1 Active ingredients of compounds

Construction of network diagrams of compounds and corresponding targets

The compounds and corresponding targets in the DYY formula were imported into Cytoscape software to draw a compound-target network diagram (see Fig. 2). In this study, degree was selected as a measure of node importance. With the help of the Network Analyzer plug-in in Cytoscape software, the topology parameters of the network were calculated and analysed from the perspective of network node importance. Degree refers to the number of edges associated with a node. The greater the degree of a node is, the larger the node area in the graph. That is, the larger the node area is, the greater the importance of the node in the network. The compounds in Fig. 2 and their corresponding targets were used as network nodes.
Figure 2 shows that one compound can act on multiple target genes, and multiple compounds can also act on one target gene at the same time. Among the compounds, MOL000422 (kaempferol), MOL000098 (quercetin), MOL003896 (7-Methoxy-2-methyl isoflavone), MOL004328 (naringenin), MOL000392 (formononetin) and MOL000358 (beta-sitosterol) occupied the largest area on the graph among all compounds and were important core compounds.

Fig. 2



Compounds and corresponding targets network diagram. The green arrows in the figure represent the MOL numbers of the compound, and the red arrows represent the top five compounds with the largest area. The pink rectangles represent the target genes predicted by the compound. Lines represent the relationship between nodes. The larger the graph area is, the more connections there are to the node, and the more important the node is

Acquisition of disease target

A total of 251 genes related to COVID-19 were obtained by searching the Genecards database. The relevance score was used as the selection criterion to obtain the top 30 genes (see Table 2).

No	Gene symbol	Description	Relevance score
1	TNF	Tumor necrosis factor	33.08
2	IL6	Interleukin 6	31.28
3	CXCL8	C–X–C motif chemokine ligand 8	31.05
4	CD40LG	CD40 ligand	30.56
5	IL10	Interleukin 10	30.33
6	IFNG	Interferon gamma	27.48
7	CRP	C-Reactive protein	25.76
8	STAT1	Signal transducer and activator of transcription 1	22.73
9	MBL2	Mannose binding Lectin 2	22.1
10	TP53	Tumor protein P53	19
11	CCL2	C–C motif chemokine Ligand 2	18.13
12	IL2	Interleukin 2	17.68
13	CCL5	C–C motif chemokine Ligand 5	16.71
14	IFNA1	Interferon alpha 1	16.65
15	EGFR	Epidermal growth factor receptor	16.29
16	CXCL10	C–X–C motif chemokine ligand 10	15.3
17	TGFB1	Transforming growth factor beta 1	14.98
18	IL1B	Interleukin 1 beta	13.78
19	ACE2	Angiotensin I converting enzyme 2	12.32
20	CSF2	Colony stimulating factor 2	11.95
21	PPARG	Peroxisome proliferator Activated Receptor Gamma	11.93
22	CCR5	C–C motif chemokine Receptor 5 (Gene/Pseudogene)	11.37
23	CXCL9	C–X–C motif Chemokine Ligand 9	11.3
24	GPT	Glutamic–pyruvic Transaminase	11.12
25	MAPK1	Mitogen-activated Protein Kinase 1	11.09
26	CASP3	Caspase 3	10.88
27	IFNB1	Interferon beta 1	10.77
28	ALB	Albumin	10.68
29	FGF2	Fibroblast growth factor 2	10.53
30	SFTPD	Surfactant protein D	10.47

Intersection of drug targets and disease targets

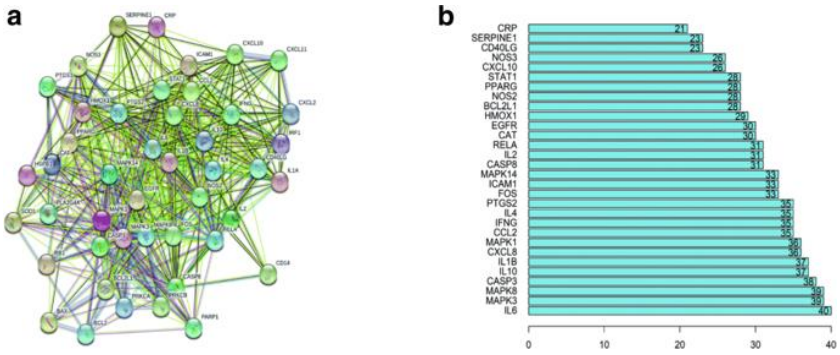
The above DYY drug target genes were intersected with COVID-19 disease targets to obtain possible genes associated with DYY treatment of COVID-19. The results showed that there was a total of 45 genes associated with DYY treatment of COVID-19 (see Additional file 2: Fig. S2).

PPI analysis and core target screening

The STRING database was used to draw a PPI network diagram of DYY for COVID-19 (see Fig. 3a). As shown in Fig. 3a, the network diagram consisted of 45 nodes and 581 edges, for which the average node degree was 25.8, and the PPI enrichment p-value was < 1.0e−16. The above PPI network was processed using R language, and the top 30 core genes were selected (see Fig. 3b). Figure 3b shows that the top 30 core genes had a node degree greater than 21, and the top genes, such as IL6, MAPK3, MAPK8, CASP3, IL10, IL1B,

CXCL8, MAPK1, CCL2, IFNG and IL4, had a higher number of connections than other genes, all showing 35 or more connections.

Fig. 3

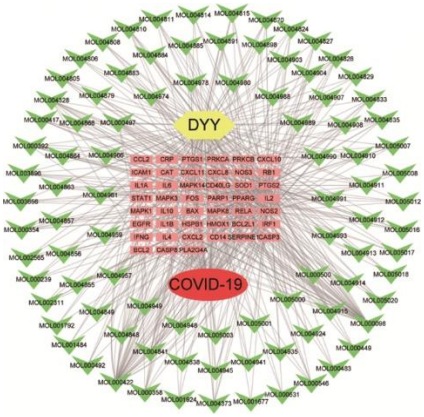


Protein interaction diagram and core gene bar chart. In the protein interaction diagram (**a**), the nodes represent proteins, and the connections represent interactions between proteins. The more connections there are, the greater the degree of connection. The node degree value indicates the number of connections between any node in the diagram and other nodes. In the core gene bar chart (**b**), the abscissa represents the number of genes, and the ordinate represents the name of the gene

Construction of network visualization

The active ingredients of DYY, the targets corresponding to the active ingredients, and the targets predicted for COVID-19 were imported into Cytoscape software to build a drug-target-disease network diagram (see Fig. 4). The network had a total of 139 nodes (including 94 compound nodes and 45 gene nodes) and 546 connections.

Fig. 4

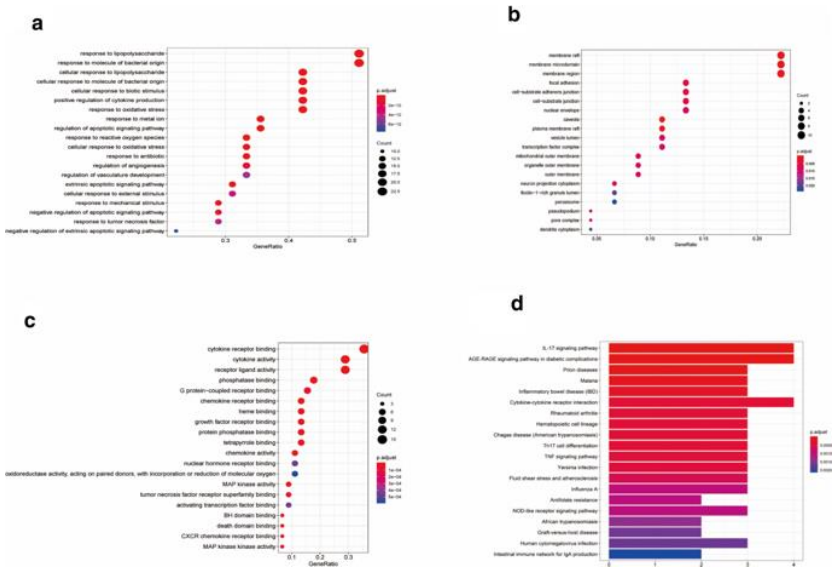


Drug-target-disease regulation network diagram. The yellow polygon in the figure represents the drug (DYY), the red circle represents the disease (COVID-19), the green arrows represent the compounds, and the pink boxes represent the targets of action

GO analysis and KEGG pathway enrichment analysis

The R language and DAVID database were used for GO enrichment analysis by using the above-mentioned targets of DYY to treat COVID-19, and the number of biological process (BP), cellular component (CC), and molecular function (MF) entries was 1,506, 33 and 83, respectively. The top 30 biological processes were screened and are represented as graphical bubbles (see Fig. 5a–c). The KEGG pathway enrichment analysis identified 40 signaling pathways, and the top 20 entries were selected and are represented by a bar graph (see Fig. 5d).

Fig. 5

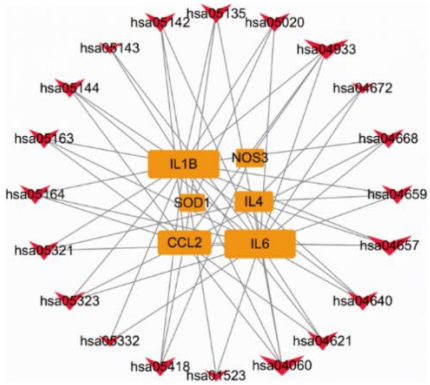


GO analysis and KEGG pathway enrichment analysis graphs. In the bubble charts in (**a–c**), the ordinate represents the names of the BP, CC, and MF terms, respectively, and the abscissa represents the degree of enrichment. In (**d**), the ordinate represents the names of the pathways, and the abscissa represents the number of genes enriched in the pathway. The P value indicates the significance of enrichment. The smaller the P value is, the higher the significance of enrichment, and the redder the colour on the graph

Construction of the KEGG relationship network

The top 20 pathways involved in DYY treatment of COVID-19 and the genes enriched in these pathways were imported into Cytoscape software to build a KEGG relationship network diagram (see Fig. 6). In Fig. 6, the pathways and the genes enriched in the pathways were used as network nodes, and we selected the top three pathways (hsa04657 (IL-17 signaling pathway), hsa04933 (AGE-RAGE signaling pathway in diabetic complications), and hsa0406 (cytokine–cytokine recev] ptor interaction pathway)) and top three target genes (IL6, ILIB, CCL2) enriched in these pathways according to degree.

Fig. 6



KEGG relationship network diagram. The red arrows indicate the pathway IDs. The larger the arrow is, the greater the number of genes connected to the pathway. The orange box indicates the name of the gene. The larger the box is, the greater the number of pathways connected

Molecular docking verification of core compounds and core target genes

The results obtained by the molecular docking software are shown in Table 3. The letters x, y and z were used to represent the size and position of the pocket. The final selected pocket is shown in bold in the column titled ‘Pocket size’. The results of the docking of the receptor and ligand are shown under ‘Docked complex’, and the residues docked with the small-molecule ligand are shown as yellow sticks. The structure

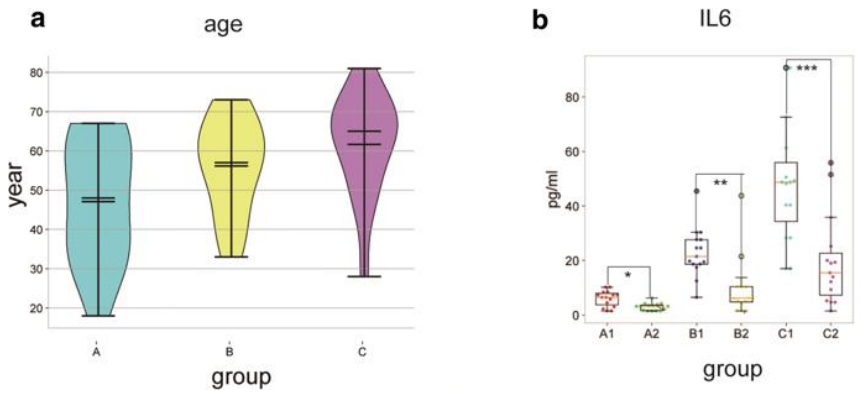
with the initial ligand and the predicted protein pocket were processed by Discovery Studio 3.5 Client software, and the docked complex was processed by PyMOL software. As seen from Table 3, the scores for the five core compounds (kaempferol, quercetin, 7-Methoxy-2-methyl isoflavone, naringenin, formononetin) and protein crystal structures corresponding to the core target genes (IL6, IL1B, CCL2) were all greater than -5 kcal/mol, indicating that the compound had a certain affinity for the protein crystal structure. The interactions between some ligands (small-molecule compounds) and receptors (proteins) are shown in Additional file 3: Fig. S3. Additional file 3: Fig. S3 shows that the small-molecule compounds were tightly bound to the protein residues via various interactions.

Compound	Compound 2D structure	Target and PDB ID	Structure with initial ligand	Prediction of protein pocket	Pocket size	Docked complex	Affinity(kcal/mol)
kaempferol		IL6 (6s22)			x=-27.809076 y=2.104481 z=-19.675366		-8.9
quercetin		IL6 (6s22)			x=-27.809076 y=2.104481 z=-19.675366		-9.3
7-Methoxy -2-methyl isoflavone		IL6 (6s22)			x=-27.809076 y=2.104481 z=-19.675366		-7.6
naringenin		IL6 (6s22)			x=-27.809076 y=2.104481 z=-19.675366		-8.6
formononetin		IL6 (6s22)			x=-27.809076 y=2.104481 z=-19.675366		-8.0
kaempferol		IL1B (5cel)			x=-24.396000 y=61.232000 z=-81.474000		-8.0
quercetin		IL1B (5cel)			x=-24.396000 y=61.232000 z=-81.474000		-7.8
7-Methoxy -2-methyl isoflavone		IL1B (5cel)			x=-24.396000 y=61.232000 z=-81.474000		-6.9
naringenin		IL1B (5cel)			x=-24.396000 y=61.232000 z=-81.474000		-8.0
formononetin		IL1B (5cel)			x=-24.396000 y=61.232000 z=-81.474000		-7.0
kaempferol		CCL2 (6ctw)			x=-10.090407 y=10.937556 z=5.736963		-7.2
quercetin		CCL2 (6ctw)			x=-10.090407 y=10.937556 z=5.736963		-7.5
7-Methoxy -2-methyl isoflavone		CCL2 (6ctw)			x=-10.090407 y=10.937556 z=5.736963		-7.1
naringenin		CCL2 (6ctw)			x=-10.090407 y=10.937556 z=5.736963		-7.2
formononetin		CCL2 (6ctw)			x=-10.090407 y=10.937556 z=5.736963		-6.9

Clinical validation of the core target IL6

Of the 45 patients selected, 15 were mild cases (group A), 15 were moderate cases (group B), and 15 were severe cases (group C). The age distribution of each group of patients and changes in IL6 levels before and after treatment are shown in Fig. 7a, b, respectively. Compared with the severe cases, Fig. 7a shows that the mild and moderate cases were younger. Figure 7b shows that a majority of the patients had different levels of IL6 elevation before treatment (the normal reference value of IL6 is 0–7 pg/ml), and the increase in IL6 was most pronounced in severe cases. After treatment, IL6 decreased in all groups, and differences within each group before and after treatment were statistically significant.

Fig. 7

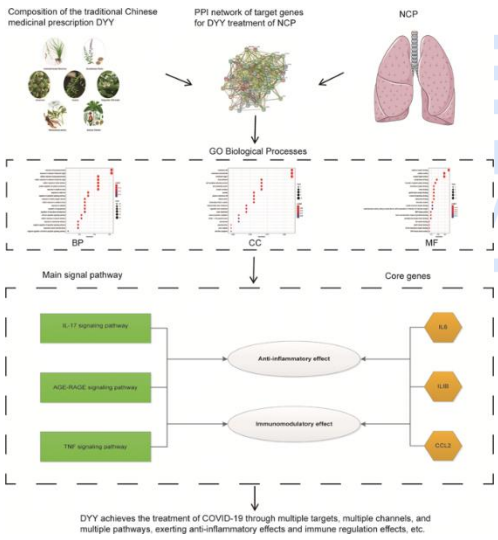


Graph of patient age distribution and IL 6 levels. In (**a**), the abscissa represents group, and the ordinate represents age. In (**b**), the abscissa represents the group, and the ordinate represents the level of IL6, where A1, B1, and C1 represent patients before treatment while A2, B2, and C2 represent patients after treatment in each group. Data are expressed as the mean ± S.D. (n = 15 per group); *p < 0.01, **p < 0.001, and***p < 0.001. the after treatment groups(A2, B2, C2) vs. the before treatment groups(A1, B1, C1)

Mechanism of action of DYY in the treatment of COVID-19

Based on the above studies, the specific mechanism of the action of DYY in the treatment of COVID-19 is shown in Fig. 8.

Fig. 8



Mechanism of action of DYY in the treatment of COVID-19

Discussion

As of April 6, 2020, the cumulative number of COVID-19 confirmed cases worldwide has exceeded 1.2 million. However, no vaccine or definitive antiviral drugs are available for the prevention and treatment of COVID-19. Therefore, it is crucial to find out medicines with confirmed curative effects for the prevention and treatment of COVID-19 as soon as possible to improve the patient's condition and prevent the ongoing outbreak of COVID-19.

In this study, we first searched and screened a database of traditional Chinese medicine database to obtain 174 DYY compounds and 7053 corresponding target genes. Ent-epicatechin, quercetin, mairin, beta-sitosterol, sitosterol, and stigmasterol are common compounds of two or more Chinese medicines. Studies have shown that quercetin can reduce apoptosis induced by hypoxia and rescue phosphorylation of AMPK [15]. Beta-sitosterol has antipyretic, analgesic, anti-inflammatory, and antioxidant functions and plays roles in cough and phlegm elimination, immune regulation and tissue repair [16, 17]. Stigmasterol is present in the

membrane [18] and has anti-inflammatory effects [19]. The compound-target network diagram (Fig. 2) shows that there was a complex network relationship between the compounds and the targets. Kaempferol, quercetin, 7-Methoxy-2-methyl isoflavone, naringenin, formononetin, and beta-sitosterol had the largest number of targets and were core compounds.

By searching the disease database, we found a total of 251 genes related to COVID-19. Drug target genes and disease related genes were intersected, and a total of 45 target genes for DYY treatment of COVID-19 were finally obtained (Additional file 2: Fig. S2). These genes were analysed by PPI analysis to obtain the corresponding network diagram (Fig. 3a). Figure 3a shows that the target genes of DYY for the treatment of COVID-19 were not independent, and there was a certain relationship among these genes. The core gene map in Fig. 3b shows that the top-ranking genes were IL6, MAPK3, MAPK8, CASP3, IL10, IL1B, CXCL8, MAPK1, CCL2, IFNG, IL4, etc. These genes were mainly concentrated in the inflammatory response, immune modulation, and cellular stress processes, which indicated that they might play a key role in DYY treatment of COVID-19. It is well known that IL6, IL10, IL1B, and IL4 are all members of the interleukin family. Interleukins play an important role in transmitting information, regulating immune cells, mediating T and B cell activation, and responding to inflammation [20, 21]. CCL2 and CXCL8 belong to the chemokine family and are important inflammatory cytokines. They play an important role in the migration of Tregs to inflammatory tissues [22] and in immune regulation in the body [23]. MAPK3, MAPK8, and MAPK1 are members of the MAPK family and can participate in responses to potentially harmful abiotic stress stimuli [24].

At the same time, a network of drug active ingredients, target genes corresponding to the active ingredients and disease targets was constructed as shown in Fig. 4. We can see that one compound can act on multiple target genes. Similarly, one target gene can also correspond to multiple compounds. That is, multiple compounds can act on a common target. Based on the above analysis, we concluded that multiple active ingredients in the traditional Chinese medicine prescription DYY can act on COVID-19 through multiple targets.

Through functional enrichment analysis of target genes for DYY treatment of COVID-19, GO biological process and KEGG pathway enrichment maps were obtained (see Fig. 5). It can be seen from Fig. 5 that in the GO terms, the BP terms (Fig. 5a) were mainly associated with the cell's response to processes such as lipopolysaccharide, molecule of bacterial origin, biotic stimulus, cytokine production, oxidative stress, and adaptive signaling pathways. CC terms (Fig. 5b) were mainly associated with various membranes, including membrane raft, membrane microdomain, membrane region, plasma membrane raft, nuclear envelope, and mitochondrial outer membrane, etc.; the terms were also associated with focal adhesion, cell-substrate adherens junction, cell-substrate junction, etc. The MF terms (Fig. 5c) were mainly associated with various receptors (cytokine, chemokine, growth factor, CXCR chemokine, G protein-coupled, nuclear hormone receptors), binding functions (phosphatase, heme, protein phosphatase, tetrapyrrole, BH domain, death domain, tumor necrosis factor receptor superfamily) and various cytokine, ligand, and kinase activities (cytokine, receptor ligand, MAP kinase, chemokine, oxidoreductase). The pathways involved in the KEGG enrichment pathway (Fig. 5d) were mainly the IL-17 signaling pathway, AGE-RAGE signaling pathway, cytokine–cytokine receptor interaction, TNF signaling pathway, and NOD-like receptor signaling pathway. The diseases involved were mainly infectious and immune diseases. Infectious diseases included viral infectious diseases (prion diseases, influenza A, human cytomegalovirus infection, etc.), parasitic infectious diseases (malaria, Chagas disease, African trypanosomiasis, etc.) and bacterial infectious diseases (Yersinia infection). Immune diseases included inflammatory bowel disease, rheumatoid arthritis, and graft-versus-host disease.

As shown in Fig. 6, hsa04657 (IL-17 signaling pathway), hsa04933 (AGE-RAGE signaling pathway in diabetic complications), and hsa0406 (cytokine–cytokine receptor interaction pathway) enriched the highest number of genes, indicating that these pathways may play an important role in the mechanism of action of DYY in the treatment of COVID-19. The IL-17 signaling pathway is involved in the body's immune response [25, 26]

and inflammatory response [27]. The AGE-RAGE signaling pathway has important protective effects on bones and the heart and participates in oxidative stress response [25] and fibrosis transduction [22]. The cytokine–cytokine receptor interaction is a key pathway for regulating the cellular inflammatory response [28]. IL6, ILIB, CCL2 and other genes occupy a large rectangular area, indicating that there are more pathways connected to these genes. Therefore, it can be speculated that these genes play a key role in the mechanism of action of DYY in the treatment of COVID-19. IL6, ILIB, and CCL2 represent a wide range of inflammatory mediators and pathways. Many animal and human experiments have demonstrated that IL6 has a wide range of anti-inflammatory effects [29]. ILIB has analgesic, immunomodulatory, anti-hypoxia, and anti-inflammatory functions. CCL2 is an important inflammasome-associated chemokine [30]. Inhibition of CCL2 can reduce the infiltration of peripheral inflammatory cells such as monocytes and neutrophils [9]. NOS3 is a vasoprotective gene [31] that regulates vascular tone, blood pressure and platelet aggregation [32]. Research reports have shown that NOS3 can affect metabolism in the urea cycle of the methylation pathway, which is essential for preventing systemic inflammation [33].

By combining the core target gene bar chart (Fig. 3b) and the KEGG relationship network diagram (Fig. 6), we can see that IL6 is one of the most critical genes for anti-inflammatory and immune regulation in COVID-19 patients treated with DYY. Based on the comparison of COVID-19 patients before and after treatment with DYY, the IL6 level of COVID-19 patients increased to different degrees when they were admitted to the hospital but decreased after treatment, further confirming that DYY may play an important role in anti-inflammatory and immune regulation and may have other effects in the treatment of COVID-19 patients.

Conclusions

In summary, we speculate that DYY may play an anti-inflammatory and immunoregulatory role in COVID-19 by acting on multiple target proteins, such as IL6, ILIB, and CCL2. The role of DYY involves a variety of biological processes, mainly signaling pathways such as the IL-17 signaling pathway, cytokine–cytokine receptor interaction, and AGE-RAGE signaling pathway, involved in diabetic complications. In short, DYY plays a role in COVID-19 treatment through multiple targets, multiple channels, and multiple pathways, making it worthy of clinical application and promotion. However, only part of the specific mechanism of action of DYY has been clinically verified, and further verification is needed in subsequent experiments.

Availability of data and materials

The data used to support the results of this study can be obtained from the first author upon reasonable request.

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Abstract

Purpose

Lianhuaqingwen (LH) as traditional Chinese medicine (TCM) formula has been used to treat influenza and exerted broad-spectrum antiviral effects on a series of influenza viruses and immune regulatory effects Ding et al. (2017). The goal of this study is to demonstrate the antiviral activity of LH against the novel SARS-CoV-2 virus and its potential effect in regulating host immune response.

Methods
The antiviral activity of LH against SARS-CoV-2 was assessed in Vero E6 cells using CPE and plaque reduction assay. The effect of LH on virion morphology was visualized under transmission electron microscope. Pro-inflammatory cytokine expression levels upon SARS-CoV-2 infection in Huh-7 cells were measured by real-time quantitative PCR assays.

Results
LH significantly inhibited SARS-CoV-2 replication in Vero E6 cells and markedly reduced pro-inflammatory cytokines (TNF-α, IL-6, CCL-2/MCP-1 and CXCL-10/IP-10) production at the mRNA levels. Furthermore, LH treatment resulted in abnormal particle morphology of virion in cells.

Conclusions
LH significantly inhibits the SARS-COV-2 replication, affects virus morphology and exerts anti-inflammatory activity *in vitro.* These findings indicate that LH protects against the virus attack, making its use a novel strategy for controlling the COVID-19 disease.

Introduction

Coronaviruses are a group of enveloped viruses named for theircoronary appearance with positive single-stranded RNA genomes [2].In addition to six known strains of coronaviruses that are infectious tohumans, a novel coronavirus (SARS-CoV-2) was detected recently inWuhan, China [3, 4]. Like the other two highly pathogenic cor-onaviruses SARS-CoV and MERS-CoV, SARS-CoV-2 also caused severerespiratory illness and even death. Moreover, the population's sus-ceptibility to these highly pathogenic coronaviruses has contributed tolarge outbreaks and evolved into the public health events, highlightingthe necessity to prepare for future reemergence or the novel emergingviruses [5].Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 is initiated byzoonotic transmission likely from bats and spreads rapidly among hu-mans [6]. The basic reproduction number (R0) of person-to-personspread is about at 2.6, which means that the SARS-CoV-2 infected casesgrow at an exponential rate. As of February 07, 2020, 57,620 cases ofthe SARS-CoV-2 have been reported in China, including 26,359 sus-pected cases, and a sustained increase is predictable. The initial patientcluster with confirmed SARS-CoV-2 infection was reported Wuhanpneumonia with unknown aetiology, which bore some resemblance toSARS-CoV and MERS-CoV infections and was associated with ICU ad-mission and high mortality. Moreover, High concentrations of cytokines were recorded in plasma of patients requiring ICU admission, such asGCSF, IP10, MCP1, MIP1A, and TNFα, suggesting that the cytokinestorm was associated with disease severity [7]. A retrospective clinicalstudy indicated the risk of fatality among hospitalized cases at 4.3 % insingle-center case series of 138 hospitalized patients [8], and the in-fection fatality risk could be below 1% or even below 0.1 % in a largenumber of undetected relatively mild infections [9]. However, It ischallenging to judge the severity and predict the consequences with theinformation available so far. Since no specific antiviral treatment forCOVID-19 is currently available, supportive cares, including sympto-matic controls and prevention of complications remain the most criticaltherapeutic regimens, especially in preventing acute respiratory distresssyndrome [10]. Although the control of SARS-CoV-2 still presentsmultiple challenges in the short term, more potent antiviral drugs areurgent to be developed [4].At present, some drugs are effective in eliminating SARS-CoV-2 andimproving symptoms. The most promising antiviral drug for SARS-CoV-2 is remdesivir that is currently under clinical development for thetreatment of Ebola virus infection [11]. However, the efficacy andsafety of remdesivir for SARS-CoV-2 pneumonia patients need to beassessed by further clinical trials. In addition, in the prevention andtreatment of COVID-19, Tranditonal Chinese medicines have receivedbroad adoption, especially in treating cases of mild symptoms [12].Lianhuaqingwen (LH), a Chinese patent medicine composed of 13herbs, has played a positive role in the treatment of SARS-CoV-2. Aretrospective analysis of clinical records was conducted in the SARS-CoV-2 infected patients at Wuhan Ninth Hospital and CR & WISCOGeneral Hospital. LH combination could significantly relieve cardinalsymptoms and reduce the course of the COVID-19 [13], making itsuccessively included in theGuideline for the Diagnosis and Treatment ofNovel Coronavirus (2019-nCoV) Pneumonia (On Trials, the Fourth/Fifth/Sixth/Seventh Edition) issued by National Health Commission of thePeople's Republic of China and also recommended by 20 provincialhealth commissions including Hubei, Beijing, and Shanghai as well asNational Administration of Traditional Chinese Medicine for the treat-ment of COVID-19. Moreover, LH exerted broad-spectrum effects on aseries of

influenza viruses by inhibiting viral propagation and reg-ulating immune function and achieved similar therapeutic effectivenesswith Oseltamivir in reducing the course of H1N1 virus infection[1, 14,15]. Notably, the anti-influenza activity of LH in infected micemight depend on the regulation of cytokines, particularly in cytokinestorm associated cytokines, such as IP-10, MCP-1, MIP1A, and TNF-α[1]. In the present study, we evaluated the antiviral and anti-in-flammatory efficiency of LH against a clinical isolate of SARS-CoV-2from Guangzhou in vitro.2. Materials and methods2.1. Cell lines and virusThe African green monkey kidney epithelial (Vero E6) cells and thehuman hepatocellular carcinoma (Huh-7) cells were cultured inDulbecco's ModifiedEagle's medium (DMEM, Gibco, USA) supple-mented with 10 % fetal bovine serum (FBS) at 37 °C. A clinical isolatedSARS-CoV-2 virus (Genebank accession no. MT123290.1) was propa-gated in Vero E6 cells, and viral titer was determined by 50 % tissueculture infective dose (TCID50) according to the cytopathic effect by useof Reed-Muench method [17]. All the infection experiments were per-formed in a biosafety level-3 (BLS-3) laboratory.2.2. Reagent preparationLH capsule (Lot No.B2001019) was obtained from YilingPharmaceutical Co. Ltd. (Shijiazhuang, China). UPLCfingerprints of LHconsist of 32 common peaks. 9 of 32 common peaks are identified. Thesimilarities in 10 batches of LH Capsules samples were all above 0.96(Supplementary Fig. 1). The black powder of raw material of LH wasfirst dissolved in dimethyl sulfoxide (DMSO) to 240 mg/mL. Aftershaking for 30 min at room temperature, the LH solution was dilutedwith serum-free DMEM to 24 mg/mL as a stock solution and stored at−20 °C before using. Remdesivir was kindly provided by Prof. JiancunZhang from Guangzhou Institutes of Biomedicine and Health, ChineseAcademy of Sciences and was dissolved in DMSO to 100 mM and storedat−20 °C before using. DMEM with 2% FBS was used as the dilutionbuffer in the follow-up experiments.2.3. Cytotoxicity assayThe cytotoxic effects of the LH on Vero E6 and Huh-7 cells wereevaluated by Methyl Thiazolyl Tetrazolium (MTT) assay. Briefly,monolayers of Vero E6 cells and Huh-7 cells in 96-well plates wererinsed with phosphate-buffered saline (PBS) followed by incubationwith indicated concentrations of LH. After 72 h, the cells were stainedwith MTT solution at 0.5 mg/mL for 4 h. The supernatants were thenremoved, and the formed formazan crystals were dissolved in 200μLDMSO. The absorbance was measured at 490 nm using MultiskanSpectrum reader (Thermo Fisher, USA). The 50 % cytotoxic con-centration (CC50) was calculated by the GraphPad Prism 7.0 software.2.4. Cytopathic effect (CPE) inhibition assayThe Vero E6 cell monolayers were grown in 96-well plates and in-oculated with 100 TCID50of coronavirus strains at 37C for 2 h. Theinoculum was removed, and the cells were subsequently incubated withindicated concentrations of LH or the positive control remdesivir.Following the 72 h of incubation, the infected cells shown 100 % CPEunder the microscope. The percentage of CPE in LH-treated cells wererecorded. The 50 % inhibition concentration (IC50) of the virus-inducedCPE by LH was calculated by the Reed-Muench method [17].2.5. Plaque reduction assayThe Vero E6 cell monolayers in 6-well plates were infected with 50plaque-forming units (PFU) of SARS-CoV-2 for 2 h at 37 °C. After in-cubation, the cell monolayers were covered with agar overlay (finalconcentration: 0.6 % agar, 2% FBS, indicated concentrations of LH orremdesivir). The plates were then incubated for 48 h at 37 °C with 5%CO2. Subsequently, the agar overlays were removed, and the cellmonolayer wasfixed with 10 % formalin, stained with 1% crystalviolet, and then the plaques were counted and photographed.2.6. RNA isolation and reverse transcriptase-quantitative PCR analysis(RT-qPCR)The Huh-7 cell monolayers in 12-well plate were rinsed with PBSand then exposed to coronavirus at a multiplicity of infection (MOI) of 1for 2 h at 37 °C. The inoculum was removed and replaced with theindicated concentrations of LH or mock-treated with DMEM supple-mented with 2% FBS for subsequent 48 h incubation at 37 °C with 5%CO2. The cells were then harvested for RNA isolation and qPCR asdescribed previously [16]. The primer and probe sequences used foranalysis are listed in Supplementary Table 1. The relative mRNA ex-pression was calculated using the 2−△△Ctmethod with GAPDH as aninternal reference gene.2.7. Electron microscopeMonolayers of Vero E6 cells in 6-well plates were incubated withSARS-CoV-2 at a MOI of 0.001 for 2 h at 37 °C. The virus inoculum wasthen removed and replaced with DMEM medium supplemented with2% FBS containing LH (600μg/mL) or remdesivir (5μM). At 48 h p.i.,the cells werefixed, dehydrated and embedded as described previously [18]. Ultrathin sections (70 nm) of embedded cells were prepared,deposited onto Formvar-coated copper grids (200 mesh), stained withuranyl acetate and lead citrate, and then observed under JEM-1400PLUS transmission electron microscopy (Japan Electron Optics La-boratory Co., Ltd., JEM-1400 PLUS).2.8. Statistical analysesStatistical analysis was performed using GraphPad Prism 7.0 soft-ware. The differences in mRNA expression levels of cytokines werecompared using a one-way analysis of variance (ANOVA). Values ofp < 0.05 was considered to be statistically significant.3. Results3.1. Antiviral activity of LH on SARS-CoV-2 in

vitroThe cell viability after LH or remdesivir treatment was determinedby MTT assay in both Vero E6 and Huh-7 cells. LH showed unapparentcytotoxicity for both cell lines at concentrations up to 600μg/mL(Fig. 1A, C). The positive control remdesivir showed no cytotoxicity tocells at a concentration of 50μM(Fig. 1B, D).To investigate the antiviral effect of LH against SARS-CoV-2 virus,the Vero E6 cells were infected with 100 TCID50of virus and incubatedwith LH at various concentrations for 72 h. As shown inFig. 2A, LHinhibited the replication of SARS-CoV-2 virus with an IC50value of411.2μg/mL by CPE assay (Fig. 2A). Meanwhile, treatment with LHfollowing infection also had a dose-dependent inhibitory effect onplaque formation of the SARS-CoV-2 virus (Fig. 2C). We selected re-mdesivir as the positive control in our study and the results showed thatremdesivir potently inhibited virus-induced CPE with an IC50of 0.651μM and a total plaque formation inhibition at 5μM(Fig. 2B, C).To further confirm the efficacy of LH in inhibiting SARS-CoV-2 virusreplication in cells, we detected the viral particles in ultrathin sectionsof infected cells under electron microscopy. At 48 h p.i., viral particleswere found in cytoplasm, intracellular vesicles, endoplasmic reticulum,and cell membrane and presented spherical crown-like appearance,which was typical coronavirus morphology (Fig. 3B, G). LH (600μg/mL) and positive control remdesivir (5μM) treatment resulted in areduction of the number of virions compared with mock-treated in-fected cells (Fig. 3G–J). It was interesting to note that some virions inthe surface of LH-treated cells presented spindle sharp which was incontrast to the typical spherical particles in the mock-treated cells(Fig. 3I).3.2. Inhibition of SARS-CoV-2-induced cytokine and chemokine expressionby LH in vitroTo determine the effect of LH on the expression of cytokines andchemokines induced by SAR2-CoV-2, the mRNA expression levels ofTNF-α, IL-6, CCL-2/MCP-1, and CXCL-10/IP-10 were detected andcompared between the LH-treated and mock-treated Huh-7 cells. The results showed that the elevated expressions of these four cytokineswere significantly inhibited by LH treatment in a concentration-de-pendent manner (Fig. 4).4.

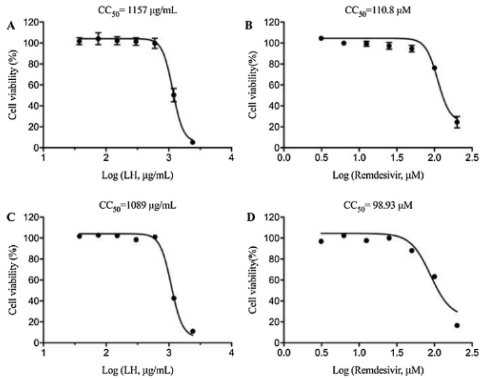

Fig. 1. Cytotoxic effect of the LH and remdesivir on Vero E6 cells (A, B) and Huh-7 cells (C, D). Data are presented as mean ± SD. The experiments were performed in triplicate.

Discussion

Starting from December 2019, a pandemic of respiratory illnesscaused by a novel coronavirus named SARS-CoV-2 is sweeping themainland of China. This virus has spread to several foreign countries,threatening to trigger a global outbreak. Several antiviral agents can beenvisaged to control or prevent viral infections by antiviral assay invitro [14, 17]. However, the efficacy and safety of novel candidatesneed validations in vivo, even for those clinically approved medicines,which means that it will take months to years for clinical practices. At present, symptomatic and supportive treatments remain key to clinicalpractices. Thus, Traditional Chinese Medicines (TCM) carried both theantiviral effect and the symptomatic relief might bring more clinicalbenefits [12]. As a classical TCM prescription for respiratory diseases,

LH is the only approved medicine in the treatment of SARS and influenza. After the outbreak of SARS-CoV-2, LH as a representative TCMprescription was recommended again in the latest Guideline for theDiagnosis and Treatment of Novel Coronavirus (2019-nCoV) Pneu-monia issued by National Health Commission of the People's Republicof China The purpose of this study was to demonstrate whether thetherapeutic effects of LH on the COVID-19 targeting virus replicationand immunological regulation as it did on the infection caused by in-fluenza viruses.Our previous study showed that LH exhibited in vitro anti-influenzaactivity with IC50ranging from 200 to 2000μg/mL [1]. Here we de-monstrated that LH also has a comparable antiviral potency against theSARS-CoV-2 virus with an IC50value of 411.2μg/mL (Fig. 2). Transmission electron microscopy (TEM) has been a potent tool to observevirus entry, virus particle assembly, viral ultrastructure, and buddingfrom the plasma membrane [17]. To understand the antiviral

details ofLH, EM pictures were taken from each group. Abundant virus particlesassembled at the surface of membrane, cytoplasm, and plasma vesiclesin the SARS-CoV-2 infected cells, decreased in the treatment of LH at600ug/mL. Notably, slight deformation of virus particles was seen inthe LH treatment, which required us to make further studies.

Highly pathogenic coronaviruses such as SARS-CoV and MERS-CoVcause fatal pneumonia, which is mainly associated with rapid virusreplication, massive inflammatory cell infiltration and elevated proin-flammatory cytokine/chemokine responses. Although the pathophysiology of fatal pneumonia caused by highly pathogenic coronaviruseshas not been completely understood, accumulating evidence suggeststhat the cytokine storm plays a crucial role in causing fatal pneumonia[18]. Excessive amounts of proinflammatory cytokines were reported(e.g., IL-1β, IL-6, IL-12, IFN-γ, IP-10, and MCP-1) in the serum of SARS patients [18], similar in the serum of MERS patients [19]. ChaolinHuang et al. confirmed the occurrence of the cytokine storm in theCOVID-19 patients in ICU rather than those in non-ICU patients [7].Based on the excessive cytokines responses, Suxin Wan et al. claimedthat IL-6 and IL-10 levels could be used as one of the bases for pre-dicting the outcome and prognosis of the COVID-2019 [20]. In thisstudy, host cells infected with HCoV-229E and SARS-COV-2 increasedthe cytokine release such as TNF-α, IL-6, CCL-2/MCP-1, and CXCL-10/IP-10, which was suppressed by LH in a dose-dependent manner. Thechange of cytokine profiles suggested that LH might have a potentialeffect on the inhibition of cytokine storm induced by SARS-COV-2,which also needed to be validatedin vivo.

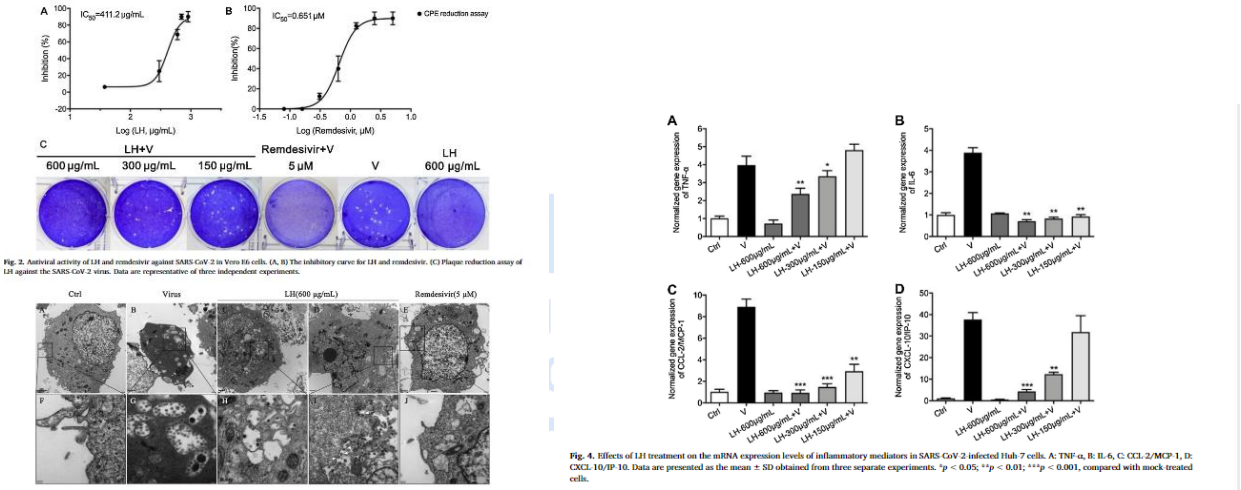

Fig. 3.Virions in the ultra sections of infected Vero E6 cells under electron microscope. (A, F) uninfected cells, (B, G) mock-treated SARS-CoV-2 virus infected cells,(C, D, H, I) infected cells with LH treatment, (E, J) infected cells with remdesivir treatment. White arrows indicated the spindle sharp of viral particles in infected cells with LH treatment.

4. Conclusion

Since the launch of LH, it has been widely used as a broad spectrumof antiviral agent in the clinical practice, especially for various re-spiratory virus infections. Previous studies have shown that LH a broadspectrum of effects on a series of influenza viruses by interfering withboth viral and host reactions. Although LH significantly relieved theclinical symptoms of the COVID-19, the underlying mechanism of an-tiviral effects on coronavirus, especially in the SARS-COV-2, was stillelusive. In this study, we demonstrated that LH exerted its anti-cor-onavirus activity by inhibiting virus replication and reducing the cytokine release from host cells, which supported the clinical applicationof LH in combination with existing therapies to treat COVID-2019.

Declaration of Competing Interest

The authors declare that they have no known competingfinancialinterests or personal relationships that could have appeared to influ-ence the work reported in this paper.

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Appendix

A. Supplementary dataSupplementary material related to this article can be found, in theonline version, at doi:https://doi.org/10.1016/j.phrs.2020.104761.References

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[11]S. Mulangu, et al., A randomized, controlled trial of ebola virus diseaseFig. 4.Effects of LH treatment on the mRNA expression levels of inflammatory mediators in SARS-CoV-2-infected Huh-7 cells. A: TNF-α, B: IL-6, C: CCL-2/MCP-1, D:CXCL-10/IP-10. Data are presented as the mean ± SD obtained from three separate experiments. *p< 0.05; **p< 0.01; ***p< 0.001, compared with mock-treatedcells. therapeutics, N. Engl. J. Med. 381 (24) (2019) 2293–2303.

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Natural Methods to Protect Your Respiratory System from Infection During the Current Flu and Coronavirus Season. Heiner Fruehauf, phd, lac March 7, 2020

1. Reduce sugar intake. White and brown sugar causes our bodies to become more acidic, which benefits the growth of bacteria. Synthetic sugar alternatives such as Splenda are harmful to the nervous system and should also be avoided. Use maple syrup, local honey, or coconut sugar for sweetening.

2. Increase your intake of naturally occurring trace minerals, which make the body more alkaline and have the potential to prevent infection. For instance, use Bio Nativus' Ionic Trace Minerals (a trace mineral extract made from concentrated water from the Great Salt Lake). Add 5-15 drops to every glass of water or liquid you drink, up to 5x/day.

3. Gargle with saltwater after brushing your teeth, preferably with a full-spectrum natural salt such as Real Salt.

4. Get a skin brush and start brushing your skin after showering in the morning, especially the head, neck and chest areas. This will stimulate lymphatic circulation and increase your general feeling of vitality and well-being.

5. Apply essential oils before going out in public, either in the form of an anti-viral massage oil (applied to the chest, neck and lower arm areas) or an anti-viral "perfume" (applied to the area below the nostrils). During the bubonic plague in the middle ages, essential oils prevented the professions of perfumers (who exclusively worked with oils on a daily basis) and thieves (who fortified themselves with oils before burglarizing abandoned houses) to become ravaged by the pandemic. Since different viruses thrive in the environment every year, the types of appropriate oils also vary from year to year. Of the many types of essential oils available on the market, I have found that the following oils have proven to be most effective for the prevention and treatment of upper respiratory infections in early 2020:

• Eucalyptus • Tea Tree • Niaouli • Lemon• Cinnamon leaf • Clove • Rosemary • Thyme

• Frankincense • Myrtle

Any or all of the oils described above can be mixed together to produce an essential oil blend. Massage oils should incorporate 30 drops of essential oils per ounce (blended into a base of high-quality olive oil or apricot seed oil). "Perfumes" should be mixed 50/50 with a base oil like jojoba oil or apricot seed oil, and used by applying 1-2 drops of the blend to the area below the nostrils. Alternatively, according to a recent report I received, Chinese medicine practitioners during the 1938 epidemic in China successfully swabbed their nostrils with vinegar before seeing afflicted patients.

6. Additionally, the internal application of Chinese herbs has been proven effective in the prevention and treatment of both the COVID-19 and SARS strains during the 21st century coronavirus epidemics in mainland China. For my recent suggestions to natural medicine practitioners flooded by the question of how to best

protect oneself during the current season of respiratory vulnerability see my separate article, “Initial Thoughts on Coronavirus Prevention and Treatment with Chinese Medicine”.

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REVIEW ARTICLE

Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: experts’ consensus statement

Abstract

Since the outbreak of 2019 novel coronavirus infection (2019-nCoV) in Wuhan City, China, by January 30, 2020, a total of 9692 confirmed cases and 15,238 suspected cases have been reported around 31 provinces or cities in China. Among the confirmed cases, 1527 were severe cases, 171 had recovered and been discharged at home, and 213 died. And among these cases, a total of 28 children aged from 1 month to 17 years have been reported in China. For standardizing prevention and management of 2019-nCoV infections in children, we called up an experts’ committee to formulate this experts’ consensus statement. This statement is based on the Novel Coronavirus Infection Pneumonia Diagnosis and Treatment Standards (the fourth edition) (National Health Committee) and other previous diagnosis and treatment strategies for pediatric virus infections. The present consensus statement summarizes current strategies on diagnosis, treatment, and prevention of 2019-nCoV infection in children.

Background

In December, 2019, a cluster of pneumonia cases, who were later proven to be caused by a novel coronavirus (named as “2019-nCoV”), emerged in Wuhan City, Hubei Province,

China. By January 30, 2020, 9692 confirmed cases and 15,238 suspected cases have been reported around 31 prov- inces and cities in China. Among the confirmed cases, 1527 were severe cases, 171 had recovered and been discharged at home, and 213 died. Twenty-eight confirmed cases aged from 1 month to 17 years had been reported in China [1]. Coronavirus (CoV) belongs to the Coronaviridae family, Nidovirales order. CoVs are divided into four genera: α-, β-, γ-, and δ-coronavirus. α- and β-coronaviruses only infect mammals, whereas γ- and δ-coronaviruses mainly infect birds, with a few infecting mammals. Human CoVs include α-coronaviruses (229E and NL63), β-coronaviruses (OC43 and HKU1), the Middle East respiratory syndrome-related coronavirus (MERS-CoV), severe acute respiratory syndrome-related coronavirus (SARS-CoV), and 2019- nCoV. The 2019-nCoV belongs to the β-coronavirus genus [2], which includes bat-SARS-like (SL)-CoVZC45, bat- SL-CoVZXC21, SARS-CoV, MERS-CoV, and 2019-nCoV. Current studies have revealed that 2019-nCoV may originate from wild animals, but the exact origin remains unclear. 2019-nCoV infected patients are the main infection sources. However, we also should attach importance to asymptomatic cases which may play a critical role in the transmission process. Respiratory droplets and contact are the main transmission routes [3]. Close contact with symptomatic cases and asymptomatic cases with silent infection are the main transmission routes of 2019-nCoV infection in children. People of all ages are susceptible to 2019-nCoV. The elderly and those with underlying chronic diseases are more likely to become severe cases. Thus far, all pediatric cases with laboratory-confirmed 2019-nCoV infection were mild cases, and no deaths had been reported. For standardizing the prevention and treament of 2019-nCoV infections in children, we called up an experts’ com- mittee to formulate this consensus statement. This statement is based on the Novel Coronavirus Infection Pneumonia Diagnosis and Treatment Standards (the fourth edition) (National Health Committee) and other previous diagnosis and treatment strategies for pediatric virus infections.

Clinical manifestations

Based on the current epidemiological data, the incubation period of 2019-nCoV infections ranges from 1 to 14 days, mostly ranging from 3 to 7 days. Current reported data of pediatric cases revealed that the age of disease onset ranged from 1.5 months to 17 years, most of whom had a close contact with infected cases or were family cluster cases [4]. Infected children might appear asymptomatic [5] or present with fever, dry cough, and fatigue, and few have upper res- piratory symptoms including nasal congestion and running nose; some patients presented with gastrointestinal symp- toms including abdominal discomfort, nausea, vomiting, abdominal pain, and diarrhea. Most infected children have mild clinical manifestations.

They have no fever or symptoms of pneumonia with a good prognosis. Most of them recover within 1–2 weeks after disease onset. Few may progress to lower respiratory infections. No newborns delivered by 2019-nCoV infected mothers have been detected positive; and no newborn cases have been reported yet. It should be noted that clinical manifestations in pediatric patients should be further defined after collecting more pediat- ric case data. Furthermore, the number of confirmed infected cases will increase after a wide use of pathogen analysis. Data from adults reveal that severe cases often develop dyspnea one week after disease onset. Severe cases may rap- idly progress to acute respiratory distress syndrome (ARDS), septic shock, refractory metabolic acidosis, and coagulation dysfunction [6, 7]. Although no deaths in children have been reported up to now, the potential risk of death should be highlighted. Though clinical symptoms in pediatric patients are relatively milder compared with those in adult patients, ARDS and death cases also occurred in infected children during the SARS and MERS epidemics [8–11].

Auxiliary examinations Laboratory examination [3]

1. In the early phase of the disease, white blood cell count is normal or decreased, with decreased lymphocyte count; liver enzymes, muscle enzymes, and myohemo- globin levels are increased in some patients.

2. Most patients display elevated C-reactive protein level and erythrocyte sedimentation rates, and normal proc- alcitonin levels.

3. Severe cases show high D-dimer levels and progres- sively decreased blood lymphocytes counts.

4. Samples from throat swabs (better using nasopharyn- geal swab in children), sputum, lower respiratory tract secretions, stool and blood, etc. are tested positive for 2019-nCoV nucleic acids.

Chest imaging examination [3]

Suspected cases or confirmed cases should undertake chest X-ray examination as soon as possible. Chest CT scan is required when necessary. In the early stage of disease, chest images show multiple small plaques and interstitial changes, which are obvious in the lung periphery, further deteriorate to bilateral multiple ground-glass opacity and/or infiltrat- ing shadows. Lung consolidation may occur in severe cases. Pleural effusion is rarely seen.

Diagnosis

Suspected cases

2019-nCoV should be suspected in patients who meet any one of the criteria in the epidemiological history and any two of the criteria in clinical manifestations.

Epidemiological history

1. Children with a travel or residence history in Wuhan city and neighboring areas, or other areas with persistent local transmission within 14 days prior to disease onset;

2. Children with a history of contacting patients with fever or respiratory symptoms who have a history of contact with patients from Wuhan city and neighboring areas, or other areas with persistent local transmission within 14 days prior to disease onset;

3. Children who are related with a cluster outbreak or close contact with 2019-nCoV infected cases;

4. Newborns delivered by confirmed 2019-nCoV-infected mothers.

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Clinical manifestations

1. Fever, fatigue, dry cough; some pediatric patients may have low-grade fever or no fever;

2. With above-mentioned chest imaging findings (refer to the section of Chest imaging examination);

3. In the early phase of the disease, white blood cell count is normal or decreased, or with decreased lymphocyte count;

4. No other pathogens are detected which can fully explain the clinical manifestations.

Confirmed cases Suspected cases who meet any one of the following criteria [3]:

1. Respiratory tract or blood samples tested positive for 2019-nCoV nucleic acid using RT-PCR;

2. Genetic sequencing of respiratory tract or blood samples is highly homologous with the known 2019-nCoV.

Clinical classifications

1. Asymptomatic infection (silent infection) Children tested positive for 2019-nCoV, but without manifestations of clinical symptoms or abnormal chest imaging findings.

2. Acute upper respiratory tract infection Children with only fever, cough, pharyngeal pain, nasal congestion, fatigue, headache, myalgia or dis- comfort, etc., and without signs of pneumonia by chest imaging or sepsis.

3. Mild pneumonia Children with or without fever, respiratory symptoms such as cough; and chest imaging indicating pneumonia, but not reaching the criteria of severe pneumonia.

4. Severe pneumonia Meeting any of the following criteria [3, 12–15]:

(1) Increased respiratory rate: ≥ 70 times/min (< 1 year), ≥ 50 times/min (≥ 1 year) (after ruling out the effects of fever and crying);

(2) Oxygen saturation < 92%; (3) Hypoxia: assisted breathing (moans, nasal flaring, and three concave sign), cyanosis, intermittent apnea;

(4) Disturbance of consciousness: somnolence, coma, or convulsion;

(5) Food refusal or feeding difficulty, with signs of dehydration.

5. Critical cases Those who meet any of the following criteria and require ICU care:

(1) Respiratory failure requiring mechanical ventilation; (2) Shock; (3) Combined with other organs failure.

Early identification of critical cases

According to the experiences in diagnosis and treatment of community-acquired pneumonia in children, children with a history of contact with severe 2019-nCoV infected cases, or with underlying conditions (such as congenital heart disease, bronchial pulmonary hypoplasia, respiratory tract anomaly, with abnormal hemoglobin level, severe malnutri- tion), or with immune deficiency or immunocompromised status (under long-term use of immunosuppressants) who meet any one of the following criteria may become severe cases:

1. Dyspnea: respiratory rate > 50 times/min for 2–12 months old; > 40 times/min for 1–5 years old; > 30 times/min in patients over 5 years old (after ruling out the effects of fever and crying);

2. Persistent high fever for 3–5 days; 3. Poor mental response, lethargy, disturbance of con- sciousness, and other changes of consciousness;

4. Abnormally increased enzymatic indexes, such as myo- cardial enzymes, liver enzymes, lactate dehydrogenase;

5. Unexplainable metabolic acidosis; 6. Chest imaging findings indicating bilateral or multi-lobe infiltration, pleural effusion, or rapid progression of con- ditions during a very short period;

7. Infants younger than 3 months; 8. Extrapulmonary complications; 9. Coinfection with other viruses and/or bacteria.

Differential diagnosis [3]

Differential diagnosis should be made to distinguish from influenza virus, parainfluenza virus, adenovirus, respira- tory syncytial virus, rhinovirus, human metapneumovirus, SARS coronavirus, and other known viral infections, as well as mycoplasma pneumoniae and chlamydia pneumo- nia and bacterial pneumonia. The coinfection of 2019-nCoV with other viruses and/or bacteria should be considered in diagnosis.

General treatment

The general treatment strategies include bed rest and sup- portive treatment; ensuring sufficient calory and water intake; maintaining water electrolyte balance and homeo- stasis; monitoring vital signs and oxygen saturation; keep- ing respiratory tract unobstructed and inhaling oxygen when necessary; measuring blood routine, urine routine, C-reac- tive protein, and other blood biochemical indexes including liver and kidney function, myocardial enzyme spectrum, and coagulation function according to patients' conditions. Blood gas analysis and timely re-examination of chest imag- ing should be performed when necessary.

Symptomatic treatment

The patients with high fever should be actively controlled. If patients' body temperature exceeds 38.5 °C with obvi- ous discomfort, physical cooling (warm water bath, use of antipyretic patch, etc.) or antipyretic drug treatment should be performed. Common drugs include: ibuprofen orally, 5–10 mg/kg every time; acetaminophen orally, 10–15 mg/kg every time. Keep children quiet and administrate sedatives immediately when convulsions or seizure occur.

Oxygen therapy

When hypoxia appears, effective oxygen therapy should be given immediately including nasal catheter, mask oxy- gen. Nasal high-flow oxygen therapy, and non-invasive or invasive mechanical ventilation should be undertaken when necessary.

Antiviral therapy Interferon-α [3, 16–28]

Interferon-α can reduce viral load in the early stage of infection which can help to alleviate symptoms and shorten the course of disease. Based on our clinical research and World Journal of Pediatrics experiences of using interferon-α in treating bronchiolitis, viral pneumonia, acute upper respiratory tract infection, hand foot mouth disease, SARS, and other viral infections in children, the recommended usage is as follows:

1. Interferon-α nebulization: interferon-α 200,000– 400,000 IU/kg or 2–4 μg/kg in 2 mL sterile water, nebulization two times per day for 5–7 days;

2. Interferon-α2b spray: applied for high-risk populations with a close contact with suspected 2019-nCoV infected patients or those in the early phase with only upper res- piratory tract symptoms. Patients should use 1–2 sprays on each side of the nasal cavity, 8–10 sprays on the oro- pharynx, the dose of interferon-α2b per injection is 8000 IU, once every 1–2 hours, 8–10 sprays/day for a course of 5–7 days.

Lopinavir/litonavir [3, 29, 30]

Lopinavir/litonavir has been tried to apply to the treatment of adult patients with 2019-nCoV pneumonia, but its effi- cacy and safety remain to be determined.

Usage of other agents Antibiotics [3, 12]

Avoiding irrational use of antibiotics, especially in combina- tion with broad-spectrum antibiotics. Paying close attention to the changes of conditions in children with coinfection of bacterial or fungal infection; actively collecting samples for pathogen analysis and timely or rational use of antibiotics or anti-fungal drugs.

Arbidol [31], oseltamivir [32] and other anti-influenza drugs

Arbidol is administrated for adults infected with 2019-nCoV; however, its efficacy and safety remain unclear. Oseltamivir and other anti-influenza agents can be applied for patients coinfected with other influenza virus.

Other drugs [3, 12] Glucocorticoids

The use of glucocorticoids should be based on the severity of systemic inflammatory response, degree of dyspnea, with or without ARDS, and the progress status of chest imag- ing results. Glucocorticoids can be used in a short period (3–5 days). The recommended dose of methylprednisolone should not exceed 1–2 mg/kg/day.

Immunoglobulin

Immunoglobulin can be used in severe cases when indicated, but its efficacy needs further evaluation. Treatment of severe and critically ill cases [3, 12]

On the basis of symptomatic treatment, we should actively prevent and treat complications, underlying diseases, sec- ondary infection, and provide organ function support as indicated.

Respiratory support

Children who undergo non-invasive mechanical ventilation for 2 hours without improvements in conditions, or cannot tolerate non-invasive ventilation, with increased airway secretions, severe cough, or hemodynamic instability, should be subjected to invasive mechanical ventilation promptly. The invasive mechanical ventilation should adopt low tidal volume “lung protective ventilation strategy” to reduce ventilator related

lung injury. If necessary, prone position ventilation, lung recruitment, or extracorporeal membrane oxygenation (ECMO) can be applied.

Circulation support

On the basis of full fluid resuscitation, improve microcircu- lation, use vasoactive drugs, and monitor hemodynamics if necessary.

Traditional Chinese medicine

This disease belongs to the epidemic disease category of Traditional Chinese Medicine and results from contract- ing epidemic pathogens. Different regions can refer to the following plans for dialectical treatment according to the patient's conditions, local climate features, and physical characteristics of children.

Clinical treatment period 1. Asymptomatic infection:

(1) Therapeutic methods: strengthening the healthy and dispelling pathogenic factors;

(2) Recommended prescription and drugs: modi- fied Yupingfeng powder in combination with Buhuanjin Zhengqi powder composed of 9–12 g of Zhihuangqi (Prepared Astragalus), 6–9 g of Chaobaizhu (Roasted Rhizoma Atractylo- dis Macrocephalae), 3–9 g of Houpo (Officinal Magnolia Bark), 6–9 g of Cangzhu (Atractylodes lancea), 6–9 g of Chenpi (Pericarpium citri reticu- latae), 3–6 g of Jiangbanxia (Ginger processed pinellia), 6–9 g of Huoxiang (Agastache rugosus), 6 to 9 g of Fuling (Poria cocos), and 3–6 g of Zhi- gancao (Prepared Liquorice Root).

2.Old and dump tightening the lung

(1) Clinical manifestations: aversion to cold, fever or no fever, dry cough, sore throat, nasal congestion, tiredness and fatigue, nausea and retching, loose stool, pale tongue or reddish tongue with whitish- greasy fur, floating, and soft pulse;

(2) Therapeutic methods: dispersing lung to promote pathogenic factors, detoxify, and dispel dampness;

(3) Prescription and drugs: modified Qingqi decoction composed of 6–9 g of Cangzhu, 3–9 g of Houpo, 6–9 g of Chenpi, 6–12 g of Huoxiang, 3–9 g of Banxia, 3–9 g of Xingren, 9–15 g of Suye, 6–9 g of Jiegeng, 6–9 g of Guanzhong, 6–9 g of Fuling, 3–6 g of Shengjiang, and 3–6 g of Gancao.

3. Plague poison obstructing lungs:

(1) Clinical manifestation: fever persists or chill and fever alternate; cough with little or yellow phlegm; shortness of breath holds back; abdomi- nal distension constipation. The tongue is red, while the moss is yellow and greasy or yellow and dry. Slide number of arteries and veins;

(2) Therapeutic methods: detoxification, opening and closing, clearing the lungs, and dampness;

(3) Prescription and drugs: modified Xuanbai Chengqi decoction composed of 6–9 g of Huoxi- ang, 10 g of Cangzhu, 3–6 g of Zhimahuang, 3 -9 g of Chaoxingren, 15–30 g of Shengshigao, 10 g of Gualou, 3–6 g of Jiujun (to be added later in preparation), 6–9 g of Huangqin, 6–9 g of Ful- ing, 6–9 g of Danpi, 6–9 g of Shichangpu, and 3–6 g of Chuanbei.

4. Inner blocking causing unconsciousness and collapse:

(1) Clinical manifestation: dyspnea, lethargy, rest- lessness, cold and sweat in limb, dark purplish tongue, thick and slimy fur or dry fur, big floating and unstable pulse, cyanosis in fingerprints, and reaching for the Mingguan point (distal phalanx)

(2) Therapeutic methods: opening the blocking and solidification dysfunction, detoxifying, and reviv- ing the unconscious;

(3) Prescriptions and drugs: modified Shenfu decoc- tion plus Shengmai drink composed of 3–6 g of Renshen (radix ginseng), 6–12 g of fuzi (radix aconiti Praepareta) (to be decocted one hour first). 6–12 g of Shanzhuyu (Fructus Corni), 10 g of Maimendong (Radix ophiopogonis), and 3–6 g of Rougui (Cinnamomum cassia), to be taken with Angong Niuhuang Pill.

5. Qi deficiency of both the lung and spleen.

(1) Clinical manifestation: feeble cough, lassitude and asthenia, spontaneous sweating, poor appetite, loose stool, pale tongue with whitish and slippery fur, thready, and weak pulse;

(2) Therapeutic methods: nourishing the lungs and strengthening the spleen, nourishing qi, and dehu- midifying;

(3) Prescription and drugs: modified LiuJunZi decoc- tion composed of 15 g of Zhihuangqi (Prepared Astragalus), 10 g of Xiyangshen (American Gin- seng), 10 g of Chaobaizhu (Roasted Rhizoma Atractylodis Macrocephalae), 6 g of Fabanxia (Rhizoma Pinelliae preparatum), 6 g of Chenpi (Pericarpium citri reticulatae), 3 g of Chuan- bei (Tendril-leaved fritillary bulb), 15 g of Ful- ing (Poria cocos), 6 g of Huoxiang (Agastache rugosus), and 3 g of Sharen (Fructus amomi) (to be added in later).

Psychotherapy

Psychological counseling plays an important role in disease recovery. If patients (especially older children) show mood swing, fear, or psychological disorders, active psychological intervention and treatment are needed.

Release and discharge criteria [3]

Confirmed patients can be discharged from isolation or transferred to the corresponding departments for treatment of other diseases if all the following criteria are met:

1. The body temperature returns to normal longer than 3 days;

2. The respiratory symptoms improve obviously;

3. The detection of respiratory pathogenic nucleic acid is negative for two consecutive times (the sampling inter- val is at least 1 day).

Suspected patients can be discharged from isolation when the detection of respiratory pathogenic nucleic acid is nega- tive for two consecutive times (the sampling interval is at least 1 day).

Prevention [33–35]

Novel coronavirus infection is a new communicable dis- ease with an emergent outbreak that affects all popula- tions. 2019-nCoV infection has been classified as category B infectious disease legally but managed as category A infectious disease. It is paramount to implement infection control practices by infection source controlling, transmis- sion route blocking, and susceptible population protection.

Controlling infection sources

Patients infected with 2019-nCoV are the main infection sources. Children infected by novel coronavirus should be isolated at home or admitted to designated hospitals under the guidance of healthcare workers depending on the severity of their medical conditions. Try to provide single rooms for isolated children, and reduce the chance of con- tact with the co-residents. There are enormous demands for room ventilation, necessary cleaning, and disinfection work for the articles used by children. Equally crucial is the need of equipment with disposable masks and properly disposal after use when taking care of the sick.

Blocking transmission routes

1. Preventing transmission by respiratory droplets and contact: Cover mouth and nose with napkin or towel when coughing or sneezing. Wash hands for children frequently, or teach children seven-step washing technique. Try not to touch mouth, nose, or eyes before cleaning hands thoroughly after returning from public places, after covering the mouth when coughing, before eating or after using toilet; regularly disinfecting toys by heating at 56 °C for 30 min, 75% alcohol or chlorine- containing disinfectants, and ultraviolet rays.

2. Reduce exposure to infection: Avoid public transport at epidemic areas, and wear masks when going to crowded or poorly ventilated public places; avoid touching or eat ing wild animals, and going to markets selling with live animals.

3. Children's health monitoring: Children with a history of close contacts of infected patients need to be monitored for body temperature and clinical features routinely. When presenting with suspicious symptoms, children should be taken to a designated hospital for screening. Newborns delivered by infected mothers must complete a pathogen test and be isolated in a single ward or at home according to their medical conditions.

Boosting immunity

Balanced diet, oral health, adequate exercise, regular rest, avoiding excessive fatigue, and boosting immunity are the powerful measures to preventing infection, as well as main- taining emotional stability and mental health. Vaccination is an effective way to prevent virus infection. The research and development of anti-virus vaccines has been carried out in China at present.

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69. Sinha SK, Prasad SK, Islam MA, Gurav SS, Patil RB, AlFaris NA, Aldayel TS, AlKehayez NM, Wabaidur SM, Shakya A. Identification of bioactive compounds from *Glycyrrhiza glabra* as possible inhibitor of SARS-CoV-2 spike glycoprotein and non-structural protein-15: a pharmacoinformatics study. J Biomol Struct Dyn. 2020 Jun 18:1-15. doi: 10.1080/07391102.2020.1779132.

Abstract

At present, the world is facing a pandemic named as COVID-19, caused by SARS-CoV-2. Traditional Chinese medicine has recommended the use of liquorice (Glycyrrhiza species) in the treatment of infections caused by SARS-CoV-2. Therefore, the present investigation was carried out to identify the active molecule from the liquorice against different protein targets of COVID-19 using an *in-silico* approach. The molecular docking simulation study of 20 compounds along with two standard antiviral drugs (Lopinavir and Rivabirin) was carried out with the help of Autodock vina software using two protein targets from COVID-19 i.e. spike glycoprotein (PDB ID: 6VSB) and Non-structural Protein-15 (Nsp15) endoribonuclease (PDB ID: 6W01). From the observed binding energy and the binding interactions, glyasperin A showed high affinity towards Nsp15 endoribonuclease with uridine specificity, while glycyrrhizic acid was found to be best suited for the binding pocket of spike glycoprotein and also prohibited the entry of the virus into the host cell. Further, the dynamic behavior of the best-docked molecules inside the spike glycoprotein and Nsp15 endoribonuclease were explored through all-atoms molecular dynamics (MD) simulation study. Several parameters from the MD simulation have substantiated the stability of protein-ligand stability. The binding free energy of both glyasperin A and glycyrrhizic acid was calculated from the entire MD simulation trajectory through the MM-PBSA approach and found to high binding affinity towards the respective protein receptor cavity. Thus, glyasperin A and glycyrrhizic acid could be considered as the best molecule from liquorice, which could find useful against COVID-19.Communicated by Ramaswamy H. Sarma.

Introduction

The recent pandemic 2019 novel coronavirus, also known as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Zhou, Yang, et al., 2020) has resulted in 295,101 deaths with a fatality rate of 6.85% and has infected a total of 4,307,287 individuals around the globe, as of 9:28 am CEST, 15 May 2020 (World Health Organization, 2020). The spectrum of SARS-CoV-2 infection is found to have a very wide range of severity, from asymptomatic carriage to mild respiratory tract infection and severe to fatal pneumonia (Singhal, 2020). Besides this, SARS-CoV-2 is characterized by very high morbidity and high mortality, which emphasizes the urgent medical as well as a public health need for validation, development and approval of effective prophylactic and therapeutic interventions against the COVID-19 (Zhu et al., 2020).

The coronaviruses (CoV) are single-stranded enveloped, positive-sense RNA with the largest genome size that ranges approximately from 26 to 32 kilobases as observed till date. The CoV uses its spike (S) glycoprotein (180 kDa) to bind its receptor, and mediate membrane fusion and virus entry. Indeed, S proteins are typical homo trimeric class I fusion proteins, and protease cleavage is required for activation of the fusion potential of S protein. A two-step sequential protease cleavage starts with activation of S proteins CoVs, which involves prime cleavage between two subunits (i.e. S1 and S2) and activates cleavage on S2' site (Belouzard et al., 2009; Cotten et al., 2013; Ou et al., 2020; Wan et al., 2020). Depending on virus strains and cell types, CoV S proteins may be cleaved by one or several host proteases, including furin, trypsin, cathepsins, transmembrane protease serine protease-2 (TMPRSS-2), TMPRSS-4, or human airway trypsin-like protease (HAT). Availability of these proteases largely mediating attachment and membrane fusion on target cells (Kang et al., 2020). Upon the entry, the viral particle is encoded and ready for translation ORF 1a and 1b into polyproteins pp1a (4382 amino acids) and pp1ab (7073 amino acids) that are processed by proteases 3-C-like protease ($3CL^{pro}$) and papain-like protease (PL^{pro}). Subsequently, these polyproteins are cleaved into at least 16 non-structural proteins (Nsps), which assembles and form the replication-transcription complex (Pillaiyar, Meenakshisundaram, & Manickam, 2020). Importantly, the homology modelling revealed that SARS-CoV-2 S and SARS-CoV S share the same functional host cell receptor i.e. angiotensin-converting enzyme 2 (ACE-2) (Zhang et al., 2020). Besides, the SARS-CoV-2 RBD-SD1 106 fragment (S residues from 319-591) have reported having ACE-2 binding site (Wrapp et al., 2020). The affinity with which ACE-2 bounds to SARS-CoV-2 S ectodomain is 10- to 20-fold higher than ACE-2 binding to SARS-CoV S. Due to the high binding affinity of SARS-CoV-2 S for human ACE-2, the SARS-CoV-2 exhibits greater ability to transmit from person-to-person (Wan et al., 2020). Furthermore, the genomic RNA, acting as a messenger RNA (mRNA) has been

found to play a critical role in the initial RNA synthesis of the infectious cycle, template for replication, transcription and also act as a substrate for packaging (Ricagno et al., 2006; Snijder et al., 2016). The Nsp15 is a nidoviral RNA uridylate-specific endoribonuclease (NendoU) and possess C-terminal catalytic domain, which is specific for uridine acting on both, single- and double-stranded RNA (Elfiky, 2020a). Reports reveal that the NendoU activity of Nsp15 is the main contributor for the protein interference with innate immune response and thus, it is said that Nsp15 plays a pivotal role in the biological progression of coronavirus (Bhardwaj et al., 2008). Therefore, the drugs under investigation, if targets the conserved sites can be likely to block the entry, replication and proliferation of the virus and thus may exhibit a wide spectrum of activity (Adeoye et al., 2020; Elfiky, 2020b; Joshi et al., 2020; Pan et al., 2020; Prajapat et al., 2020; Tian et al., 2020; Wu et al., 2020).

Although, scientific fraternity and interdisciplinary research groups are putting their sincere efforts to combat pathogenic SARS-CoV-2 by applying variable holistic approaches to understand the possible features of pathogenesis, for the effective prognosis and early identification/detection of cases along with the development of effective therapeutic interventions including prevention, prophylactics, vaccines and treatment measures against COVID-19, a public health emergency of international concern. In principle, the cost-effective and time-efficient computational technique provides a powerful network-based tool (Cheng et al., 2018) to test a novel hypotheses conceptualizing systematic drug repositioning strategy for rapid identification of therapeutic lead and/or potential leads combinations effective against 2019-nCoV/SARS-CoV-2 from existing approved and/or preclinical drugs (Kandeel & Al-Nazawi, 2020; (Pillaiyar, Meenakshisundaram, Manickam, et al., 2020; Serafin et al., 2020; Sinha et al., 2020; Zhou, Hou, et al., 2020).

A promising published report on glycyrrhizin, a bioactive constituent of liquorice (*Glycyrrhiza glabra* L. Family: Fabaceae), which is a key medicine of Traditional Chinese Medicine (TCM) system, indicates its role in the treatment of patients suffering from an infection caused by SARS-CoV (Cinatl et al., 2003). Moreover, according to TCM theory, liquorice is primarily effective for fatigue and debilitation, asthma with coughing, excessive phlegm, respiratory infections and for relieving drug toxicity (Grienke et al., 2014; Wang et al., 2013). Further, bioactive compounds of liquorice have been reported with antimicrobial, antiviral and immunoregulatory features. In brief, glycyrrhizic acid has been reported for its efficacy against HIV-1 chronic hepatitis C virus, coxsackievirus A16 and enterovirus 71 and Kaposi sarcoma-associated herpesvirus (Curreli et al., 2005; De Clercq, 2000; Sabouri Ghannad et al., 2014; Wang et al., 2013). The licochalcone A and isoliquiritigenin has been effective in preventing the acute lung injury induced of pathogenic origin (Liu et al., 2019). Liquiritin has also been reported for their anti-viral potential against HIV (Grienke et al., 2010).

Application of computational resource and power in drug discovery research to screen molecular databases has become faster and less expensive approach towards the scientific community worldwide (Okimoto et al., 2009). The structure-based screening of chemical or phytochemical databases already reached at a new height due to the availability of large numbers of crystal target. With the help of excellent algorithms and pharmacoinformatics tools molecular docking can predict the almost true conformational state of a protein-ligand complex and predicts the binding affinity of the small molecules about correctly (Meng et al., 2011). In the current investigation, we have conceptualized the structure-based antiviral screening of natural products from liquorice with the aim to obtained structurally potential inhibitors by targeting the crystal structure of prefusion SARS-CoV-2 spike glycoprotein and Nsp15 endoribonuclease from SARS-CoV-2, which expedites the discovery of leads in treatment against COVID-19. Initially, the phytochemicals are screened through molecular docking approach. Further, the molecular dynamics (MD) simulation study is carried out to explore the dynamic behavior of the molecules. Finally, the affinity of the small molecules is checked through the MM-PBSA (Molecular Mechanics Poisson–Boltzmann Surface Area) based binding energy calculation.

Material and methods

Molecular docking simulation

Virtual screening of phytochemicals through pharmacoinformatics approach has become a pivotal tool in the drug discovery research. Availability of crystal structure of protein molecules has given a new dimension towards the structure-based screening of small to large chemical databases. To explore promising anti-COVID molecules, a set of 20 reported bioactive compounds of liquorice (Glycyrrhiza species) were retrieved from the PubChem database (Kim et al., 2016). In brief, the names search for bioactive compounds of *Glycyrrhiza glabra* which has been documented for their antitumor, antimicrobial, antiviral and immunoregulatory efficacies viz. glycyrrhizinic acid (CID_14982), licochalcone A (CID_5318998), licochalcone B (CID_5318999), licochalcone C (CID_9840805), licochalcone D (CID_10473311), licochalcone E (CID_46209991), licochalcone F (CID_44130137), licochalcone G (CID_49856081), glyasperin A (CID_5481963), glyasperin B (CID_480784), glyasperin C (CID_480859), glyasperin D (CID_480860), isoliquiritinapioside (CID_6442433), 1-methoxyphaseollidin (CID_480873), dehydroglyasperin C (CID_480775), kanzonol Q (CID_11253965), liquiritin (CID_503737), hedysarimcoumestan B (CID_11558452), 5,6,7,8-tetrahydro-2,4-dimethylquinoline (CID_5321849), 5,6,7,8-tetrahydro-4-methylquinoline (CID_185667) (Bode & Dong, 2015; Jiang et al., 2020; Kim & Ma, 2018; Liu et al., 2019; Prajapati & Patel, 2015; Yang et al., 2017); was carried out in PubChem database. The resulting 3D structures in sdf file format were directly used in the generation of input pdbqt files for Autodock vina. The 2D format of all molecules were downloaded and transformed to 3D coordinates and subsequently optimized with Openbabel2.3.2 GUI. The molecular docking simulation study was performed on the Autodock vina program (Trott & Olson, 2010), which is the most widely used and trusted steadfast open-source docking simulation tool. Recently submitted two crystal structure of SARS-CoV-2 i.e. spike glycoprotein [PDB ID: 6VSB (Wrapp et al., 2020)] and non-structural protein 15 [PDB ID: 6W01 (Kim et al., 2020)] were downloaded from the Research Collaboratory for Structural Bioinformatics-Protein Databank (RCSB-PDB). Both crystal structures were prepared using AutoDock Tools (ADT) (Morris et al., 2009) by repairing the missing atoms, the addition of polar hydrogens and the Gastigier charges. Finally, both structures were saved in the pdbqt format after assigning the AD4 (AutoDock 4) (Morris et al., 2009) atom type. To select the binding site of both proteins the already available information was considered. The region around the bound citric acid in the Nsp15 was considered as the active site. In the case of Spike glycoprotein, it is mentioned that amino acid residues in a range of 319 to 591 recombinantly expressed and measures the ACE2 binding using biolayer interferometry (Wrapp et al., 2020). Hence the coordinate of Ser325 amino residue was considered as the position of the grid box. To confine the active site of the protein molecule, the grid box information was obtained from the ADT with the help of above information. In case of PDB ID: 6W01, grid box was generated around the bound citric acid having co-ordinate of −63.624, 72.524 and 28.280 for -x, -y and -z-axis respectively. On the other hand, for PDB ID: 6VSB, the grid coordinate was considered as 204.457, 199.799 and 246.898 along the x-, y- and z-axis respectively. The grid box size for both crystal structures was determined by manual inspection and set as 50x50x50. The configuration file was created by adding the grid, receptor and ligand information before the execution of molecular docking. To adjudge the molecular docking study, two standard drug molecules, Lopinavir and Rivabirin (Cao et al., 2020) were considered as control molecules throughout the study. In recent past, a number of findings have been reported the efficacy of Lopinavir, a protease inhibitor and Ribavirin, a nucleoside analogue in suppressing the shedding of SARS-CoV-2 (Hung et al., 2020; Lim et al., 2020; Sheahan et al., 2020). All 20 phytochemicals along with above two drug molecules were also prepared by adding polar hydrogen and Gasteiger charges in ADT. Each molecule was saved as pdbqt format for Autodock vina input after detection and set of torsion angles. The molecular docking outcomes were analyzed based on binding energy and binding interactions profile. Highest binding energy among the drugs was used as a threshold to select the best phytochemicals for both the receptors. The binding interactions between ligands and the receptor were assessed through the protein-ligand interaction profile (PLIP) (Salentin et al., 2015).

Molecular dynamics simulation

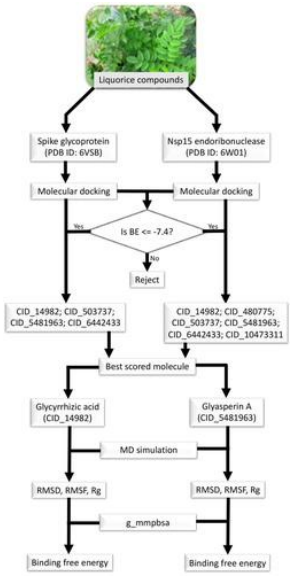
Molecular dynamics (MD) simulation study is one the crucial and effective tools to explore the dynamic nature of the protein-ligand complex and the relative stability. The highest binding affinity scorer ligand in respect to each protein i.e. Glycyrrhizic acid for spike glycoprotein and glyasperin A for Nsp15 endoribonuclease complex with respective protein was selected for further MD simulation study. The MD simulation was done on Gromacs-2018.2 software tool (www.gromacs.org) installed at the Lengau CHPC server, Cape Town, South Africa. This study was performed for 100 ns with a 2fs of time step at a 1 atm constant pressure and 300 K constant temperature. To generate the protein topology the all-atoms CHARMM36 force field was considered. During the study, each protein-ligand complex system was created within the cubic box of 1 Å from the center of the system. The system was solvated by the TIP3P water model. Sufficient numbers of Na^+ and Cl^- ions were added to neutralize the simulation system and further minimized the system by employing the steepest descent algorithm of 10,000 steps. To address the long-range distances the van der Waals and electrostatic interaction cutoff range were set to 0.9 and 1.4 nm respectively. The SwissParam tool (Daina et al., 2017) was used to generate ligand topology. After each of 1ps interval, the trajectory information was saved for further analysis. The entire system was equilibrated through the NVT (constant number of particles, volume, and temperature) followed by NPT (constant number of particles, pressure, and temperature) ensemble approaches to distribute the solvent and ions equally around the protein-ligand complex. Further root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF) and radius of gyration (Rg) were calculated to determine the molecular complex stability in terms of conformation and performance. The binding free energy was then determined through the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method with the help of g_mmpbsa tool (Islam & Pillay, 2019, 2020). Theory and details of this method have been given in our previous publication (Bhowmick et al., 2019; Parida et al., 2020).

Result and discussion

Virtual screening

The structure-based screening is one of the crucial approaches to explore small to large molecular databases to find promising molecules for a specific target. In the current study, an effort was taken to find out anti-spike glycoprotein and Nsp15 endoribonuclease molecules from a set of 20 phytochemicals through molecular docking and MD simulation studies. The flow diagram of the work is given in Figure 1. The binding site of the SARS-CoV-2 spike glycoprotein was considered around the Ser325 which is already reported as active site residue (Yu et al., 2020). In the case of Nsp15 endoribonuclease the binding site was selected around the co-crystal ligand. All 20 phytochemicals along with Lopinavir and Ribavirin were docked in the spike glycoprotein and Nsp15 endoribonuclease.

Figure 1. Work flow of screening of phytochemicals against spike glycoprotein and Nsp15 endoribonuclease.



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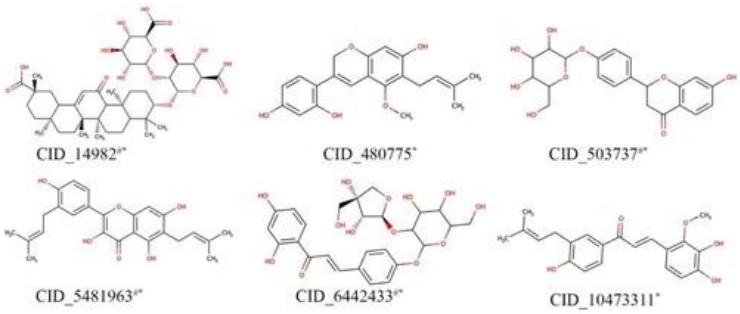
Details of each of phytochemicals and binding energies against the spike glycoprotein and Nsp15 endoribonuclease are given in Table S1 (Supplementary file). The binding energy of Lopinavir and Ribavirin was found to be −7.4 and −5.6 Kcal/mol respectively when docked in the spike glycoprotein. Moreover, the Lopinavir and Ribavirin were docked in the Nsp15 endoribonuclease and binding energy was found to be −8.3 and −6.6 Kcal/mol respectively. To select best phytochemical, the threshold binding energy for spike glycoprotein and Nsp15 endoribonuclease was considered as −7.4 and −8.3 Kcal/mol, respectively. From Table S1 (Supplementary file) it can be seen that CID_14982, CID_503737, CID_5481963 and CID_6442433 were satisfied above criteria for both spike glycoprotein and Nsp15 endoribonuclease. Moreover, CID_480775 and CID_10473311 were found to have binding energy less than −8.30 on docking in the Nsp15 endoribonuclease. Hence, CID_14982, CID_503737, CID_5481963 and CID_6442433 were found to be promising molecules for both enzymes. In addition to the above CID_480775 and CID_10473311 were found promising against Nsp15 endoribonuclease. Two-dimensional representation of all six promising molecules is given in Figure 2.

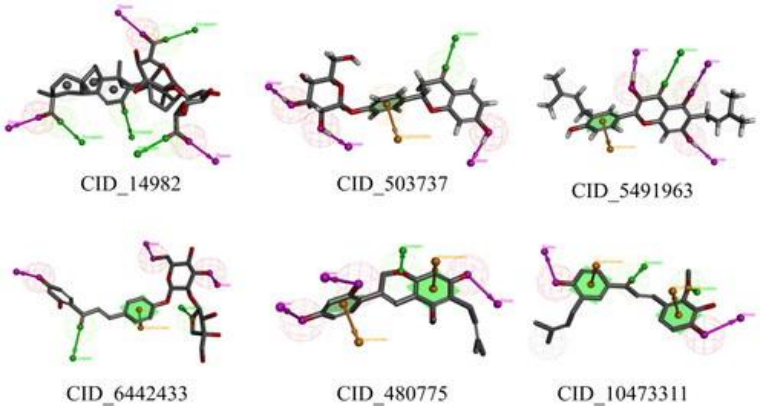
Figure 2. Two-dimensional representation of final molecules. #Spike glycoprotein inhibitors; *Nsp15 endoribonuclease inhibitors.



All the above proposed final molecules were found structurally diverse. All molecules consist of several pharmacophoric features including hydrogen bond (HB) acceptor (HBA), HB donor (HBD), hydrophobic (Hy), etc., those might be crucial for potential binding interactions with the catalytic amino acids at the active site. In brief, the HBD and HBA features in CID_14982 (Figure 3) were found crucial in hydrogen bond interactions with Gln298, His625, Gln321 and Arg319, while the Hy region was found to be important in the interaction with Ile624 and Val620 residues in the spike glycoprotein. In the case of Nsp15 endoribonuclease of the SARS-COV-2, the HBD and HBA features were revealed as important in interactions with residues Tyr343, His235, His250, while the hydrophobic interactions formed with residues Tyr343. Likewise, the HBD, HBA,

aromatic ring features in the other five compounds (Figure 3) were found to be crucial for the interactions with similar residues at the binding site of both the proteins. To further assess the binding interactions of each of the molecule were explored with respective enzymes. The stability of the molecules inside the protein was explored through all-atoms MD simulation. Finally, the binding free energy was calculated through the MM-PBSA approach.

Figure 3. Two-dimensional representation of the pharamacophoric features of final molecules.



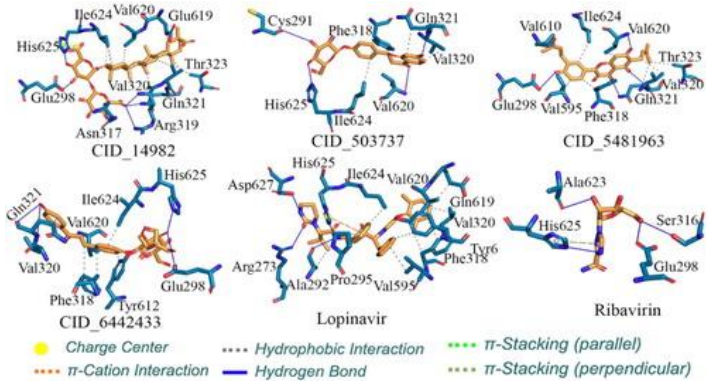
Binding interaction analysis

The binding interaction profile of all proposed molecules was explored to analyze the catalytic amino acid residues responsible for holding the molecules inside the receptor cavity of Spike glycoprotein and Nsp15 endoribonuclease. The Protein-Ligand Interaction Profiler (PLIP) webserver (Salentin et al., 2015) was used to explore two-dimensional binding interactions. The binding mode in surface view of proposed phytochemicals and considered drug molecules was explored using the PyMol. The binding energies of all six proposed molecules and considered drug molecules are given in Table 1.

Binding interactions analysis of the natural bioactive ligand with SARS-CoV-2 spike glycoprotein

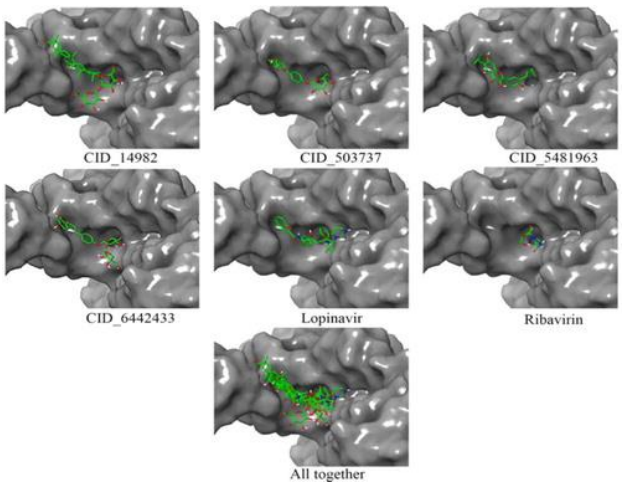
All four proposed spike glycoprotein inhibitors along with Lopinavir and Ribavirin were docked in the spike glycoprotein and binding interactions are given in Figure 4. On successful docking, the complex between spike glycoprotein and Lopinavir was explored and found that the Lopinavir properly positioned into the catalytic site assembled by Arg273, Asp627, His625, Ala292, Val320, Val595, Val620, Gln619, Tyr612, Ile624, Phe318 and Pro295 amino acids with binding energy −7.4 Kcal/mol. Amine group of tetrahydro pyrimidine of lopinavir showed H-bonding with Asp627, carboxyl with Arg273 and amide with His625, Ala292. The phenyl ring of Lopinavir formed π-π parallel stacking with His625. The triazole nitrogen of Ribavirin showed H-bonding with His625, hydroxymethyl group of tetrahydrofuran with Glu298, Ser316 and the hydroxyl group of tetrahydrofuran with Ala623 amino acid with binding energy −5.6 Kcal/mol. The triazole ring of Ribavirin formed π-π perpendicular stacking with His625. Docking study on the SARS-CoV-2 spike glycoprotein revealed that the glycyrrhizic acid (CID_14982) exhibited the best binding mode among all the ligand and standard drugs. It was properly positioned into the binding pocket of spike glycoprotein constructed by His625, Glu298, Arg319, Gln321, Gln321, Val320, Tyr343, Thr323, Ile624 and Asn317 amino acids with a binding energy of −9.2 Kcal/mol. The glycyrrhizic acid has two oxane (tetrahydropyran) ring substituted with five hydroxyl and two carboxyl groups buried properly into the binding pocket of S1 subunit and showed H-bonding with His625, Glu298, Arg319, Gln321, Val320 and three π-cation interaction with His625. It also has 7 methyl groups which provide a proper grip between hydrophobic pockets. The equal distribution of polar (i.e. 5 -OH and 2 -COOH group) and non-polar (i.e. 7 methyl group) provide a good balance between hydrophilicity and hydrophobicity (CID_14982 in Figure 4).

Figure 4. The binding interaction of glycyrrhizic acid (CID_14982), liquiritin (CID_503737), glyasperin A (CID_5481963), isoliquiritinapioside (CID_6442433), Lopinavir and Ribavirin in the spike glycoprotein.



The hydroxyl group of chromenone ring of glyasperin A (CID_5481963) showed H-bonding with Val620, Val320, carboxy of chromenone ring with Gln321 and other hydroxyl groups of the phenyl ring with Glu298 amino acid residue with binding energy −7.9 Kcal/mol (CID_5481963 in Figure 4). The liquiritin (CID_503737) has chromenone ring substituted by hydroxyl group showed H-bonding with Gln321, the carboxyl group of chromenone ring with Val620 and the hydroxyl group of oxane moiety with Cys291, His625 amino acid residues with a binding energy of −7.7 Kcal/mol (CID_503737 in Figure 4). The phenyl ring substituted by hydroxyl group showed H-bonding with Gln321, Val320, the hydroxyl group of oxane ring with Glu298, His625, the hydroxyl group of a furan ring with Glu298 and carboxyl group of isoliquiritinapioside (CID_6442433) showed H-bonding with Val620 amino acid residue with a binding energy of −7.4 Kcal/mol (CID_6442433 in Figure 4). From the above observations it can be explained that all proposed molecules for the spike glycoprotein hold similar or better binding interactions in comparison to the both Lopinavir and Ribavirin. The binding mode in three-dimensional space of all molecules were extracted which are given in Figure 5. It can be seen that all molecules perfectly fitted inside the receptor cavity of spike glycoprotein. The important amino residues and their involvement in binding interactions with spike glycoprotein inhibitors are given in Table 2.

Figure 5. Demonstrates the binding mode in 3D-surface-image of glycyrrhizic acid (CID_14982), liquiritin (CID_503737), glyasperin A (CID_5481963), isoliquiritinapioside (CID_6442433), Lopinavir and Ribavirin in the active site of spike glycoprotein of the SARS-CoV-2.

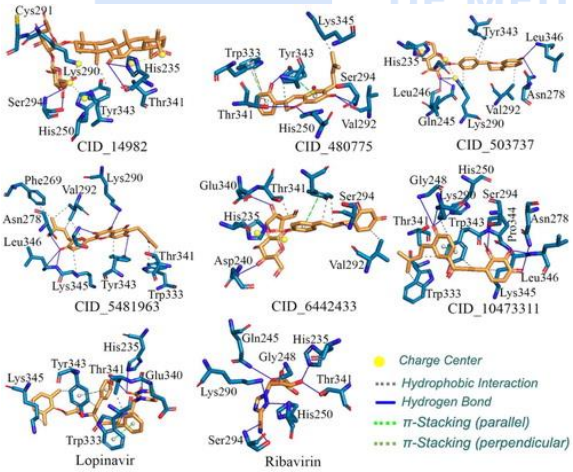


Binding interactions analysis of natural bioactive ligand with SARS-CoV-2 Nsp15 endoribonuclease

Best six phytochemicals found through virtual screening were docked in the Nsp15 endoribonuclease and binding energy is given in Table 1. Important amino acids found in binding interaction formation with

proposed molecules are given in Table 3. Both standard Lopinavir and Ribavirin was docked and binding energy found to be −8.3 and −6.6 Kcal/mol respectively. Lopinavir was properly positioned into the catalytic site constructed by His235, Thr341, Glu340, Tyr343, Trp333 and Lys345 amino acids with a binding energy of −8.3 Kcal/mol. Pyrimidine of Lopinavir showed H-bonding with Glu340 and carboxyl with His235, Thr341. The phenyl ring of Lopinavir formed π-π parallel stacking with Trp333 and π-π perpendicular stacking with Tyr343 (Figure 6). Ribavirin was found to interact into the binding pocket with a binding energy of −6.6 Kcal/mol (Figure 6). The simulation study on the crystal structure of Nsp15 endoribonuclease revealed that glyasperin A (CID_5481963) have chromenone ring substituted with three hydroxyl and 3-methylbut-2-enyl group and a phenyl ring which is substituted with a hydroxyl and a 3-methylbut-2-enyl group showed H-bonding with Tyr343, Lys290 Leu346, Asn278 and Lys345 with a binding energy of −9.2 Kcal/mol (Figure 6). Glyasperin A showed the highest stable binding mode i.e. lowest binding energy among all the docked test ligand and standard drug Lopinavir and Ribavirin. Glyasperin B, C and D have 2, 4-dihydroxy phenyl ring attached to chromen ring but in Glyasperin A one hydroxy of phenyl is substituted with a 3-methylbut-2-enyl group at 3rd position which showed additional hydrophobic interaction with Phe269 and Val292, due to which glyasperin A exhibited better binding affinity. The isoliquiritinapioside (CID_6442433) have furan ring substituted with hydroxyl group showed H-bonding with Glu340, Asp240 and Ser294 showed π stacking and hydrophobic interaction with isoliquiritinapioside (Figure 6) (−9.0 Kcal/mol). The hydroxyl group of chromenone ring of liquiritin (CID_503737) showed H-bonding with Leu346, Asn278, Leu246, Lys290, Gln245, two π-cation interactions with Lys290, His235 amino acid residues (−8.8 Kcal/mol) (Figure 6). The hydroxyl group of chromene ring of dehydroglyasperin C (CID_480775) showed H-bonding with Ser294, Val292 and Thr341, His250 with a binding energy of −8.4 Kcal/mol. The phenyl ring of dehydroglyasperin C formed π-π parallel stacking with Tyr343 and π-π perpendicular stacking with Trp333 (Figure 6). Structure of dehydroglyasperin C has resembled with glyasperin A, but due to lack of one 3-methylbut-2-enyl group on phenyl ring decreases its hydrophobic interaction and also a binding affinity with protein.

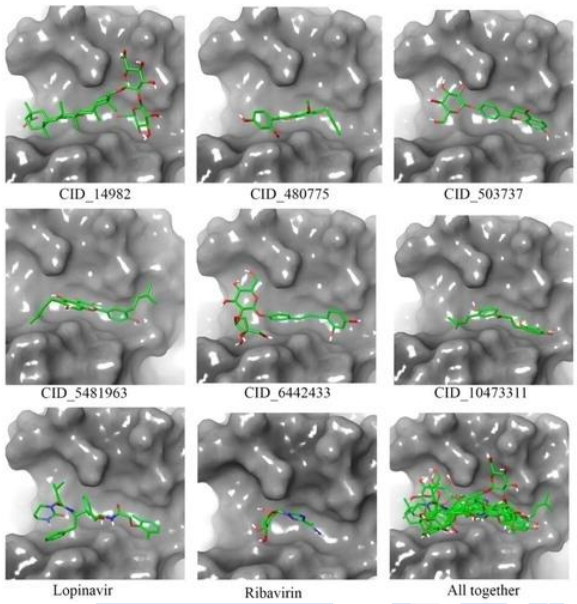
Figure 6. The binding interaction of glycyrrhizic acid (CID_14982), dehydroglyasperin C (CID_480775), liquiritin (CID_503737), glyasperin A (CID_5481963), isoliquiritinapioside (CID_6442433), licochalcone D (CID_10473311), Lopinavir and Ribavirin in the active site of Nsp15 endoribonuclease of the SARS-CoV-2.



The glycyrrhizic acid (CID_14982) have oxane ring-substituted with hydroxyl and carboxyl groups exhibited H-bonding with Ser294, Cys291, His250 and Thr341 amino acids with binding energy −8.3 Kcal/mol and three π-cation interactions with Lys290, His235, His250 (Figure 6). Licochalcone D showed H-bonding with Lys290, Asn278, Gly248, Leu346, His250, Pro344 (−8.3 Kcal/mol) and phenyl ring formed π-π parallel stacking with Trp343 (Figure 6). The isoliquiritinapioside and glycyrrhizic acid have oxan ring, and liquiritin and dehydroglyasperin C have chromenone ring showed good binding with active site located between the two β-sheets, carries residues Lys290, Tyr343, His235. The Lys290 in which His235 has been proposed to constitute the catalytic triad and Tyr343 is believed to govern Uridyl specificity. Moreover, a US patent (US005843990A) signifies the use of pyran-chromenone compounds in inhibiting the growth or replication of a viruses, which is not limited to herpes Simplex virus (types 1 and 2), HIV-1, HIV-2, cytomegalovirus, Varicella Zoster virus, papillomavirus, feline leukaemia virus, avian sarcoma viruses like hepatitis types A-E,

Rous sarcoma virus, influenza virus, measles, rubella and mumps viruses (Baker, 1998). The position of the binding pose in three-dimensional space (Figure 7) was clearly explained all six molecules perfectly fitted inside the receptor cavity of Nsp15 endoribonuclease.

Figure 7. The binding mode of glycyrrhizic acid (CID_14982), dehydroglyasperin C (CID_480775), liquiritin (CID_503737), glyasperin A (CID_5481963), isoliquiritinapioside (CID_6442433), licochalcone D (CID_10473311), Lopinavir and Ribavirin in the active site of Nsp15 endoribonuclease of the SARS-CoV-2.



Molecular dynamics simulation

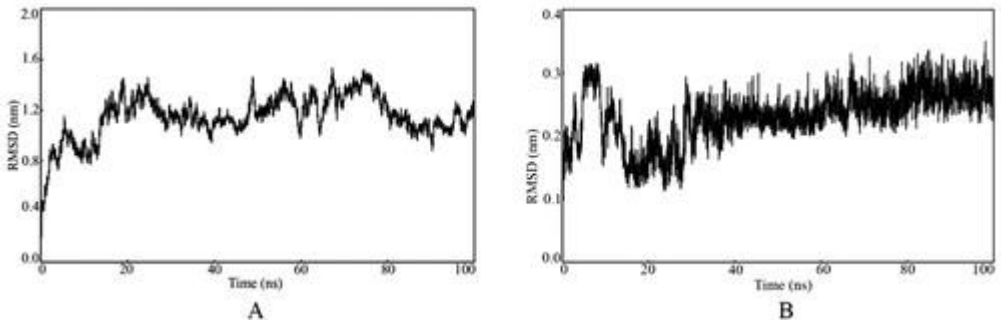
In order to check the stability of glycyrrhizic acid-glycoprotein and glyasperin A-Nsp15 Endoribonuclease complexes, an all-atoms MD simulation of time span of 100 ns was performed. On successful completion of the MD simulation the entire trajectory of each complex was considered to explore the RMSD, RMSF and Rg. The average, maximum and minimum value of RMSD, RMSF and Rg were obtained from the entire frames and given in Table 4.

Table 4. Maximum, minimum and average values of RMSD, RMSF, Rg and MM-PBSA based binding free energy of final proposed molecules complex with SARS-CoV-2 protein(s).

Root mean-squares deviation (RMSD)

The protein backbone RMSD is one of the critical parameters obtained from the protein-ligand complex which gives the overall information about the stability and insight into the structural conformation in the dynamic states during the MD simulation. The system equilibration in terms of stability can be explained through the RMSD analysis. The lower range of RMSD along with consistent variation throughout the simulation can be inferred the stability of the protein backbone. On the other hand, the higher RMSD and (or) high fluctuation to the native structure indicates comparatively low stability of the protein-ligand complex. It is always preferable to accept the protein biomolecule with the lower range of RMSD but higher deviated RMSD can also be acceptable which might indicate that the protein is probably undergoing large or some sort of conformational change during the simulation. The RMSD of each frame for both complexes was calculated and it is given in Figure 8. The average, maximum and minimum RMSD values of both complexes are given in Table 4.

Figure 8. Protein backbone vs time of MD simulation. **A:** Spike (S) glycoprotein bound with glycyrrhizic acid. **B:** Nsp15 endoribonuclease bound with of glyasperin A.

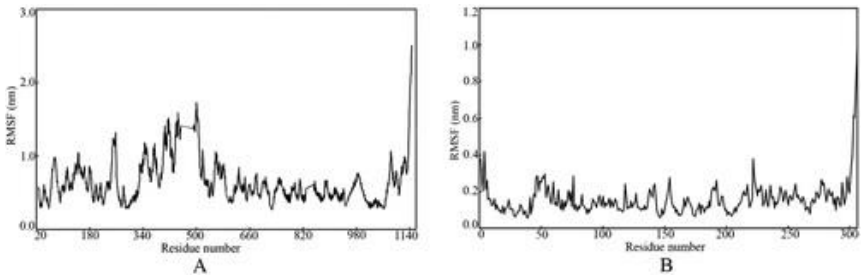


The average RMSD value was found to be 1.149 and 0.231 nm for the backbone of spike glycoprotein and Nsp15 endoribonuclease respectively. It is also important to note that not a single frame of glycoprotein and Nsp15 endoribonuclease backbone was deviated higher than 1.531 and 0.350 nm in comparison to the respective native structure when bound with glycyrrhizic acid and glyasperin A, respectively. On close inspection, it can be seen that spike glycoprotein backbone bound with glycyrrhizic acid fluctuated from beginning to about 20 ns and afterwards it was equilibrated until the end of the simulation. A similar pattern was also observed in case of Nsp15 endoribonuclease backbone bound with glyasperin A. Above observation clearly explained the stability of the protein-ligand complexes during the all-atoms MD simulation.

Root mean-squares deviation (RMSF)

The individual amino residue in the protein-ligand complex plays a critical role in complex stability. The fluctuation of the amino residues can be inferred by the RMSF parameter which explains the average deviation of each amino residue over time from the reference position. More precisely it can be said that it analyzes the specific part of the protein structure that are fluctuating from its mean structure. The amino acid or group of amino acids with high RMSF value indicate the greater flexibility attained by the complex, whereas lower RMSF indicates lesser flexibility for the complex. The RMSF of individual amino residues of both glycoprotein and Nsp15 endoribonuclease bound glycyrrhizic acid and glyasperin A is given in Figure 9. The average, maximum and minimum RMSF values are given in Table 4.

Figure 9. The RMSF of individual amino acids. **A:** Spike (S) glycoprotein bound with glycyrrhizic acid. **B:** Nsp15 endoribonuclease bound withglyasperin A.

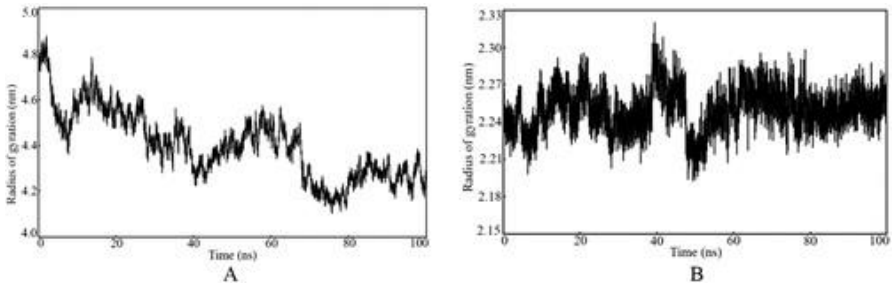


The average RMSF was found to be 0.634 and 0.148 nm for spike glycoprotein and Nsp15 endoribonuclease, respectively. In case of spike glycoprotein bound with glycyrrhizic, the amino acids were found consistent except in the range 200 to 500. The fluctuation of the above amino acids might be due to lack of inter- and intra-molecular binding interactions. On the other hand, the RMSF plot of amino acids belongs to the Nsp 15 endoribonuclease bound with glyasperin A was found consistent. Due to the free end of both proteins, the end amino acid was found to fluctuate highest in comparison to others.

Radius of gyration (Rg)

The compactness and rigidity of the protein-ligand complexes can be assessed through the Rg parameter obtained from MD simulation trajectory. It can be defined as the mass-weighted root-mean-square distance of a collection of atoms from their common center of mass (Baig et al., 2014). Therefore, the overall dimensions and the alteration in the macromolecular structure during the MD simulation can be explored by the Rg parameter. The Rg values for each frame of both complexes were calculated and plotted against the time simulation, and it is given in Figure 10. Moreover, to explore in more details the average, maximum and minimum Rg values were calculated and showed in Table 4.

Figure 10. The Rg values of each frame plotted against the time of simulation. **A:** Spike (S) glycoprotein bound with glycyrrhizic acid. **B:** Nsp15 endoribonuclease bound with glyasperin A.

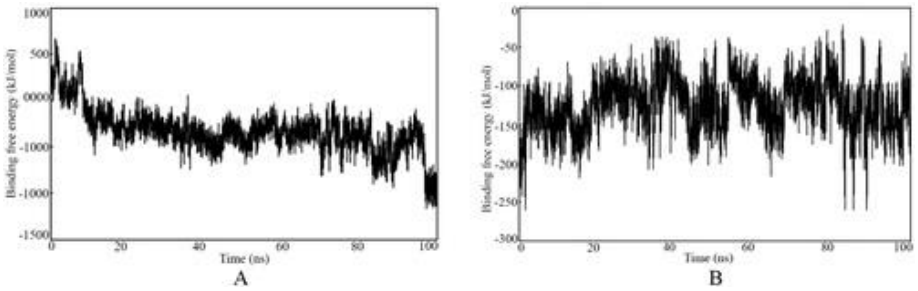


The Rg value was varied from 4.104 and 4.877 nm, and 2.193 and 2.320 nm for the spike glycoprotein and Nsp15 endoribonuclease, respectively. The Rg parameter of the spike glycoprotein system bound with glycyrrhizic acid was gradually decreasing from about 4.8 nm and finally equilibrated around 2 nm. In the case of Nsp15 endoribonuclease bound with glyasperin A, the Rg was consistent throughout the simulation except for few fluctuations around 40 ns. Hence, the trend of Rg plot against the time simulation and low fluctuation of the same undoubtedly explain that residual backbone and folding of both spike glycoprotein and Nsp15 endoribonuclease have remained consistent after binding with glycyrrhizic acid and glyasperin A, respectively.

Binding free energy through MM-PBSA approach

The binding energy calculated through the MM-PBSA approach is considered to be more accurate than binding energy calculated by any other means including molecular docking. The binding energy of any small molecule can give an idea about the affection towards the macromolecule. To explore the affinity of both glycyrrhizic acid and glyasperin A towards the glycoprotein and Nsp15 endoribonuclease respectively, the binding free energy was calculated from the entire trajectory of MD simulation through the MM-GPBSA approach. Higher negative binding energy explains more affinity towards the receptor. The binding free energy of each molecule was plotted against the time of simulation and it is given in Figure 11. The average, minimum and maximum binding energy of all frames were also calculated and it is given in Table 4.

Figure 11. **A:** Binding free energy of glycyrrhizicacidbound with Spike (S) glycoprotein. **B:** Binding free energy of glyasperin Abound with Nsp15 endoribonuclease.



From Table 4, it can be seen that average binding free energy was found to be −331.723 kJ/mol and −124.036 kJ/mol for glycyrrhizic and glyasperin A, respectively. In the case of glycyrrhizic, few frames at the beginning of the MD simulation were found with positive binding energy but after about 7 ns all frames showed high negative value. New orientation and conformation gained by the complex after 7 ns might be the reason for change binding energy of the molecule. The binding free energy of glyasperin A was found to be almost constant throughout the simulation. Not a single frame was found to have positive binding free energy. Hence, the binding energy of both molecules suggests that glycyrrhizic and glyasperin A possess a strong affinity to inhibit the spike glycoprotein and Nsp15 endoribonuclease, respectively.

Conclusion

The pharmacoinformatics approaches such as molecular docking and MD simulation studies were carried out to explore a set of molecules belong to the natural products. All the selected molecules including two anti-viral drugs, Lopinavir and Rivabirin were docked in the COVID-19 targets such as spike glycoprotein and Nsp15 endoribonuclease. The binding energies from the molecular docking study and binding interaction were explored in details. Several crucial amino residues were found to interact with all the molecules. A total of six phytochemicals were found promising compounds based on comparative analysis of binding interactions and binding energy with Lopinavir and Ribavirin against spike glycoprotein and Nsp15 endoribonuclease. Further, high binding energy scored one molecule from each of spike glycoprotein (glycyrrhizic acid) and Nsp15 endonuclease (glyasperin A) were used for the MD simulation in complex with the respective target molecule. Many parameters were calculated from the MD simulation and found that both molecules were retained inside the protein in the dynamic states. Finally, the binding free energy of both molecules was calculated from the MD simulation trajectories. High negative binding free energy value of both molecules substantiated their strong affinity towards the target molecule. It can be concluded that that glyasperin A might block the Nsp15 endoribonuclease activity with uridine specificity and glycyrrhizic acid connect well with the widespread binding pocket of spike glycoprotein due to its bulky nature. Moreover, it can be said that the glycyrrhizic acid disturbed the connection of the virus with ACE-2 receptor at entry-level and after entry into host cell glyasperin A inhibits the replication process of the virus. Hence, both proposed molecules might be important molecules to control the COVID-19 subjected to experimental validation.

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Abstract

The TCM protocol in the Diagnosis and Treatment Protocol for corona virus disease 2019 (COVID-19) (Trial Version 7) has been updated from previous versions. The protocol was formulated under the direct leadership of the National Administration of Traditional Chinese Medicine, based on the experience of a panel of experts, supported by evidence from fever clinics and from the outcomes of emergency (EM) observation rooms and inpatients throughout China (especially in Wuhan, Hubei Province) in combination with the latest scientific research results and data. The present interpretation of the TCM protocol is based on an overall understanding of the revised content, and aims to guide and standardize its clinical application to provide a reference for clinicians.

71. Sun P, Zhou WS. Acupuncture in the Treatment of COVID-19 : An Exploratory Study. 2020;(June):1–7. Journal of Chinese Medicine | Issue123 | June 2020 Acupuncture in the Treatment of COVID-19: An Exploratory Study. Peilin Sun & Wen Sheng Zhou

Abstract

The coronavirus COVID-19 has presented a serious new threat to humans since the first case was reported in Wuhan, China on 31 December 2019. By the end of February 2020 the virus has spread to 57 countries with nearly 86,000 cases, and there is currently no effective vaccination available. Chinese herbal medicine has been used in this epidemic with encouraging results, but with concerns regarding disturbance of patients' digestive function. This study aims to explore the role of acupuncture in treating COVID-19 by investigating relevant current literature along with classical Chinese medicine texts on epidemics. Based on this analysis, acupuncture points and strategies are suggested for practitioners to use as a guide to treatment.

Keywords Coronavirus, COVID-19, acupuncture, Chinese medicine, ghost points, infection, epidemic, pandemic.

Introduction

Coronaviruses (CoV) can cause severe diseases like severe acute respiratory syndrome (SARS-CoV) or Middle East respiratory syndrome (MERS-CoV). The first case of a novel zoonotic coronavirus (nCoV) was reported in Wuhan, China on 31 December 2019 and it now presents a serious threat to humans. One month later, nCov became a global emergent health issue and was renamed COVID-19 by the World Health Organization (WHO). By 29 February (the time of writing), 38 days after the lockdown in Wuhan, China has 79,394 reported cases and 2,838 deaths, while 85,641 cases have been reported globally across 57 countries with 2,933 deaths.1 Among those infected, 20 per cent are in intensive care. The WHO has already released 675 million dollars to help combat this global emergency, to cover the period from February to April, and has also gathered 300 top healthprofessionals internationally to develop a vaccine before COVID-19 becomes pandemic. Despite Chinesemedicine experts apparently not being included in these efforts, in reality many studies from affected hospitals in China have reported that Chinese medicine has been playing an important role in the battle against COVID- 19.5,6

Disappointingly, according to some Chinese medical academics, in some locations acupuncture has not featured as a treatment throughout the course of patients' infection, but only during the recovery period.7 Chinese medicine has a recorded history of over two thousand years of combating epidemics, with acupuncture playing a vital role alongside herbal medicine. For instance, Wu Youke (1580-1660) in his text Zhen Jing (Acupuncture Canons) pointed out how infectious qi attacks the human body via the mouth and nose and then penetrates inwards, as well as noting which acupoints should be employed in treatment.8 This study provides acupuncture strategies to treat COVID-19 and is based on both classical Chinese medicine theory and current literature. The aim of this article is to shed new light on this urgent health struggle, and to help acupuncture practitioners contribute to their local communities. Clinical manifestations of COVID-19 Chen et al.9 and Wang et al.10 each reported a case series based in two separate hospitals 10 miles apart in Wuhan during January 2020, which included a total of 237 subjects presenting with COVID19. Chen et al.'s study documented infected patients as presenting with signs of fever (83 per cent) and cough (82 per cent), followed by dyspnoea (31 per cent), confusion (11 per cent) and headache (8 per cent), while 1 to 5 per cent of patients exhibited sore throat, rhinorrhoea, chest pain, diarrhoea, nausea and vomiting; 68 per cent of patients were male, 51 per cent experienced chronic illness and 75 per cent developed bilateral pneumonia. Wang et al.'s study reported the main symptoms as being fever (98.6 per cent), fatigue (69.6 per cent), dry cough (59.4 per cent), myalgia (34.8 per cent) and dyspnoea (31.2 per cent), with 54.3 per cent of the patients being male. Most of the patients in the two studies received antibiotic and antiviral treatments. The authors concluded that hypertension, diabetes, cardiovascular illness and malignancy are common comorbidities of COVID-19. Wang et al. point out that the best approach to COVID-19 is to avoid becoming infected in the first

place, as the medication currently available is ineffective. A few points are worth drawing out from these two reports:

• There are big differences in the major clinical manifestations of the illness as outlined in the two studies.
• A fair number of patients showed atypical symptoms, such as diarrhoea and nausea. • Major complications appeared during hospitalisation, such as acute respiratory distress syndrome, arrhythmia and shock (Wang et al.).
• Traditional Chinese medicine texts that describe how epidemics have been fought through Chinese history can be used to address these points.

The Chinese medicine understanding of epidemic disease. The original Chinese term for epidemic, li yi (戾疫, literally 'ferocious epidemic'), has a recorded history of over two thousand years. In 524 BCE, the Zhou dynasty king Jing was admonished for his luxurious lifestyle, which he was advised put him at risk of contracting li (戾, ferocious qi).11 Mozi (4th Century BCE) also mentions li yi, which was interpreted by Johnston (2010) as 'pestilence and plague'.12 Large-scale epidemics have emerged in China dozens of times since the beginning of the first millennium, often occurring in cold and damp years as defined by five-phase philosophy.13 A large number of Chinese medicine scholars produced doctrines during or after such disasters that in time became famous. Zhang Zhong jing (150-129), who suffered the loss of many family members, composed the Shang han lun (Treatise on Cold Damage) in which he expounded that cold, wind or damp can invade the human body, penetrating from the yang channels of the outer body inwards the yin channels or organs. He stated that li yi is acute and infectious, its symptoms develop much swifter than typical shang han (cold damage), and can easily progress to a critical - even fatal - stage. It is important for physicians to intervene accurately and rapidly in such diseases to reverse the patient's situation. Prior to the Ming dynasty, most Chinese m e d i c i n e s c h o l a r s believed li yi to be caused by cold, but this idea was challenged by Ming scholars such as Wu Youke after experiencing several epidemics that swept China, such as in 1641. Wu argued that cold only presents in winter, whereas warm epidemics (wen yi 温疫) can present in all seasons, and that li yi represents an extremely merciless exogenous qi that differs from the usual six exogenous forms of qi. Wu thought any acute epidemic disease related to unseasonable warmth, and should be treated with herbal medicine. He condemned some medical professionals for mistaking epidemic yi qi for shang han and therefore failing in their duty to adequately treat patients.

Warm disease theory was to cause many controversies; for instance, Qing dynasty scholars Ye Lin and Li Guanxian thought Wu may have confused his idea of warm disease with epidemic disease due to the phonetic similarity of their characters (温 warm and 瘟 epidemic – a character that did not exist in ancient Chinese).15,16 However, 瘟 was not only shown as an entry in the Chinese rhyme dictionary Jiyun (1037)17 but was also actually specifically identified and annotated by Wu in the chapter on Miscellaneous Qi in his text Wen yi lun (Treatise on Warm Epidemics).18 This warm versus cold controversy has still not been resolved, including Wu's conclusion that herbal medicine is the only cure for epidemic disease. Regardless of whether or not Wu was correct on this point, the influence of the climate in Wuhan on the recent spread of COVID-19 can be understood using his theory. The Chinese medicine academic Tong19 as well as many other Chinese medicine scholars believe that the climate in Wuhan in December 2019, with its continuous rain and abnormal warmth, led to the epidemic by fostering cold and damp qi that impairs human yang qi, particularly in the Lung and Spleen. The facts on which this understanding is based are: 1. Patients mostly complain of fatigue, poor appetite, nausea, vomiting, fullness, diarrhoea or constipation, which points to damp-cold affecting the Spleen and Lung. 2. Patients' tongue coatings are very thick and greasy (described as fu tai 腐苔, a tongue coating that looks like rotten bean curd), indicating heavy dampness and turbidity. Tong and his team have drafted a four-stage differentiation and a treatment protocol, as follows: 1. Damp-cold stagnating the Lung; 2. Epidemic toxicity blocking the Lung; 3. Visceral obstruction causing collapse; 4. Lung and Spleen deficiency. Subsequently, Wang et al.,20 Ma et al.21 and Chen et al.22,23 have expanded on this general outline with detailed herbal prescriptions to be tailored to individual patients' conditions. The primary principles of

treatment are to warm yang, disperse cold and eliminate damp. Hubei medical experts characterised the COVID-19 virus as 'loving cold and being afraid of warmth'.

The role of acupuncture in treating COVID-19 Somewhat dispiritingly, while scholars have delivered strategies for herbal medicine, acupuncture treatment has received little attention. This article aims to explore the feasibility of acupuncture treatment for COVID-19-infected patients, and is based on published herbal strategies for this disease as well as Chinese medicine theory. Professor Sun, co-author of this article, has been practising Chinese herbal medicine and acupuncture in Europe for over 40 years. This analysis is based on his empirical observations of what is more likely to suit European patients. All Chinese medicine academics emphasise that this epidemic is characterised by damp, cold and toxicity, which easily lead to heat and stasis. Professor Sun underlines that the key to treatment is to identify whether the pattern is one of damp-cold toxin causing heat or heat-toxin mixing with damp. In patients with constitutional yang excess, dampcold accumulation can quickly turn to damp-heat. In such cases, the treatment principle should be to eliminate dampcold whilst simultaneously clearing heat. Heat toxin mixed with damp represents a different scenario; even though damp is also present, the root treatment is to clear heat and remove toxin, whilst additionally eliminating damp. If the former pattern is mistaken for the latter when treating with herbal medicine, then damp toxin could be aggravated further. In general, the primary treatment principles should be to boost Stomach and Spleen qi while at the same time 1) dispersing cold and scattering damp, 2) eliminating inner toxic qi by venting the exterior, and 3) increasing qi to eliminate turbidity. Epidemic qi attacks the body rapidly and violently, therefore clinical features can change dramatically and vary significantly between cases. Severe symptoms can develop within just a few days. The following clinical possibilities should be borne in mind: • Once damp-cold becomes significant, it can: a) block the Lungs causing dypnoea; b) attack the Pericardium causing chest tension, nausea, cold sweat and shock; c) cause Kidney yang failure, inducing haematuria, dehydration, abnormal urination and weight loss; and d) damage the Stomach and Spleen, leading to vomiting and diarrhoea. • Once damp-cold turns to heat, it will occlude the Lungs and yangming (Stomach and Large Intestine) resulting in fever, coughing, chest tension and shortness of breath, fatigue, poor appetite, nausea, vomiting, bloating, diarrhoea or constipation, eventually destroying the body's yin and evolving into endogenous wind syndrome. How can one avoid contracting such a ferocious epidemic virus? The Nei jing (Inner Classic) provides the answer: people with strong zheng (upright) qi will avoid the worst effects of epidemic infection despite the fact that everybody, no matter their age or gender, may be affected.24 Because each individual has a different physical constitution, the manifestations of the disease will vary, and so a single herbal prescription cannot be universally effective for every patient. Acupuncture is conducted with patients on a one-to-one basis, and is oriented more to provide symptomatic relief than the generic herbal decoctions applied during epidemic periods. The relevant acupuncture protocols, based on the Chinese government four-stage differentiation scheme for treating COVID-19, are outlined below. Suspected infection period Invasion of the Lung by damp-cold: beginning of infection with fever, chills, joint and muscle pain, fatigue, sore throat, bitter taste in the mouth, dry throat, a pale tongue with thin white coating and a slightly rapid floating pulse. Acupuncture prescription:

• Lieque LU-7 + Zhaohai KID-6, Waiguan SJ-5 + Zulinqi GB-41 with even method.

• Hegu LI-4, Fengchi GB-20, Zhigou SJ-6, Neiguan P-6, Feishu BL-13, Yanglingquan GB-34, Zhongwan REN-12, Fenglong ST-40 and Zusanli ST-36 with reducing method.

Damp-cold obstructing the Spleen: gastrointestinal discomfort, possibly fever, muscle pain, nausea, vomiting, diarrhoea, abdominal distension, fatigue, a pale tongue with a white greasy coating and a deep-slow or deep-delayed (chen-chi) pulse. Acupuncture prescription:

• Waiguan SJ-5 + Zulinqi GB-41, Neiguan P-6 + Gongsun SP-4 with even method.

• Zhigou SJ-6, Neiguan P-6, Feishu BL-13, Yanglingquan GB-34, Zhongwan REN-12, Fenglong ST-40, Tianshu ST-25, Yinlingquan SP-9, Zusanli ST-36 with even method.

Clinical treatment period Initial stage: Damp-cold occluding the Lung: possible fever, dry cough, bitter taste in mouth, dry throat, fatigue, chest tightness, nausea and/or vomiting, loose stools, pale or reddish tongue with white greasy coat, and a floating-soft pulse. Acupuncture prescription:

• Lieque LU-7 + Zhaohai KID-6, Neiguan P-6 + Gongsun SP-4, Waiguan SJ-5 + Zulinqi GB-41 with even method. Hegu LI-4, Chize LU-5, Zhongwan REN-12, Yanglingquan GB-34, Zusanli ST-36 and Qiuxu GB-40 with reducing method.

Intermediate stage: Damp obstructing the middle and upper burner: Cough, white or yellowish sputum, rough expectorate, chest tightness, shortness of breath, stomach distension, nausea, abdominal bloating, poor appetite, loose stools, pale or reddish tongue with a white greasy coat and a floating-soft or weak pulse. Acupuncture prescription:

• Lieque LU-7 + Zhaohai KID-6, Neiguan P-6 + Gongsun SP-4, Waiguan SJ-5 + Zulinqi GB-41 with even method.

• Chize LU-5, Feishu BL-13, Yuji LU-10, Zhongwan CV-12, Fenglong ST-40, Yanglingquan GB-34, Zusanli ST-36, Tianshu ST-25 and Qiuxu GB-40 with reducing method.

Epidemic toxin obstructing the Lung, heat dropping into yangming: High fever, cough with yellow sputum, chest tightness, shortness of breath, panting, wheezing on exertion, bloated lower abdomen, constipation, red tongue with yellow greasy or dry coat and a slippery and rapid pulse. Acupuncture prescription:

• Lieque LU-7 + Zhaohai KID-6, Neiguan P-6 + Gongsun SP-4 with even method.

• Chize LU-5, Feishu BL-13, Shanzhong REN-17, Yuji LU-10, Hegu LI-4, Quchi LI-11, Tianshu ST-25, Fenglong ST-40 and Neiting ST-44 with reducing method.

Severe stage: Internal obstruction causing collapse, yin and yang separating: Severe breathing difficulty, asphyxia (mechanical ventilation may be needed), unconsciousness, restlessness, sweaty and cold extremities, a dark purple tongue with thick or dry coat, and a large floating rootless pulse. Acupuncture prescription:

• Baihui DU-20, Guanyuan REN-4, Qihai REN-6, Zusanli ST-36, Feishu BL-13, Shanzhong REN-17 and Sanyinjiao SP-6 with reinforcing method, and moxibustion on Guanyuan CV-4 and Qihai CV-6.

• Tianshu ST-25 and Fenglong ST-40 with reducing method.

Recovery stage: Lung and Spleen qi deficiency, deficiency of yuan (original) qi: Shortness of breath, fatigue, poor appetite, nausea, abdominal distension and fullness, asthenictype constipation, sticky loose stools, a pale swollen tongue with a greasy white coat and a deep, slow pulse. Acupuncture prescription:

• Guanyuan REN-4, Qihai REN-6, Zusanli ST-36 and Taixi KID-3 with reinforcing method and moxibustion at Guanyuan REN-4 and Qihai REN-6.

• Feishu BL-13, Pishu BL-20 and Shenshu BL-23 with reinforcing method.

Qi and blood deficiency, Liver and Kidney yin deficiency: Shortness of breath, fatigue, poor appetite, insomnia, asthenic-type constipation, flushing, night sweats, dry mouth, restless, dizziness, weak knees, scanty urine, a pale red tongue with scanty or flaking coat, thin and weak pulses. Acupuncture prescription:

• Guanyuan REN-4, Qihai REN-6, Zusanli ST-36, Sanyinjiao SP-6, Taixi KID-3, Yingu KID-10 and Ququan LIV-8 with reinforcing method.

• Taichong LIV-3 and Neiguan P-6 with even method.

Acupuncture point categories

The acupuncture prescriptions in this article are based on published papers on the Chinese medicine treatment of COVID-19, Chinese state-broadcast information combined with the classical Chinese medicine theory that has been used to deal with numerous epidemics over thousands of years. However, acupuncture is by its very nature an individualised therapy. Practitioners must take into consideration each patient's individual condition and constitution, and tailor the above prescriptions accordingly. It is recommended that practitioners bear in mind the following point categories during treatment. Ghost points There are many methods of acupuncture practised globally, each of which has its main focus area, such as musculoskeletal issues or emotional problems.13 Ghost point needling is one method that is relevant in the treatment of epidemics. Before the 1st century CE, the term yi (疫) was associated with ghosts or demons (gui 鬼), as in the term yi gui which appears in Lunyu (Analects of Confucius) by Confucius (551–479 BCE), where it is often rendered 'hungry ghost' in the English translations of the text.25 Uncontrollable infectious diseases - li yi - were at this time regarded as evil qi and associated with ghosts.26 Shaman doctors commonly used the term gui (ghost) as a term for unexplained illness and disease. Of course, symptoms of mental illness can be part of the clinical presentation during acute infectious disease. Ghost points initially appeared in the Shang dynasty (1520-1030 BCE) and

were used for fighting epidemics in the Zhou dynasty (1030-727 BCE), for example for treating coma during the Warring States Period (475-221 BCE). These points were later recommended by Sun Simiao (581- 682) for treating mental-emotional illness such as madness.27 The concept gui (ghost) should be seen within its historical context. Throughout most of the Han-Tang period, frequent wars and epidemics resulted in great social and economic stress.28 Cao Zhi (192-232 CE), the prince of the state of Wei expressed his grief for friends who died during an epidemic in 217 CE in Shuo yi qi (Speaking Epidemic Qi), and also described people's devastation and hopelessness during that disaster.8 The 'ghost qi' from unseasonable weather thus not only caused deadly epidemics but also consequent fear. That is, people have more anxiety during epidemic periods whether they become infected or not - which the needling of the ghost points can effectively counter. Unfortunately, since the systematic development of Chinese medicine in the Han dynasty and the cultural revolutions in the 20th century, medical scholars began to avoid use of practices involving the term 'ghost' in order to distant themselves from shamanism.15 Some modern authors believe that ghost points are actually related to the treatment of yin fire.29

Obviously, when yin and yang separate, yang floats upwards and outwards; this creates a volatile, unbalanced state, which ferocious epidemic qi can easily take advantage of (and might explain why certain body types are infected more easily than others). However, it is the opinion of the authors of this article that these points can still be valid in the fight against today's epidemic threat, especially Shaoshang LU-11 and Yinbai SP-1, which can be applied throughout the whole treatment period of COVID19. They are both jing-well points and are located at the end of the Lung and the beginning of the Spleen channels (the major organs primarily attacked by COVID-19). Their functions are as follows:

• Shaoshang (Lesser Shang, also known as Gui Xin, Ghost Trust) LU-11: Clears the Lung and purges fire, expels evil, treats cough and dyspnoea due to exogenous pathogenic qi occluding the Lung, as well as sore and swollen throat, nasal congestion and epistaxis.

• Yinbai (Hidden White, also known as Gui Lei or Ghost Fortress) SP-1: The Zhenjiu Jiayi jing (Systematic Classic of Acupuncture & Moxibustion) recommends this point for treating dyspnoea, asthma, abdominal distension, heat and fullness in the chest, violent diarrhoea, dyspnoea when lying supine, cold feet, epigastric glomus, nausea and vomiting, and poor appetite. Confluent points The confluent points of the Extraordinary Vessels are located in the limbs and can be exceptionally effective for opening the channels and easing body tension. However, they should be treated in a strict order.30 The upper burner organs are the first targeted and obstructed by epidemic qi, followed by the middle burner and finally the lower burner. Therefore for acute infection by COVID-19, opening up the blockage in the upper burner should be the first priority. The actions of these points can be summarised as follows:

• Lieque LU-7 and Zhaohai KID-6: regulate qi and blood in the chest, thorax and upper abdomen, and balance the Ren Mai (Conception Vessel) and Yin Qiao Mai (Yin Motility Vessel)

• Neiguan P-6 and Gongsun SP-4: regulate abdominal qi and blood, and balance the Chong Mai (Penetrating Vessel) and the Yin Wei Mai (Yin Linking Vessel)

• Waiguan SJ-5 and Zulinqi GB-41: Release exterior tension and clear heat from the Liver and Gall Bladder, harmonise Yang wei mai (Yang Linking Vessel) the Dai Mai (Girdle Vessel) and Shao Yang channel collaterals.

Conclusion

This is a public domain data-based exploratory study which has limitations in terms of having no empirical evidence. Despite this, it is based on review and analysis of extensive documentation. As currently there is no cure for or vaccination to prevent COVID-19, exploring possible therapies to contribute to this recent global health crisis could prove vital. While results from Chinese herbal treatment in this area have been encouraging, they have involved unintended consequences, such as disturbing patients' Stomach and Spleen function. Historically, acupuncture has been used effectively to treat epidemic infectious diseases, and despite historical neglect, it could become a crucial weapon in the battle against COVID-19 and other future epidemics. Of course, practitioners should ensure that they are properly protected when working with infected patients, which means wearing a protective suit and administering acupuncture in a hospital environment (which brings

its own challenges). Inspiring evidence of the role of acupuncture has been appearing since the beginning of March 2020. Professor Zou Xu is a critical care medical expert from Guangdong TCM hospital. As one of the supporting medical staff in Wuhan Leishenshan hospital, he always takes acupuncture needles during his ward inspections to help COVID-19 infected patients, especially those with acute symptoms such as shortness of breath, coughing, dizziness, insomnia, restlessness, palpitations, diarrhoea or vomiting. The effect of his acupuncture was often instantaneous. A 72 year old female patient with high blood pressure and diabetic chronic illness complained of a lower back ache, whereupon Zou needled the point Taixi KID-3 and the patient was able to stand upright immediately. Zou explains that acupuncture can improve the patients' oxygen supply and consumption, helping them regain yuan-original qi while blocking the toxicity attacking the Lung. Most importantly, acupuncture is not aiming to destroy the epidemic qi, but instead it can influence the conditions of its survival in the body.31 Zou's team was in charge of 16 patients, of which six patients volunteered for Chinese medicine treatment alone; as of 1st March 2020, all six have fully recovered and have been discharged from hospital.32 In another 'Report from the Front Line in Wuhan', Professor Liu Li Hong has also documented the work of his team treating patients with COVID-19 in Wuhan, emphasising the importance of acupuncture in helping patients immediately with symptoms such as stuffiness in the chest, shortness of breath, abdominal discomfort, itchy throat, cough, dizziness, pain and sweating.33 Acknowledgements Wen Sheng Zhou gives thanks to M. J. Fleming for his editorial help. Authors sincerely thank Daniel Maxwell and the Journal of Chinese Medicine for their tremendous effort in publishing this article. Peilin Sun has been engaged in clinical practice and teaching of traditional Chinese medicine for more than 40 years and has written and published many articles and textbooks on the subject. He is a professor at the Instituut voor Complementaire Zorg Opleidingen in Belgium (www. ICZO.be), visiting professor and PhD supervisor at Nanjing University of Chinese Medicine and maintains a private clinical practice in Belgium. Wen Sheng Zhou, has a BA in Chinese language and literature in China and an MSc in acupuncture from the University of Westminster (UK). She is currently studying for her PhD with Nanjing University of Chinese Medicine and practises acupuncture in London (UK).

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Abstract

Corona Virus Disease 2019 (COVID-19) broke out in 2019 and spread rapidly around the world. There is still no specific antiviral therapy to the current pandemic. In China, historical records show that Traditional Chinese Medicine (TCM) is effective in prevention and enhancing the resistance to pandemic with unique insights. To fight with COVID-19, National Health and Commission of PRC has recommended some TCM in the guideline, such as HuoxiangZhengqi, LianhuaQingwen ShufengJiedu and XueBijing, and actually displayed a remarkable effect in clinical treatment strategic for COVID-19. We review studies to provide an in-depth understanding into the effect of TCM, and also introduce the possible mechanism involved in COVID-19 treatment.

Background

Currently, the disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was named Corona Virus Disease 2019 (COVID-19), have been spread around the world with over 2,430,000 confirmed cases and nearly 160,000 deaths (up to 21 April). Notably, the situation is getting worse and worse.

By comparing the infection patterns of coronavirus hosts in other vertebrates, SARS-CoV-2 was found to be similar to those of SARS-CoV and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), SARS-CoV-2 could also be transmitted among humans. It can infect human cells by utilizing human angiotensin converting enzyme 2 (ACE2) as a receptor. Clinical presentation of COVID-19 is fever, fatigue, and dry cough and some patients show with nasal congestion, runny nose, inappetence, diarrhea and pneumonia on

computed tomography (CT). Some severe cases can rapidly develop into acute respiratory distress syndrome (ARDS), refractory metabolic acidosis, septic shock, and coagulation dysfunction [1].

Unfortunately, there is no current any specific antiviral treatment for patients with suspected or confirmed COVID-19. According to the experiences in the SARS treatment, National Health Commission (NHC) of the PRC recommended TCM as strategies for COVID-19 treatment. From the current results, TCM has exhibited positive effects in combating with COVID-19. This article reviews focuses on the effects of 4 TCM in COVID-19 treatment: HuoxiangZhengqi, LianhuaQingwen, ShufengJiedu and XueBijing, and summarize the mechanism of these drugs on COVID-19, to provide a deeper insight of therapeutic benefits of these TCM.

HuoxiangZhengqi ameliorate symptoms through anti-inflammatory effects

HuoxiangZhengqi (HXZQ) formula contains almost ten Chinese herbs. It is applied to traditional syndrome differentiation for cold, fever, nausea and vomiting, abdominal distension, diarrhea, also showed good effects in pediatric in dyspepsia to achieve the harmony inside and outside, the efficacy of antiemetic diarrhea. In NHC guidelines, HXZQ was recommended for clinical features with aversion to cold with no sweating, headache, full chest diaphragm, urine frequency, yellow urine, etc.

According to the report from COVID-19 patients, the rise of creatine kinase (CK) and lactate dehydrogenase (LDH) was said to be related to lung cell damage and systemic symptoms [1]. In SARS treatment, HXZQ showed good effects in improving CK, LDH and oxygenation indexes [2]. It implied that HXZQ could improve oxygenation indexes and systemic symptoms through down-regulating the level of CK and LDH, might be a possible mechanism in treating COVID-19.

COVID-19 could cause strongly immune reaction. COVID-19 patients showed that in the peripheral blood inflammatory cytokines such as, IL-2, IL-6, IL-10 and Tumor Necrosis Factor α (TNF-α) increased and $CD4^+$, $CD8^+$, $CD16^+$, $CD19^+$ and $CD45^+$ T cells were decreased [3], but an increase in Th17 cell proportion [4]. In animal model, HXZQ could regulate $CD4^+$ and $CD8^+$ cells and suppression on TNF-α level [5]. It indicates that HXZQ have the function of anti-inflammation and immune regulation in COVID-19 through suppress inflammatory factors and regulate immune response. In studies based on network pharmacology and molecular docking, researchers found that the core compounds of HXZQ such as quercetin, isorhamnetin, irisolidone, have a stronger binding ability to SARS-CoV-2 3CL (Mpro) than that of remdesivir with COVID-19. They could combine with ACE2 binding to PI3K-Akt signaling pathway to affect viral replication, thus exerting therapeutic effect on COVID-19. Which is worthy of further research and helps to provide theoretical guidance [6].

LianhuaQingwen protect lung from pneumonia via inhibiting pro-inflammatory cytokines production

LianhuaQingwen (LHQW) formula is composed of 13 Chinese herbs, which was approved in the SARS treatment in 2003. It has become an effective treatment for SARS-CoV, MERS-CoV, H_1N_1, H_3N_2, and H_7N_9. Analysis of COVID-19 treatment with LHQW indicate that LHQW could significantly relieve clinical symptoms in patients with fever, weakness, cough and reduce the course of the COVID-19 [7]. The molecular docking results showed that the key components are kaempferol, quercetin, luteolin, glycyrrhetinic acid, stigmasterol, indigo had good binding ability with SARS-CoV-2 3CL (Mpro) and ACE2, acts on COVID-19 through multiple components, multiple targets, and multiple pathways [8].

Lung, which is the target organ of COVID-19, according to the TCM theory, “damp and toxin plague” is the main cause of COVID-19 etiology, even cause fatal pneumonia. Increasing evidence points out that cytokine storm displays a key role in causing fatal pneumonia [9]. In pulmonary oxidative lesions models, LHQW could significantly reduce pathological changes, including alveolar septum thickening, capillary congestion, interstitial edema, peripheral bronchial lymphocyte infiltration and neutrophils, the mechanism might be related to the levels of malonaldehyde, LDH, glutathione peroxidase, and super oxide dismutase were

regulated by LHQW, which play significant role in pathogenesis of lung injury [10]. LHQW could inhibits the replication of SARS-COV-2 in vitro, and significantly reduced pro-inflammatory cytokines production (IL-6, TNF-α), which might mediate strong immune response or even cytokine storm [11].

In children with mycoplasma pneumoniae pneumonia, after treatment with LHQW, the $CD3^+$, $CD4^+$, and $CD8^+$ T cell subsets in patients were significantly altered, and IL-6, c-reactive protein (CRP) in serum and procalcitonin (PCT) levels significantly reduced [12]. In COVID-19 patients treat with LHQW, the total effective rate was 74.55%, and 28 patients were cured after 3 days. After 7 days of treatment, the total effective rate was 92.73% and 39 patients were totally cured, main symptoms patients experienced including fever, cough, fatigue and chest tightness were significantly reduced [7].

ShufengJiedu act on COVID-19 through multiple targets and multiple inflammatory signaling pathways

The main components of ShufengJiedu (SFJD) are polygonum cuspidatum, forsythia, radix isatidis, bupleurum root, rhizoma corydalis, verbena, reed root, liquorice. Previous research suggested that SFJD can alleviate the clinical symptoms of patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD) and shorten the hospitalization time [13]. SFJD not only had the function of inhibiting virus proliferation and anti-inflammatory, but also has certain immune regulation function. Whatever in vivo or vitro, SFJD had a function of inhibiting airway inflammatory responses via regulating NLRP3 inflammasome and then down-regulating the level of IL-18 and IL-1β which similar to these effects of oseltamivir [14].

SFJD combined with western medicine treatment in COVID-19 have been gained significant improvement in pneumonia associated symptoms [15]. The combination of SFJD and Arbidol was better than Arbidol alone in the treatment of COVID-19, which could significantly shorten the symptoms improvement time and negative conversion time of the clinical patients [16]. In deeper studies, after SFJD treatment, partial pressure of oxygen in lung tissue increased, the level of lactic acid decreased, inflammatory cytokines such as IL-1β, IL-6 and TNF-α were inhibited [17]. Latest molecular docking results showed that quercetin, kaempferol, luteolin, these core compounds in SFJD had high affinity with target proteins. Similar to LHQW, these chemical compounds involved a variety of biological processes and pathways to treat COVID-19 by combining with key target proteins IL-6, ALB, and MAPK3, which supported the clinical application with COVID-19 [18].

XueBijing injection reduce multiple organ damage caused by COVID-19 through anti-inflammation and improving immune function

With the approving for marketing in 2004 by Chinese authorities, Xuebijing (XBJ) injection has been used in H1N1, H7N9, dengue fever, MERS as well as ebola. From previous report, XBJ could antagonize endotoxin, anti-inflammation, improving immune function and microcirculation, and regulating coagulation disorders [19, 20]. COVID-19 patients often occur respiratory distress, coagulation disorders and microcirculation disorders, especially in patients with systemic inflammatory response syndrome or/and multiple organ failure, timely use of XBJ can effectively reverse the situation and reduce the fatality. Currently, Chinese researchers are now conducting a prospective analysis of the clinical efficacy of XBJ on COVID-19. Hydroxysafflor yellow A, chlorogenic acid and salvianolic acid B were major compositions in XBJ by molecular docking [21], through “multi-component, multi-target, multi-pathway” to play the role of anti-inflammatory, vascular endothelial protection and immunity. XBJ could inhibit inflammatory cytokines such as IL-1, IL-6, IL-8, IL-17 and TNF-α [22]. By increasing the Th1/Th2 ratio, XBJ injection could improve the proportion of Th1 cells in septic rats [23], promote the apoptosis of $CD4^+$ $CD25^+$ T cells (Tregs) [24, 25], and further improve the immune function.

Potential mechanism of 4 TCM in COVID-19 treatment

To date, NHC has issued 7 editions guidelines of diagnosis and treatment for COVID-19. In each edition, TCM has been recommended for COVID-19 treatment based on different stage and symptom differentiation. TCM

has shown good effects in combating with COVID-19, early intervention of TCM in COVID-19 treatment could increase cure rate, shorten disease course and reduce mortality cases. According to the guidelines, 4 TCM in this paper and main ingredients and traditional indications versus COVID-19 are as follow (Tables 1, 2).

Table 1 4 TCM recommended by guidelines of treatment for COVID-19

Table 1 4 TCM recommended by guidelines of treatment for COVID-19

From: The potential insights of Traditional Chinese Medicine on treatment of COVID-19

	Stage of disease	Symptom
HXZQ	Medical observation period	Hypodynamia with gastrointestinal upset
LHQW	Medical observation period	Hypodynamia with fever
SFJD	Medical observation period	Hypodynamia with fever
XBJ	Clinical treatment period	Several cases and critical cases

Table 2 Main ingredients and traditional indications versus COVID-19

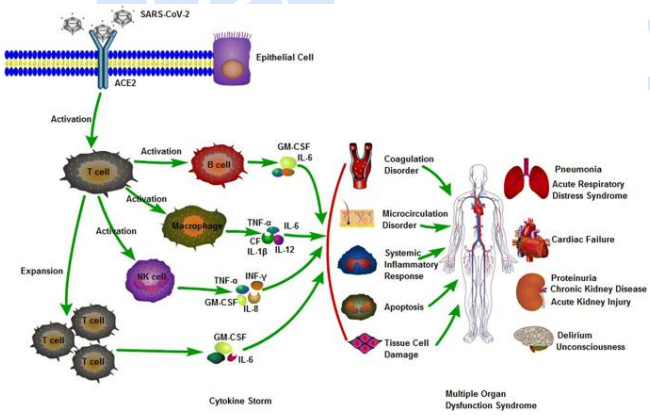
Table 2 Main ingredients and traditional indications versus COVID-19

From: The potential insights of Traditional Chinese Medicine on treatment of COVID-19

	Main ingredients	Traditional indications	COVID-19
HXZQ	Ageratum, poriacocos, perilla, angelica, orange peel, radix platycodonis, atractylodes, magnolia officinalis, pinellia, liquorice	Gastrointestinal cold, influenza and upper respiratory tract infections, viral enteritis, diarrhea	Hypodynamia accompanied by gastrointestinal upset, cold without sweating, headache and heaviness, limb pain, thirst with no desire to drink, yellow urine, frequent micturition
LHQW	Forsythia, honeysuckle, ephedra, male fern rhizome, houttuyniae, pogostemon cablin, rheum, rhodiola, menthol, liquorice	Fever, aversion to cold, muscular soreness, stuffy nose runny nose, cough, headache, pharyngoxerosis and pharyngalgia	Hypodynamia and fever
SFJD	Polygonum cuspidatum, forsythia, isatidis, bupleurum, rhizoma corydalis, verbena, reed root, liquorice	Fever, aversion to wind, pharyngalgia, headache, stuffy nose runny nose, cough	Hypodynamia and fever
XBJ	Paeoniae, angelica rhizoma Chuanxiong, flos carthami, salviae miltiorrhizae	Fever, dyspnea, palpitation, dysphoria, infection, viscera damage	Dyspnea, high fever or alternating cold and heat, cough with less phlegm, coma, etc.

So far as we know, COVID-19 could cause mortal systemic complication with strongly immune response or cytokine storm, further cause multiple organ dysfunction syndrome (MODS), which is the main cause of mortality in COVID-19 (Fig. 1).

Fig. 1



Pathogenesis of COVID-19. SARS-CoV-2 is binding to ACE2 receptor via infecting epithelial cell, with the activation of immune cell, they release a large number of cytokines, and then produce cytokine storm, resulting in MODS

TCM can regulate the inflammatory response of the body through “multi-component, multi-target, multi-pathway” to improve the immunity of the body, so as to ameliorate symptoms, prevent complications, and achieve indirect suppression of the virus. Through the prediction of molecular docking, major chemical constituents and possible targets of 4 TCM in COVID-19 were found (Table 3). According to the analysis and illumination from the latest literatures, we summarized possible mechanism and related targets of LHQW in treating with COVID-19 and showed it in Fig. 2.

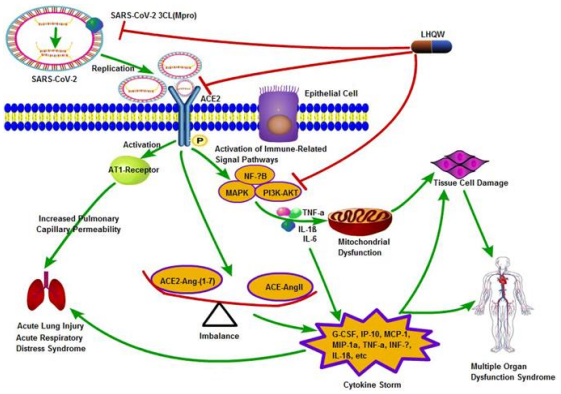
Table 3 Major chemical constituents and possible targets of TCM in COVID-19

Table 3 Major chemical constituents and possible targets of TCM in COVID-19

From: The potential insights of Traditional Chinese Medicine on treatment of COVID-19

	Major chemical constituents	Possible targets	References
HXZQ	Quercetin, isorhamnetin, irisolidone, kaempferol, wogonin, baicalein	PTGS2, HSP90AB1, CAMSAP2	[5, 6]
LHQW	Kaempferol, quercetin, glycyrrhetinic acid, stigmasterol, indigo	SARS-CoV-2 3CL (Mpro), ACE2, MAPK, PI3K-AKT, NF-κB	[8]
SFJD	Quercetin, kaempferol, luteolin	IL-6, ALB, MAPK3	[18]
XBJ	Hydroxysafflor yellow A, chlorogenic acid, salvianolic acid B	NF-κB, HIF-1, VEGF	[21]

Fig. 2



Mechanism of LHQW in treating with COVID-19. LHQW exhibit functions on COVID-19 via "multi-component, multi-target, multi-pathway". Firstly, major chemical constituents in LHQW could combine with SARS-CoV-2 3CL (Mpro), inhibiting the SARS-COV-2 replication. Secondly, there is an imbalance of ACE-Ang-II and ACE2-Ang-(1-7), which can lead to overwhelming pro-inflammatory cytokines with cytokine storm, LHQW could regulate balance. Thirdly, LHQW could regulate immune-related signal pathway (MAPK, NF-κB, PI3K-AKT, ect), reduce the production of pro-inflammatory cytokines

Conclusion

With the emergence of COVID-19, three cases of zoonotic coronavirus disease have been identified in this century. However, COVID-19 has caused more deaths to date than SARS and MERS. Accumulated experiences from thousands of years in the treatment of epidemic, TCM is worth learning. TCM has holistic therapy theory, it could balance Yin and Yang, enhancing human body resistance to eliminate epidemic factors. That's why China government recommended TCM in combating COVID-19 timely.

Although, laboratory studies on the effect of TCM are far behind the clinical application in COVID-19 treatment, further studies in molecular mechanisms are expected to clarify the effect of TCM on COVID-19. In this study, combining with the latest research, this work highlights the prospect of therapeutic effects and mechanism of 4 TCM in COVID-19 treatment. The therapeutic effects of 4 TCM in COVID-19 potentially focus on: anti-inflammatory, inhibiting pro-inflammatory cytokine production and cut off cytokine storm, regulating immune response, protecting organ damage. By the continuing expansion of this pandemic, we anticipate more and more good messages about anti-SARS-CoV-2 activity of TCM will be discovered to benefit with COVID-19 patients and finally overcome the current epidemic around the corner.

Availability of data and materials

Not applicable.

Abbreviations

COVID-19: Corona virus disease 2019

TCM: Traditional Chinese Medicine

CT: Computed tomography

ARDS: Acute respiratory distress syndrome

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

ACE2: Angiotensin-converting enzyme 2

MERS-CoV: Middle east respiratory syndrome coronavirus

NHC: National health commission

HXZQ: HuoxiangZhengqi

CK: Creatine kinase

LDH: Lactate dehydrogenase

TNF-α: Tumor necrosis factor α

LHQW: LianhuaQingwen

IL: Interleukin

CRP: c-reactive protein

PCT: Procalcitonin

SFJD: ShufengJiedu

AECOPD: Acute exacerbation of chronic obstructive pulmonary disease

ERK: Extracellular regulated protein kinases

TGF-β: Transforming growth factor-β

NF-κB: Nuclear factor-κb

MAPK: Mitogen activated protein kinase

XBJ: Xuebijing

PI3K-AKT: Phosphatidylinositol 3-kinase protein kinase B

PTGS2: Prostaglandin-endoperoxide synthase 2

HSP90AB1: Heat shock protein 90 kDa alpha B1

CAMSAP2 Calmodulin-regulated spectrin-associated proteins 2

HIF: Hypoxia inducible factor

VEGF: Vascular endothelial growth factor

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Abstract

Purpose: Huashi Baidu formula (HSBDF) was developed to treat the patients with severe COVID-19 in China. The purpose of this study was to explore its active compounds and demonstrate its mechanisms against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through network pharmacology and molecular docking.

Methods: All the components of HSBDF were retrieved from the pharmacology database of TCM system. The genes corresponding to the targets were retrieved using UniProt and GeneCards database. The herb-compound-target network was constructed by Cytoscape. The target protein-protein interaction network was built using STRING database. The core targets of HSBDF were analyzed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The main active compounds of HSBDF were docked with SARS-CoV-2 and angiotensin converting enzyme II (ACE2).

Results: Compound-target network mainly contained 178 compounds and 272 corresponding targets. Key targets contained MAPK3, MAPK8, TP53, CASP3, IL6, TNF, MAPK1, CCL2, PTGS2, etc. There were 522 GO items in GO enrichment analysis ($p < .05$) and 168 signaling pathways ($p < .05$) in KEGG, mainly including TNF signaling pathway, PI3K-Akt signaling pathway, NOD-like receptor signaling pathway, MAPK signaling pathway, and HIF-1 signaling pathway. The results of molecular docking showed that baicalein and quercetin were the top two compounds of HSBDF, which had high affinity with ACE2.

Conclusion: Baicalein and quercetin in HSBDF may regulate multiple signaling pathways through ACE2, which might play a therapeutic role on COVID-19.

Introduction

Coronavirus disease (COVID-19) was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a newly discovered coronavirus with a size of 60–140 nm, located in the SARB subgene of the

Betacoronavirus family [1]. Moreover, the population's susceptibility to this highly pathogenic coronavirus led to massive global outbreak which turned into an international public health event [2]. There is a late surge of the confirmed cases in European region and region of the Americas. As on 2:00am CEST, 18 April 2020, there have been 2,160,207 confirmed cases of COVID-19, including 146,088 deaths globally [3]. A total of 83,160 cases have been confirmed in China, with 3342 deaths and a case fatality rate of 4% by now. According to an analysis of 44,672 confirmed cases out of 72,314 reported cases by the Chinese Center for Disease Control and Prevention (CDC), the crude case fatality rate of the patients with severe COVID-19 reached 49%. The fatality rate in severe patients with acute respiratory distress syndrome (ARDS) was close to 50%, and even as high as 70% if ARDS reached moderate to severe [4]. The older people or the crowd of underlying medical problems were more likely to infect with severe symptoms. The symptoms of patients were common with fever, shortness of breath, upper airway congestion, dry cough, fatigue, sputum production, myalgia/arthralgia, and breathing difficulties [5]. Critical patients can lead to pneumonia, kidney failure, and even death [5].

No vaccine for SARS-CoV-2 has been published publicly, and there was no medication specific for the treatment of COVID-19 so far [6]. It was proved that Traditional Chinese Medicine (TCM) could obviously shorten fever duration and symptomatic relief of the patients with severe COVID-19 [7–9]. Up to now, 'three medicines and three formulas' have been proven effective in treating the COVID-19. Among them, Huashi Baidu formula (HSBDF) was used as an auxiliary medicine for the treatment of patients with severe COVID-19. HSBDF was developed by Chinese Academy of Traditional Chinese Medicine [10]. HSBDF is consisted of glycyrrhiza, apricot kernel, agastache rugosus, magnolia officinalis, atractylodes, amomum tsao-ko, pinellia ternate, poria cocos, rhubarb, astragalus, lepidium seed, red peony root, and ephedra. According to the theory of TCM, the core pathogenesis of COVID-19 was the wet epidemic caused by the cold and humidity outside the lung and spleen, which was transformed into heat and lead to heat stagnation due to the imbalance of qi mechanism and endogenous stagnated heat [11]. Agastache rugosus and ephedra in HSBDF have the functions of detoxifying dampness, clearing heat, and relieving asthma [10]. HSBDF was applied to the treatment of severe patients, and proved to be effective in resisting SARS-CoV-2, eliminating inflammation, and improving immunity [10,12].

However, the mechanism of HSBDF for the treatment of COVID-19 was not clear. HSBDF contained 14 Chinese herbs, and the components of each herb were complex. The therapeutic effect of each herb or each component was not clear.

Network pharmacology was proposed as a promising method to understand herbal formulas [13] and predict potential new drugs or targets for the specific diseases [14–16]. Molecular docking is a significant pathway of structural molecular biology and the computer aided drug design in new medicines [17,18]. Study showed that SARS-CoV-2 binded to angiotensin converting enzyme II (ACE2) receptor with nearly 10–20 times higher affinity than SARS-CoV [17]. The combination of SARS-CoV-2 and ACE2 was the main cause of COVID-19. Therefore, ACE2 and SARS-CoV-2 3CL were regarded as receptors in molecular docking. In this study, we aim to utilize network pharmacology and molecular docking to understand the active compounds of HSBDF, predict their potential targets and signal pathways, and explore the association between the active compounds of HSBDF with ACE2.

Materials and methods

Identification and screening of active compounds

Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/) was used to authenticate all compounds of the fourteen Chinese medicinal herbs in HSBDF. TCMSP had the ability to identify ingredient–target networks and the large number of herbal items. It was composed of 499 Chinese herbs recorded in the Chinese pharmacopeia with 29,384

compounds and 3311 targets [19]. The names of herbs were used as the key words to retrieve all components. We screened compounds of HSBDF based on absorption, distribution, metabolism, and excretion (ADME), which were strategic processes in drug discovery and development [20]. Oral bioavailability (OB) and drug-likeness (DL) were the most significant pharmacokinetic parameters. In ADME system [21], OB was defined as 'the degree to which active ingredients are used by the body, including the dose and rate at which they enter the bloodstream' by Food and Drug Administration (FDA) [22]. The degree of OB largely determined the effect of the compound on disease. DL was used to screen out first-rank compounds and improve candidate compounds in the early period of drug development [14]. In this study, the active compounds in HSBDF were selected according to the criterion of OB ≥30% and DL ≥0.18 [23].

Identification of protein targets

The protein targets associated with active compounds were retrieved from the TCMSP database (http://lsp.nwsuaf.edu.cn/tcmsp.php), which provided information of 6511 drug molecules and 3987 targets as well as the interactions between them [19]. As an authoritative database of protein sequences, UniProt Knowledgebase (UniProtKB) comprised 54,247,468 sequence items [24]. The targets, including the gene names and gene ID, were further extracted using UniProtKB (http://www.uniprot.org).

Construction of component–target gene network

Visual component–target network was established based on aforementioned data sets through Cytoscape 3.7.2 (http://www.cytoscape.org/) to reflect the complex relationships between active compounds and their potential targets [25]. Cytoscape 3.7.2 is an open-source software platform which is used to visualize complicated networks and integrate different types of attribute data [26]. In the network, nodes represent the screened active ingredients and targets, while the connections between the nodes represent the interactions between these biological analyses. The top eight compounds were screened as ligands for molecular docking based on the degree value. The degree value of the molecular represents the number of connections between the molecular and target in the core architecture of the network [27]. The larger the value is, the more likely the component is to become the key ingredient of HSBDF.

Predicting the targets of COVID-19

GeneCards database (https://www.genecards.org/) and Therapeutic Target Database (TTD, https://db.idrblab.org/ttd/) were used to gather the information on COVID-19-associated target genes [28]. GeneCards is a comprehensive database of functions involving proteomics, genomics, and transcriptomics [29]. The keywords 'novel coronavirus pneumonia,' 'cough,' and 'fever' were utilized to screen the COVID-19-associated targets. The names of targets were collected from TTD, which provided information about the therapeutic protein targets, the corresponding ID of each targets and the targeted disease. The common targets of HSBDF and COVID-19 were then gathered as the core targets of HSBDF for COVID-19.

Construction of protein–protein interactions (PPI) network

The core targets of HSBDF were put into STRING (https://string-db.org/cgi/input.pl) to build the PPI network interaction [30]. Cytoscape V3.7.2 [26] was used to construct and visualize the PPI network. CytoNCA, a network topology analysis plug-in in Cytoscape, was used for topological analysis of PPI networks. 'Degree' referred to the number of connections of the node in the whole network, which reflected the interaction information between nodes. The value of 'Degree' was used as a reference for the importance of the core target.

GO and KEGG pathway enrichment analysis

Webgestalt (www.webgestalt.org) was used to process data and visualize the enrichment results of Gene Ontology (GO) enrichment analysis. GO enrichment analysis included biological processes (BP), molecular functions (MF), and cellular components (CC). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed using the Rpackage 'ClusterProfifiler' [31]. Statistical significance threshold of enrichment analysis was set at $p \leq .05$.

Component–target molecular docking

The three-dimensional (3D) structure of the SARS-CoV-2 3CL (PDB ID: 6lu7) was downloaded from the RCSB PDB database (https://www.rcsb.org/). The 3D structure of ACE2 (PDB ID: 1r42) was from Qingdao Ocean Science and Technology National Laboratory 2019-nCoV drug target system structure of information sharing platform (http://ncovtarget.qnlm.ac/web/mg/hm). AutoDock Tools 1.5.6 software was used to remove the water molecules, isolate proteins, add the nonpolar hydrogen and calculate Gasteiger charges for the structure and save it as a PDBQT file. PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to download the two-dimensional (2D) structures of the top eight compounds. The 2D structure was processed and transformed into PDB format through Chem3D, and they were saved in PDBQT format as docking ligands in AutoDock Tools 1.5.6 software. SARS-CoV-2 3CL protein and ACE2 were used as receptors, and the active compounds were used as ligands. The active site of molecular docking was determined by the ligand coordinate in the target protein complex. The ligand was set to be flexible and the receptor was rigid. Autodock Vina 1.1.2 was used to dock small molecules with ACE2 protein and SARS-CoV-2 3CL protein, respectively. For results of docking, a total of 20 conformations were generated each. The conformation with the best affinity was selected as the final docking conformation and visualized in Pymol 2.3. These active ingredients were compared with lopinavir, ritonavir, and remdesivir.

Results

Active compounds of HSBDG

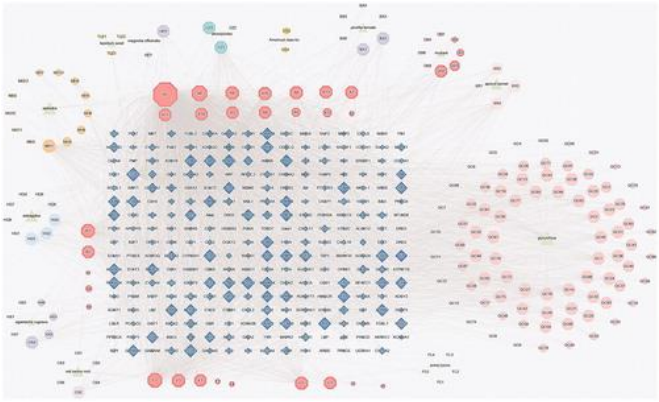
A total of 222 active compounds were retrieved in TCMSP database. These active compounds were primarily originated from glycyrrhiza (92 compounds), red peony root (29 compounds), ephedra (23 compounds), astragalus (20 compounds), apricot kernel (19 compounds), rhubarb (16 compounds), poria cocos (15 compounds), pinellia ternate (13 compounds), lepidium seed (12 compounds), agastache rugosus (11 compounds), atractylodes (nine compounds), amomum tsao-ko (eight compounds), etc. The main active components of HSBDG are shown in Table 1.

The construction of herb–compound–target network

After 44 compounds without target were removed, herb–compound–target network contained 463 nodes (including 13 herbs, 178 compounds and 272 genes) and 4081 edges (Figure 1). The larger node meant more importance. According to the degree analysis, the top eight compounds were MOL000098 (quercetin), MOL000422 (kaempferol), MOL000358 (beta-sitosterol), MOL000449 (stigmasterol), MOL000354 (isorhamnetin), MOL002714 (baicalein), MOL004328 (naringenin), and MOL000392 (formononetin), with 886°, 240°, 185°, 120°, 99°, 76°, 72°, and 72°, respectively. More details of these top eight compounds are shown in Table 2.

Figure 1. Herb-compound-target network of HSBDF (The triangle nodes are composed of all the herbs of HSBDF, which are surrounded with their particular compounds. The octagon nodes represent the

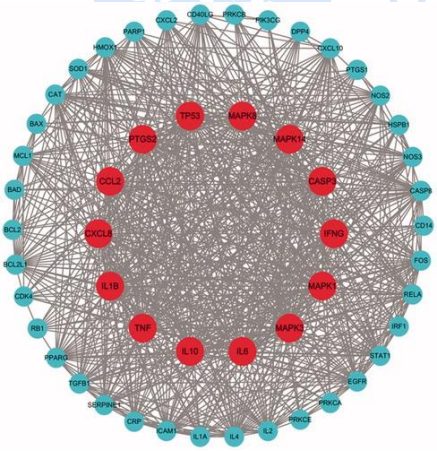
compounds of HSBDF. The rhombus nodes, arranged into a rectangular matrix, represent the relative gene targets of HSBDF).



Prediction results of virus targets and the construction of PPI network

A total of 216 possible targets of COVID-19 were screened through 'novel coronavirus pneumonia,' 'cough,' and 'fever.' These 216 targets were combined with 272 targets of HSBDF to obtain a total of 53 core targets (Figure 2, Table 3). The PPI network showed that there were strong correlations among targets and a complex interlaced network. The network contained a total of 53 nodes, which were the core targets of HSBDF in the treatment of COVID-19. Among them, MAPK3 (45), MAPK8 (44), TNF (44), IL6 (44), and TP53 (44) were considered to be hub genes (Table 3).

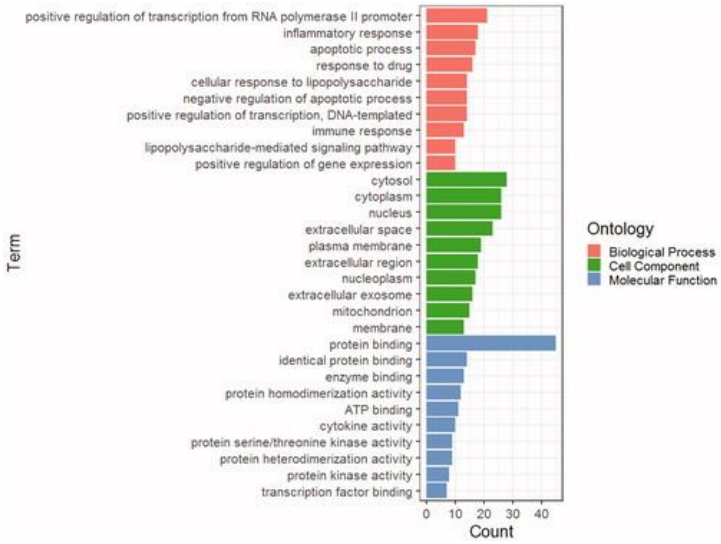
Figure 2. The PPI network of 53 nodes (genes). The larger nodes in the inner ring represent more important hub nodes. The smaller nodes in the outer ring represent the other nodes.



GO and KEGG enrichment analyze

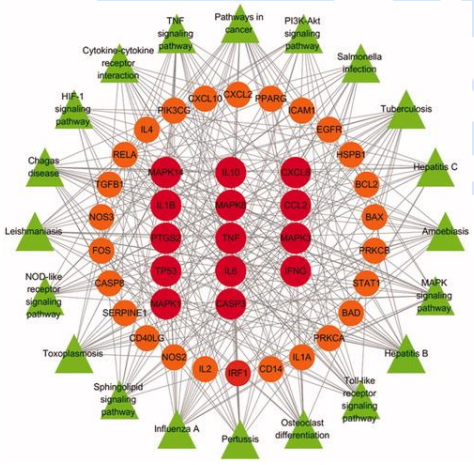
To further understand the intersection genes, GO enrichment analysis was conducted. (1) A total 328 top-ranking terms about biological processes were selected, mainly including inflammatory response, response to drug, and cellular response to lipopolysaccharide (Figure 3). The active targets essentially connected to positive regulation of transcription from RNA polymerase II promoter or apoptotic process, including IL6, TNF, and TP53. (2) According to enrichment analysis of cellular components, the targets mainly contained extracellular space, Cytosol, extracellular region, Cytoplasm, Nucleus, and so on. (3) Simultaneously, molecular function terms mainly contained protein binding, identical protein binding, enzyme binding, cytokine activity, protein homodimerization activity, etc.

Figure 3. The GO enrichment analysis of 53 nodes (BP, MF, CC).



KEGG pathway enrichment was conducted to cluster the major effects that associated to the HSBDF. A total of 20 top-ranking pathway (Figure 4) screened out ($p < .05$). The main pathway included TNF signaling pathway, PI3K–Akt signaling pathway, pathways in cancer, MAPK signaling pathway as well as some related to cancer.

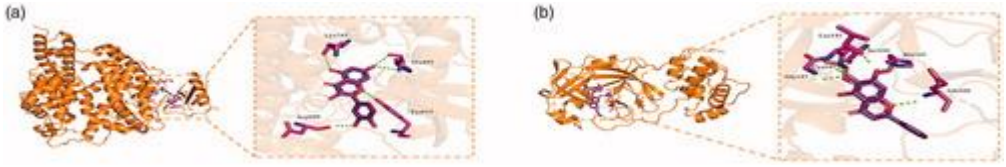
Figure 4. Target–pathway network of HSBDF. (The round nodes in the center represent the important hub nodes, and the smaller round nodes in the middle ring represent the other nodes. The outermost triangles represent the related pathways).



Results of molecular docking

Molecular docking was used to verify if the top eight compounds had a significant role in regulation of ACE2. The result showed that all the key compounds in the network had strong affinity with ACE2 protein and SARS-CoV-2 3CL protein. Baicalein (MOL002714) was the most stable active ingredient in SARS-CoV-2 3CL binding. Meanwhile, quercetin (MOL000098) showed strong association with ACE2. The small molecule quercetin showed a compact binding pattern with ACE2 protein active pocket. Quercetin formed four hydrogen bonds with the amino acid residues Lys745, Tyr613, His493, and Asp609, making quercetin and ACE2 form a stable complex (Figure 5). The binding energies of the various compounds are shown in Table 4.

Figure 5. (a) ACE2 protein-quercetin. (b) SARS-CoV-2 3CL protein-baicalein.



Discussion

In this study, we screened out eight main active components of HSBDF: quercetin, kaempferol, beta-sitosterol, stigmasterol, isorhamnetin, baicalein, naringenin, and formononetin. In molecular docking, these active ingredients were compared with lopinavir, ritonavir, and remdesivir, which were currently recommended for clinical therapeutics [32–34]. Results showed that baicalein showed strong affinity for SARS-CoV-2, and quercetin had strong affinity for ACE2 3CL. It indicated that baicalein and quercetin might play an important role in the treatment of SARS-CoV-2. Baicalein and quercetin were both flavonoids. Flavonoids reduced the barrier dysfunction induced by influenza A virus by inhibiting the NOX4/NF-κB/MLCK pathway, which might be a potential drug for the prevention and treatment of influenza A virus and pulmonary endothelial barrier dysfunction [35]. A study showed that baicalein inhibited the overactivation of the complement system in vivo and improved the acute lung injury induced by influenza a virus [36]. Since both SARS and COVID-19 were caused via binding S-protein to ACE2 [37,38], quercetin acted as a competitive antagonist to inhibit infection of SARS-CoV-2. Other research also reported that quercetin had the functions of reducing capillary brittleness, angiogenesis, detoxification, apoptosis, cell cycle, and antioxidant replication [39].

KEGG enrichment analysis showed that the key targets were mainly concentrated in TNF signaling pathway, PI3K–Akt signaling pathway, MAPK signaling pathway, HIF-1 signaling pathway, NOD-like receptor signaling pathway. The results of PPI network showed that, MAPK3, MAPK8, TNF, IL6, and TP53 were considered to be hub genes. According to these results, we think HSBDF had effect to treat SARS-CoV-2 through the following pathways. (1) TNF signaling pathway: GO enrichment analysis also showed that the major biological processes included inflammatory response in our study. TNF signaling pathway was an important pathway in inflammatory response [40], in which related factor receptors can also induce apoptosis. Quercetin acted as an anti-inflammatory by regulating the TNF signaling pathway [41]. Among the hub genes, IL6 and TNF were involved in TNF signaling pathway. IL6 and TNF were important inflammatory cytokines and commonly involved in the process of inflammation [42]. TNF induced the production of IL-6 and other cytokines, participated in the process of inflammation and oxidative stress [43]. (2) MAPK signaling pathway: Previous studies showed that MAPK signaling pathway participated in the progression of ARDS [44,45]. Many inflammatory factors such as IL-1β, TNF-α, and IL-6 were produced via MAPK signaling pathway [46]. Some anti-inflammatory medications work by targeting MAPK signaling pathway [47,48]. Quercetin was found to regulate the activation of MAPK signaling pathway in retinoblastoma, cardiomyocytes, and chorionic carcinoma cells [49]. Other studies have found that baicalin may inhibit the expression and invasion of cancer cells by inhibiting the p38 MAPK signaling pathway [50]. (3) PI3K–Akt signaling pathway: The PI3K/AKT signaling pathway regulated the activation of inflammatory response cells and the release of inflammatory transmitters to play a role in chronic inflammatory response in the lungs and airways [51]. Quercetin inhibited the PI3K–Akt signaling pathway by inhibiting the expression of AKT1 to silence the anti-apoptotic effect of lung fibroblast, thus realize the treatment of pulmonary fibrosis [52]. Baicalin also played an anti-pulmonary fibrosisrole by the PI3K–Akt pathway [53–55].

There are several limitations in our study. First, our results need to be further verified by experiments. Second, more comprehensive TCM target genes database was needed, which made the results of network pharmacology analysis more reliable. Third, even if the results of network pharmacology and molecular docking were combined, we still could not completely understand the accurate therapeutic mechanism of HSBDF. A comprehensive understanding of HSBDF and COVID-19 depended on the common development of multi-disciplines.

Conclusions

In summary, network pharmacology showed that the main active components of HSBDF, particularly baicalein, and quercetin could act on multiple targets. HSBDF had effect to treat SARS-CoV-2 mainly through the following pathways: TNF signaling pathway, PI3K–Akt signaling pathway, MAPK signaling pathway. Molecular docking showed baicalein and quercetin were the top two compounds which indicated that they might play an important role in the treatment of SARS-CoV-2.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Abstract

The outbreak of the novel coronavirus in China (SARS-CoV-2) that began in December 2019 presents a significant and urgent threat to global health. This study was conducted to provide the international community with a deeper understanding of this new infectious disease. Epidemiological, clinical features, laboratory findings, radiological characteristics, treatment, and clinical outcomes of 135 patients in northeast Chongqing were collected and analyzed in this study. A total of 135 hospitalized patients with COVID-19 were enrolled. The median age was 47 years (interquartile range, 36-55), and there was no significant gender

difference (53.3% men). The majority of patients had contact with people from the Wuhan area. Forty-three (31.9%) patients had underlying disease, primarily hypertension (13 [9.6%]), diabetes (12 [8.9%]), cardiovascular disease (7 [5.2%]), and malignancy (4 [3.0%]). Common symptoms included fever (120 [88.9%]), cough (102 [76.5%]), and fatigue (44 [32.5%]). Chest computed tomography scans showed bilateral patchy shadows or ground glass opacity in the lungs of all the patients. All patients received antiviral therapy (135 [100%]) (Kaletra and interferon were both used), antibacterial therapy (59 [43.7%]), and corticosteroids (36 [26.7%]). In addition, many patients received traditional Chinese medicine (TCM) (124 [91.8%]). It is suggested that patients should receive Kaletra early and should be treated by a combination of Western and Chinese medicines. Compared to the mild cases, the severe ones had lower lymphocyte counts and higher plasma levels of Pt, APTT, d-dimer, lactate dehydrogenase, PCT, ALB, C-reactive protein, and aspartate aminotransferase. This study demonstrates the clinic features and therapies of 135 COVID-19 patients. Kaletra and TCM played an important role in the treatment of the viral pneumonia. Further studies are required to explore the role of Kaletra and TCM in the treatment of COVID-19.

Research Highlights

83.7% of the patients had contact history in Wuhan or had been to Wuhan or had contact with people from Wuhan.

Common symptoms included fever, cough, and fatigue. Other symptoms include myalgia, fatigue, dyspnea, anorexia, etc.

Common complications of the patients include acute respiratory distress syndrome, acute cardiac injury, acute kidney injury, secondary infection and shock. ICU patients were more likely to have these complications than non-ICU patients.

Compared with non-ICU patients, ICU patients had lower lymphocyte count, and higher plasma levels of the Pt, APTT, D-dimer, LDH, PCT, ALB, CRP, AST.

All patients received antiviral therapy (kaletra or interferon), antibacterial therapy and corticosteroid and many received traditional chinese medicine. It was suggested that patients should use kaletra early.

1 INTRODUCTION

An outbreak of a series of pneumonia cases of unknown cause that began in Wuhan, China has continued since December 2019. This illness is reported to have affected more than 79 602 people so far (74 680 confirmed, 4922 suspected, 16 646 cured, and 2122 deaths). In addition to Hubei, Wanzhou, Chongqing ranks third in infection rates among all of the cities in the provinces of China. A literature review was performed, and it was found that there have been no studies regarding the characteristics of patients infected with COVID-19 in Chongqing. Clinically, the disease is characterized by fever, dyspnea, dry cough, and fatigue. Upper-respiratory tract symptoms are not prominent, but diarrhea was reported by some patients. Pulmonary imaging has shown multiple ground glass and infiltrative shadows in both lungs. Severe cases have been shown to develop into acute respiratory distress syndrome (ARDS) and septic shock. On 7 January 2019, scientists successfully isolated the pathogen that causes the pneumonia, a new type of β-coronavirus, and it was named 2019-nCoV.1 Subsequently, WHO named it coronavirus disease (COVID-19). An epidemiological survey showed that the first occurrence of COVID-19 patients was closely related to a seafood market in south China. Due to the “Spring Festival Movement” (known as the “annual migration of the population in China”), COVID-19 rapidly spread throughout the country, and the number of infected people gradually increased. The spread of COVID-19 among people has been confirmed to occur through multiple channels, such as droplets, aerosols, feces, and mouth mucus membranes.1

The aim of this study is to describe the epidemiological and clinical features, laboratory findings, radiological characteristics, treatment, and outcomes of COVID-19 patients in northeast Chongqing. It is hoped that these findings will assist the global community to more clearly understand and treat this new infectious disease.

2 METHODS

2.1 Study design and participants

This case series was approved by the Institutional Ethics Board of Chongqing University Three Gorges Hospital (also named Three Gorges Central Hospital, No: 2020-2). All of the patients were from northeast Chongqing admitted to the Chongqing University Three Gorges Hospital from 23 January to 8 February 2020 with subsequent confirmed cases of COVID-19. A total of 135 patients were enrolled in this study. Oral consent was obtained from all of the patients. The Chongqing University Three Gorges Hospital, located in northeast Chongqing, is a teaching hospital consisting of nine medical colleges and universities, including the Third Military Medical University, the Southwest Medical University, and the North Sichuan Medical College. This facility is responsible for the treatment of COVID-19 patients as assigned by the government. All of the patients with COVID-19 enrolled in this study were diagnosed according to WHO interim guidance2 and were jointly diagnosed by a multidisciplinary diagnosis and treatment team composed of infectious disease experts, respiratory medicine staff, critical care medical staff, and emergency medicine staff. The clinical outcomes (eg, discharge, mortality, and length of stay) were monitored from 8 February 2020 to the final date of follow-up.

2.2 Case collection

Clinical data of 135 COVID-19 patients were collected from 23 January 2020, when the first case in northeast Chongqing was found, to 8 February 2020. The research team collected the clinical data of patients using the electronic medical record system (HIS). General information included epidemiological histories, current medical histories, symptoms, physical signs, laboratory examination results, chest computed tomography (CT) manifestations, treatment measures, complications, admissions to intensive care unit (ICU), and other parameters. The patients were divided into mild (including normal and mild) and severe (including severe and critical) groups. The mild group had mild clinical symptoms and no pneumonia on imaging. The normal group had symptoms of fever, respiratory tract symptoms, and imaging showed pneumonia. The severe group had respiratory distress, RR ≥ 30 beats/minute in a resting state, a mean oxygen saturation of ≤93%, and an arterial blood oxygen partial pressure (PaO2)/oxygen concentration (FiO2) ≤ 300 mm Hg. The critical group had respiratory failure and required mechanical ventilation, the occurrence of shock, and the combined failure of other organs that required ICU monitoring and treatment.3

2.3 Coronavirus detection

All of the suspected cases were detected using real-time reverse transcription polymerase chain reaction (PCR), and those who were positive for the coronavirus RNA were identified as confirmed cases. Throat swab samples of the patients were collected, and COVID-19 was detected using a Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit (fluorescent PCR) (Suzhou Tianlong Biotechnology Co Ltd, Jiangsu, China). The throat swab samples, primers, fluorescent probes, reaction buffer, and enzyme mixture were prepared in a reaction system according to a certain procedure, and then amplified according to the following procedures: (a) 50°C 30 minutes; (b) 95°C 10 minutes; (c) 94°C 15 seconds → 50°C 30 seconds → 72°C 30 seconds, 5 cycles; (d) 94°C 10 seconds → 58°C 30 seconds, 35 cycles.

2.4 Statistical analysis

The categorical variables were described as frequency rates and percentages, and the continuous variables were described using the mean, median, and interquartile range (IQR) values. The continuous variables were compared using independent group t tests when the data were normally distributed; otherwise, the Mann-Whitney test was used. The proportions of categorical variables were compared using a χ 2 test. All of the statistical analyses were performed using GraphPad Prism 8. For unadjusted comparisons, a two-sided α of less than .05 was considered to be statistically significant. The analyses were not adjusted for multiple comparisons, and given the potential for type I error, the findings should be interpreted as exploratory and descriptive.

2.5 Presenting characteristics

Among the 135 hospitalized patients, 40 (29.6%) cases were divided into the severe group and 95 (70.4%) cases were divided into the mild group. The median age of all of the patients was 47 years (IQR, 36-55), and 72 (53.3%) cases were male. Compared to mild patients, severe patients were significantly older (median age 56 years [IQR, 52-73] vs 44 years [IQR, 33-49]; P < .001) and were more likely to have underlying comorbidities, such as diabetes (9 [22.5%] vs 3 [3.1%]), cardiovascular disease (6 [15%] vs 1 [1%]), hypertension (4 [10%] vs 9 [9.4%], and malignancy (3 [7.5%] vs 1 [1%]) (Table 1). A majority of 135 patients had a history of exposure in Wuhan (Figure 1).

Table 1. Demographics and baseline characteristics of patients infected with COVID-19

	All patients (n = 135)	Mild cases (n = 95)	Severe cases (n = 40)	P value
Characteristics				
Age, y	47 (36-55)	44 (33-49)	56 (52-73)	<.0001
Sex				
Men	72 (53.3%)	52 (54.7%)	21 (52.5%)	.9609
Women	63 (46.7%)	43 (45.3%)	19 (47.5)	.9609
Epidemiological				
Living/traveling in epidemic area	71 52.6%)	56 (58.9%)	15 (37.5%)	.0366
Contact positive patients	23 (17.0%)	18 (18.9%)	5 (12.5%)	.5098
Contact with suspected patients in epidemic area	19 (14.1%)	10 (10.5%)	9 (22.5%)	.1198
No clear contact history	15 (11.1%)	9 (9.5%)	6 (15%)	…
Agglomerative disease	3 (2.2%)	2 (2.1%)	1 (2.5%)	…
Current smoking	9 (6.7%)	8 (8.4%)	1 (2.5%)	…
Any comorbidity	43 (31.9%)	15 (16.3%)	28 (70%)	<.0001
Diabetes	12 (8.9%)	3 (3.1%)	9 (22.5%)	…
Cardiovascular disease	7 (5.2%)	1 (1%)	6 (15%)	…
Hypertension	13 (9.6)	9 (9.4%)	4 (10%)	…
Chronic obstructive	0	0	4 (10%)	…
Malignancy	4 (3.0%)	1 (1%)	3 (7.5%)	…
Pulmonary disease	1 (0.7%)	0	1 (2.5%)	…

	All patients (n = 135)	Mild cases (n = 95)	Severe cases (n = 40)	P value
Chronic liver disease	2 (1.5%)	1 (1%)	1 (2.5%)	...

Abbreviation: COVID-19, coronavirus disease.

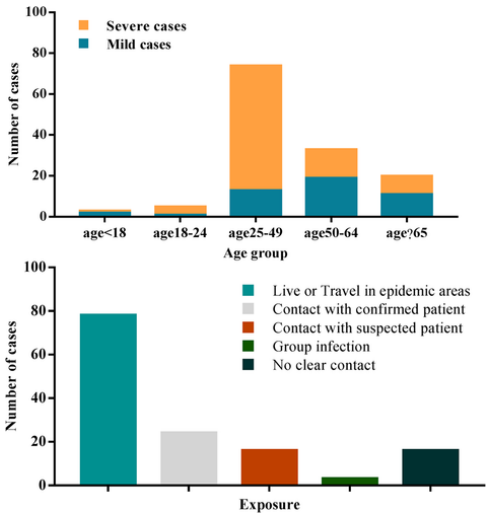

Figure 1

The epidemiology of the included COVID-19 patients. COVID-19, coronavirus disease

The most common symptoms and signs at the onset of illness were fever (120 [88.9%], primarily mild to moderate, 37.3°C-38.9°C: 70 [51.9%], 38.1°C-39°C: 37 [27.4%]), cough (102 [76.5%]), myalgia or fatigue (44 [32.5%]), and headache (24 [17.7%]). Less common symptoms were pharyngalgia (34 [25.2%]), dyspnea (18 [13.3%]), diarrhea (18 [13.3%]), chest tightness and shortness of breath (12 [8.8%]), fear of cold (14 [10.3%]), and sputum production (12 [8.8%]). The median time from first admission to transfer was 5 days (IQR, 5-13) (Table 2).

Table 2. **Signs and symptoms of patients infected with COVID-19**

	All patients (n = 135)	Mild cases (n = 95)	Severe cases (n = 40)	P value
Signs and symptoms				
Fever	120 (88.9%)	86 (90.1%)	34 (85%)	.5267
Highest temperature, °C				
<37.3	16 (11.9%)	11 (11.6%)	5 (14.3%)	.8884
37.3-38.0	70 (51.9%)	43 (45.3%)	27 (77.1%)	.0298
38.0-39.0	37 (27.4%)	35 (36.8%)	2 (5.7%)	...
>39.0	7 (5.1%)	6 (6.3%)	1 (2.9%)	...
Cough	102 (76.5'%)	67 (70.5%)	35 (87.5%)	.0606
Myalgia or fatigue	44 (32.5%)	25 (26.3%)	19 (47.5%)	.0280

	All patients (n = 135)	Mild cases (n = 95)	Severe cases (n = 40)	P value
Headache	34 (32.5%)	23 (24.2%)	11 (27.5%)	.8533
Pharyngalgia	24 (17.7%)	24 (25.3%)	0	…
Diarrhea	18 (13.3%)	5 (5.3%)	13 (32.5%)	…
Dyspnea	18 (13.3%)	0	18 (18.9%)	…
Chest tightness and shortness of breath	12 (8.8%)	9 (9.5%)	3 (7.5%)	…
Sputum production	12 (8.8%)	5 (5.3%)	7 (17.5%)	…
Fear of cold	14 (10.3%)	7 (7.4%)	7 (17.5%)	…
Loss of appetite	6 (4.4%)	0	6 (15%)	…
Palpitation	5 (3.7%)	2 (2.1%)	3 (7.5%)	…
Hemoptysis	4 (3.0%)	1 (1%)	3 (7.5%)	…
Retching	4 (3.0%)	4 (4.2%)	0	…
Days from first admission to transfer	5 (3-10)	5 (3-10)	8 (7-9)	.0873
Diastolic pressure (upon admission), mm Hg	76 (70-84)	76 (71-86)	76 (70-80)	.6352
Systolic pressure (upon admission), mm Hg	120 (111-129)	119 (111-128)	121 (112-133)	.1728
Respiratory rate (upon admission, >24 breaths per min)	12 (8.9%)	3 (3.2%)	9 (22.5%)	…

Abbreviation: COVID-19, coronavirus disease.

According to Table 3, the leukocyte counts of most of the patients were in the normal range, but the classified count showed that the lymphocyte counts of the severe patients (median = 0.8 × 109/L) were significantly lower than that of the mild patients (median = 1.2 × 109/L). Although the coagulation indexes of all of the patients were nearly in the normal range, the Pt, APTT, and d -dimer of the severe patients were higher than those of the mild patients. Compared to the mild patients, the level of albumin was lower in the severe patients (36 [33-38.5] vs 49.9 [37.4-43.6]; P < .0001), and there was no significant difference in the level of alanine aminotransferase and total bilirubin. Lactate dehydrogenase (LDH) of severe patients was significantly higher than those of the mild patients (309 [253.8-408.3] vs 212 [179.5-259]; P < .0001). Indexes related to myocardial injury, such as creatine kinase, glutamic oxaloacetylase, LDH, and C-reactive protein (CRP), increased more significantly in the severe patients. Procalcitonin was higher in the severe patients than mild patients (0.11 [0.08-0.16] vs 0.04 [0.03-0.06]; P < .0500).

Table 3. **Laboratory findings of patients infected with COVID-19 on admission to hospital**

	All patients (n = 135)	Mild cases (n = 95)	Severe cases (n = 40)	P value
White cell count, ×109/L	5.4 (4.1-7.8)	5.5 (4.0-8.0)	5.2 (4.9-6.9)	.6750
<3.5	28	24 (25%)	4 (10%)	…

	All patients (n = 135)	Mild cases (n = 95)	Severe cases (n = 40)	P value
3.5-9.5	98	65 (68%)	33 (82.5%)	.0043
>9.5	9	6 (7%)	3 (7.5%)	…
Neutrophil count, ×109/L	3.5 (2.6-4.4)	3.6 (3.0-3.9)	4.1 (3.1-5.7)	.0015
Lymphocyte count, ×109/L	1.1 (0.7-1.5)	1.2 (0.8-1.6)	0.8 (0.6-1.0)	<.0001
<1.1	68	36 (38%)	32 (80%)	.2938
≥1.1	67	59 (62%)	8 (20%)	…
Hemoglobin, g/L	133 (122-147)	134 (124-147)	130 (120-143)	.1001
Platelet count, ×109/L	158 (131-230)	170 (136-234)	147 (118-213)	.0306
<125	23 (17%)	11 (11.6%)	12 (30%)	.8198
≥125	112 (83%)	84 (88.4%)	28 (70%)	.4920
Prothrombin time, s	10.9 (10.5-11.4)	10.8 (10.4-11.3)	11.3 (10.7-11.8)	.0114
Activated partial thromboplastin time, s	26.9 (24.7-29)	26.6 (24.5-28.8)	29.7 (226.2-39.4)	.0003
d -dimer, mg/L	0.4 (0.2-0.6)	0.3 (0.2-0.5)	0.6 (0.4-1.1)	<.0001
Albumin, g/L	40.5 (37-43.4)	49.9 (37.4-43.6)	36 (33-38.5)	<.0001
Alanine aminotransferase, U/L	26 (12.9-33.15)	21.7 (14.8-36.9)	26.6 (14.5-33.3)	.7324
Aspartate aminotransferase, U/L	33.4 (27.8-43.7)	22.4 (16.9-30.5)	33.6 (25.7-44.2)	<.0001
≤40	105	80 (84%)	25 (62.5%)	.0005
>40	30	15 (16%)	15 (37.5%)	.8460
Total bilirubin, μmol/L	8.6 (5.9-13.7)	8.6 (5.6-14)	9.8 (7.8-15.6)	.0716
Potassium, mmol/L	4 (3.55-4.41)	4 (3.7-4.5)	3.8 (3.5-4.3)	.0550
Sodium, mmol/L	139 (137-141)	139 (137.2-141)	136.5 (133-138)	<.0001
Creatine, μmol/L	66 (57.8-74.5)	66 (55-79)	63.5 (51.5-74.3)	.2306
≤97	129 (95.6%)	92 (97%)	37 (92.5%)	.0835
>97	6 (4.4%)	3 (3%)	3 (7.5%)	…
Creatine kinase, U/L	82.2 (56.3-146.3)	57 (36.5-86.5)	82 (56.3-146.2)	.0016
≤200	125 (92.6%)	92 (97%)	33 (82.5%)	.0307
>200	10 (7.4%)	3 (3%)	7 (17.5%)	…

	All patients (n = 135)	Mild cases (n = 95)	Severe cases (n = 40)	P value
Lactate dehydrogenase, U/L	320.5 (248.5-385.3)	212 (179.5-259)	309 (253.8-408.3)	<.0001
≤250	77 (57%)	67 (71%)	10 (25%)	…
>250	58 (43%)	28 (29%)	30 (75%)	.0055
C-reactive protein, mg/L	10.5 (2.7-51.2)	7.7 (1.9-31.1)	91 (52.7-136.3)	<.0001
Procalcitonin, ng/mL	0.11 (0.08-0.16)	0.04 (0.03-0.06)	0.11 (0.08-0.16)	<.0001
<0.1	110 (81.5%)	89 (94%)	21 (52.5%)	<.0001
≥0.1-0.25	21 (15.6%)	6 (6%)	15 (37.5%)	.1708
≥0.25-0.5	3 (2.2%)	0	3 (7.5%)	…
≥0.5	1 (0.7%)	0	1 (2.5%)	…
Bilateral involvement of chest radiographs	135 (100%)	95 (100%)	40 (100%)	…

Abbreviation: COVID-19, coronavirus disease.

Since nearly all of the patients with COVID-19 had cough as their main early symptom, a chest CT scan was performed in all of the suspected patients. The typical pulmonary changes in the imaging results were interstitial pneumonia with primarily bilateral involvement and multiple patchy, flocculent, or strip ground glass shadow. The marginal areas of the lesions were ill-defined. There was little pleural effusion, and consolidation of the lung occurred when the disease was serious (Figure 2).

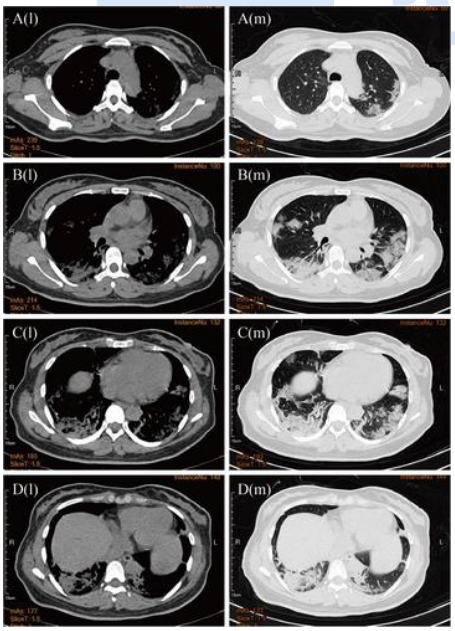

Figure 2

The character of the chest computed tomography (CT) scan in new coronavirus pneumonia patients. The letter "l" stands for "lung window", and "m" stands for "mediastinal window". The CT images were obtained at both lung and mediastinal window settings, showing the multiple patchy ground-glass density shadows on each leaf of the lungs, without bronchial obstruction and pleural effusion

2.6 Organ dysfunctions and primary interventions

Common complications of these 135 patients included ARDS (21 [15.6%]), acute cardiac injury (10 [7.4%]), acute kidney injury (5 [3.7%]), secondary infection (7 [5.1%]), and shock (1 [0.7%]). Severe patients were more likely to have these complications compared to mild patients. All of the patients received antiviral therapy (135 [100%]), and many patients received antibacterial therapy (59 [43.7%]) and corticosteroids (36 [26.7%]). Twenty-seven (67.5%) of the severe patients received noninvasive ventilation. One patient (2.5%) in the severe group was treated with invasive mechanical ventilation. In addition, traditional Chinese medicine (TCM) therapy was applied in most of the patients (124 [91.8%]). As of 8 February 2020, a total of 15 patients (11.1%) had been discharged, and one patient had died. The 28-day mortality rate was 2.5% (Table 4). There was a significant difference between the severe group and the mild group in the number of people that had received antibiotic therapy and corticosteroids ($P < .0001$). There was no significant difference between the two groups in the number of patients who used TCM ($P = .3220$) (Table 4). Actually, most of the patients were treated with a combination of Western and TCM. The application of TCM in viral pneumonia has accumulated rich experience. In recent years, the relevant research has shown that it has a good therapeutic effect on pneumonia.4, 5

Table 4. **Treatments and outcomes of patients infected with COVID-19**

	All patients (n = 135)	Mild cases (n = 95)	Severe cases (n = 40)	P value
Complications (after admission)				
Acute respiratory distress syndrome	21 (15.6%)	1 (1.1%)	20 (50%)	<.0001
Acute cardiac injury	10 (7.4%)	8 (8.4%)	2 (5%)	.7390
Acute kidney injury	5 (3.7%)	4 (4%)	1 (2.5%)	…
Secondary infection	7 (17.5%)	0	7 (17.5%)	…
Shock	1 (0.7%)	0	1 (2.5%)	…
Treatment				
Antiviral therapy	135 (100%)	95 (100%)	40 (100%)	…
Antibiotic therapy	59 (43.7%)	24 (25%)	35 (87.5%)	<.0001
Use of corticosteroid	36 (26.7%)	15 (15.8%)	21 (52.5%)	<.0001
Traditional Chinese medicine	124 (91.8%)	87 (91.5%)	37 (92.5%)	.3220
Continuous renal replacement therapy	5 (3.7%)	1 (1%)	4 (10%)	…
Oxygen support	90 (66.7%)	58 (61%)	32 (80%)	.0533
Noninvasive ventilation or high-flow nasal cannula	34 (25.2%)	7 (7.4%)	27 (67.5%)	<.0001
Invasive mechanical ventilation	1 (0.7%)	0	1 (2.5%)	…
Invasive mechanical ventilation and ECMO	0	0	0	…
Prognosis				

	All patients (n = 135)	Mild cases (n = 95)	Severe cases (n = 40)	P value
Hospitalization	120 (88.9%)	85 (89.5%)	35 (87.5%)	.9734
Discharge	15 (42.9%)	10 (1.05%)	5 (12.5%)	.9734
Death	1 (0.7%)	0	1 (2.5%)	…

Abbreviation: COVID-19, coronavirus disease.

3 DISCUSSION

To date, this report is the largest case series of hospitalized patients with COVID-19 in northeast Chongqing. There was no significant difference in the proportion of male and female patients, and infection in children was rare, which was inconsistent with the results of a study performed by Zhong et al.6 Their results showed that males were more likely to be infected than females.6 A total of 83.7% of the patients included in this study had contact history in Wuhan, had been to Wuhan, or had contact with people from Wuhan, which again verified the conclusion of human to human transmission.7 The primary symptoms were fever and cough, which agreed with the research results of Zhao et al.8, 9 Other symptoms included myalgia, fatigue, dyspnea, and anorexia. COVID-19 patients rarely developed intestinal signs and symptoms (eg, diarrhea), whereas about 20% to 25% of patients infected with MERS-CoV or SARS-CoV experienced diarrhea.10 The primary complications during hospitalization included ARDS, arrhythmia, and shock. Patchy shadows of the bilateral lungs and ground-glass shadow were typical CT signs of COVID-19. The most severe patients were older and had more basic diseases compared to mild patients.

According to the RNA detection results of COVID-19 patients in our hospital and the reports of other medical institutions, the sensitivity of the detection kit currently used in clinical is not ideal.1 However, imaging of typical pulmonary changes was seen in a vast majority of the confirmed cases. The imaging of pulmonary changes due to COVID-19, like most viral pneumonias, was pleomorphic with interstitial changes and patchy and ground glass shadows.11 The imaging of pulmonary changes was often out of step with the patient's symptoms and nucleic acid test results. The expert group from our hospital called this phenomenon the “shadow-syndrome discrepancy.” Some mild patients often had no fever, cough, dyspnea, and other symptoms. In contrast, the symptoms were mild, and multiple nucleic acid tests were negative, but the CT showed a large ground glass area in the lung. Therefore, some clinicians have suggested that a CT examination should be the first choice in the screening and diagnosis of COVID-19, and it has been suggested that patients should have a chest CT scan every 3 to 5 days to understand the changes in the lung lesions to more accurately evaluate the condition.

CRP and procalcitonin of severe patients were significantly higher than those of mild patients, but bacterial culture results showed no growth after 5 days of aerobic and anaerobic culture, suggesting that although inflammatory factors had increased, there was no significant bacterial infection. The D-dimer concentration was increased in 135 patients, especially in severe patients. This indicated the presence of a hypercoagulable state and secondary hyperfibrinolysis in vivo. In most patients, the leukocyte count was in the normal range and lymphocyte count was generally reduced, which agreed with the recent research results published by academician Zhong et al.6 This suggests that the virus may cause disease by attacking the immune system. LDH and aspartate aminotransferase generally increased, but the albumin of patients decreased. In addition, the changes in the severe patients were more obvious than the mild patients, suggesting that early liver function may be damaged in the mild patients, while liver damage in the severe patients was more obvious.

Among the 135 patients, only 1 patient died, and this case will be briefly discussed. He was a 52-year-old male with diabetes and a chronic disease, and his son had recently returned from Wuhan. His neutrophil, d -dimer count, lymphocyte, CD4+T, CD8+T, B cell, and NK cell counts were above normal levels. In addition, his

CD4+T/CD8+T counts continued to decline until death (Table 5). He eventually died of acute respiratory distress, oxygen saturation, and heart rate decline, which was consistent with the results of a study performed by Chen et al.12 In addition, Wan et al13 found that CD4+T and CD8+T were lower in severe patients, which suggested that lymphocytes were more inhibited in severe patients. Lymphocytopenia may be related to a cytokine storm caused by viral invasion. This suggests that we should pay more attention to the cellular immunity of patients and take comprehensive measures to treat patients so as to reduce mortality.

Table 5. **The clinical features of one died COVID-19 patients**

Days of hospitalization, d	White cell count, 109/L	Neutrophil count, 109/L	Lymphocyte count, ×109/L	d - Dimer, mg/L	CD4+T	CD8+T	B cell	NK cell	CD4+T/CD8+T
1	7.6	6.43	0.7	20.74	…	…	…	…	…
2	9.6	8.52	0.62	11.99	220	167	125	49	1.31
3	14.8	13.74	0.5	14.08	…	…	…	…	…
3	…	…	…	11.51	…	…	…	…	…
4	17.4	16.11	0.53	9.35	121	143	98	29	0.84
5	20.4	19.2	0.39	12.85	75	149	81	41	0.5
6	…	…	…	…	…	…	…	…	…
7	12.9	12.04	0.49	14.47	…	…	…	…	…
8	16.6	15.4	0.71	…	147	127	87	16	1.16
9	10.8	9.65	0.5	…	89	102	58	16	0.83
10	11.7	10.36	0.6	…	…	…	…	…	…

Abbreviation: COVID-19, coronavirus disease.

Currently, there are no specific treatment proposals for COVID-19 in China. At present, the primary measures to control this disease are early diagnosis, isolation, and supportive treatment for affected patients. In this study, all of the patients were treated with oxygen therapy and antiviral drugs. In addition, many patients received antibacterial therapy (59 [43.7%]) and corticosteroids (36 [26.7%]), and a few patients required invasive ventilation or even extracorporeal membrane oxygenation. According to a recent Korean scholar's report,14, 15 Kaletra was shown to be effective in the early treatment of COVID-19 patients, and the earlier Kaletra was used, the more significant the therapeutic effect. Their experience primarily came from the treatment of the MERS coronavirus in 2015, and they had established guidelines. Initially, only the severe patients were treated with Kaletra, and mild patients were not administered the drug in Wuhan, leading to a low patient cure rate. These results indicated that the application effect of Kaletra was not significant in severe patients. All of the patients in our hospital were treated with Kaletra in the early stage, with the belief that Kaletra may play a role in the inhibition of viral replication. Currently, there are 236 patients in our hospital,

and 98 of them have been cured and discharged, for a cure rate of 41.5%. The therapeutic effect is obvious. Japan has also announced that they will conduct clinical trials using Kaletra on patients with COVID-19.

In view of the high amount of cytokines induced by SARS-CoV,16, 17 MERS-CoV,18, 19 and COVID-19, 21 (52.5%) of the severe patients were treated with glucocorticoids to reduce inflammatory injury in the lungs. However, due to the limitations of existing evidence, use of glucocorticoids is still controversial. The latest clinical studies20 have suggested that glucocorticoids should not be used to treat lung injury or shock caused by COVID-19 without clinical trials. However, some studies2, 21, 22 have shown that the rational use of glucocorticoids could reduce the mortality of SARS patients with critical illness, shorten the length of stay, and not cause secondary infection and other complications. Therefore, glucocorticoids are suggested for treatment according to the "Application Recommendations of Glucocorticoids for Corona Virus Disease 2019: Recommendations for the use of Glucocorticoids for the New Coronavirus Pneumonia" issued by the Chinese Thoracic Society.23

Since 2003, TCM has been utilized to fight SARS, H1N1H7N9, MERS, EBOV, and other viral diseases.24 TCM has also been recommended for the treatment of COVID-19 in the "New Coronavirus Pneumonia Diagnosis and Treatment Plan" (trial version 5).3 Chinese patent medicines used in the treatment of COVID-19 have primarily included: Reduning injection, Suhuang Zhike capsule, and Xuebijing. In addition, the Chinese herbals used to treat COVID-19 primarily include glycyrrhiza, ephedra, bitter almond, gypsum, reed root, amomum, and trichosanthes. TCM primarily functions to clear heat and remove toxicity, to remove heat from the lungs to relieve cough, and to increase immunity.5

This study has several limitations. First, the sample size was relatively small compared to Wuhan, where the disease originated, which may have some impact on the statistical results. In general, the number of patients in this area is in the middle level of the rest of the country, except for Wuhan. Therefore, the research results were reliable. Second, most of the 135 patients were still hospitalized at the end of this study. Therefore, it was difficult to evaluate the risk factors for a poor prognosis. Long-term observation is required.

In future research, a multicenter study will be established to expand the sample size and to conduct more rigorous randomized controlled trials. In addition, the follow-up of patients who were cured and discharged will be conducted.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

SW and YH had the idea for and designed the study and had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. SW, YX, YH, YZ, YX, WF, and BL contributed to writing of the report. SW and YH contributed to the statistical analysis. RY contributed to picture drawing. All authors contributed to data acquisition, data analysis, or data interpretation, and reviewed and approved the final version.

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Abstract

To analyze the registered clinical trial protocols of traditional Chinese medicine(TCM) for the prevention and treatment of coronavirus disease 2019(COVID-19), in order to provide information for improving the quality of research design. The website of the Chinese Clinical Trial Registry(www.chictr.org.cn) and the American Clinical Trial Registry(clinicaltrials.gov) were searched to collect protocols of TCM for COVID-19. Documents were screened following the inclusion criteria, and data were extracted in regard to registration date, study objective, type of design, sponsor, patient, sample size, intervention, and evaluation index. Descriptive analysis was conducted. A total of 49 clinical trial protocols of TCM for COVID-19 were included. Primary sponsors were mainly hospitals or universities in places like Hubei, Beijing, Zhejiang and other regions. The implementation units are mainly in Hubei, Guangdong, Zhejiang, Henan and other regional hospitals. The types of study design were mainly experimental studies(40), including 30 randomized parallel controlled trials, 7 non-randomized controlled trials, 2 single arm trials and 1 consecutively recruited trial; besides, there were also 6 observational studies, 2 health service studies and 1 preventive study. The sample size reached a total of 30 562 cases, with a maximum of 20 000 for a single study and a minimum of 30. The 49 trials subjects included healthy people(3), isolation and observation cases(1), suspected cases(10),confirmed COVID-19 patients(31) and COVID-19 recovery patients(4). Of the 31 trials planned to include confirmed COVID-19 patients, 16 protocols no definite disease classification, 3 with a clear exclusion of severe subjects, 4 with common subjects, 2 with light, common or severe subjects, 1 with light and common subjects, 1 with common or severe subjects, 3 with severe subjects, and 1 with severe or critical subjects. The experimental interventions included Chinese patent medicine(Lianhua Qingwen Capsules/Granules, Huoxiang Zhengqi Dropping Pills/Oral Liquid, Babao Dan, Gubiao Jiedu Ling, Jinhao Jiere Granules, Compound Yu-xingcao Mixture, Jinye Baidu Granules, Shufeng Jiedu Capsuless, Shuanghuanglian Oral Liquid, Tanreqing Injection, Xuebijing Injection, Reduning Injection, Xiyanping Injection), Chinese medicinal decoction and taichi. The primary evaluation outcomes mainly included antipyretic time, clinical symptom relief, novel coronavirus nucleic acid turning to negative, conversion rate of severe cases and chest CT. There was a quick response of clinical research on the prevention and treatment of COVID-19 with TCM, with the current registered protocols covers the whole process of disease prevention, treatment and rehabilitation. However, issues need to be concerned, including unclear definition of patient's condition, unclear research objectives, unclear intervention process and inappropriate outcomes, etc. In addition, researchers should consider the actual difficulties and workload of doctors in epidemic response environment, and make effort to optimize the process and improve the operability of research protocols under the principle of medical ethics.

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Abstract

2019 novel coronavirus(2019-nCoV) has occurred for 2 months, and seriously affected the people's health in the world. Therefore, scientific prevention and control strategies and effective intervention measures are the only ways to solve the world problem. In the determination of intervention measures, not only the effectiveness evaluation, but also accessibility, treatment cost, inventory and production capacity and other relevant sociological issues shall be considered, especially in low and middle-income countries and regions. With the introduction of clinical epidemiological experiment design and evidence-based medicine evidence evaluation into the evaluation of curative effect of traditional Chinese medicine(TCM), TCM has officially entered the studies of syndrome regularity of new public health emergencies(such as SARS and influenza) clearly diagnosed by modern medicine for many years, as well as the development of relevant guidelines, consensus and paths. The results of curative effect show that TCM could significantly alleviate symptoms, control disease and tendency, reduce the occurrence of critical illness, and improve the clinical efficacy and the prognosis and quality of life of patients, which fully reflects the consciousness and self-confidence of traditional Chinese medicine workers. For the evidence-based evaluation of TCM intervention in new public health emergencies, the basic principles and general methods of clinical epidemiology and evidence-based medicine shall be followed to obtain high-quality evidence; besides, we shall also fully realize that clinical scientific study is carried out with the epidemic treatment as the primary task. The scientific hypothesis comes from the clinical problems unsolved. The scientific study conclusions aim to give feedbacks to clinical diagnosis and treatment regimens. The core elements of clinical trials are population(P), intervention(I), control(C), outcome(O), which are abbreviated as "PICO". The evaluation of intervention measures for new public health emergencies with traditional Chinese medicine shall have clear study objectives and a high quality, with a correct analysis method as the guarantee of real and reliable results. Then, the selection of patients, the de-finition of intervention measures and control measures, the development of end-point indicators, the clinical quality control under special epidemics, the data verification, and the data analysis methods to be adopted are all characteristics and key points that need special consideration. It is suggested that scientific experimental design, rigorous collection and scientific data analysis shall be conducted to reflect the therapeutic value of traditional Chinese medicine, so that the study results could be adopted and shared, and become the scientific evidence for China and even the global to republish the diagnosis and treatment regimens.

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Abstract

The situation of Corona Virus Disease 2019 (COVID-19) is still severe at present. In order to better fight against the epidemic and give full play to the advantages of traditional Chinese medicine, we explored the feasibility of acupuncture therapy in the intervention of COVID-19 through analyzing the relevant literature in both ancient and modern time. Additionally, we analyzed the intervention scheme of acupuncture for COVID-19 developed by China Association of Acupuncture and Moxibustion and supplemented the protocol of the intervention with auricular acupuncture. It was proposed that the advantages of acupuncture and moxibustion should be fully displayed while Chinese herbal medications have been applied in the treatment of COVID-19. During treatment, acupuncture physicians should be rationally allocated to a certain proportion so as to adequately utilize comprehensive therapeutic approaches and guarantee people's safety to the greatest extent. Eventually, the clinical therapeutic effect may be improved, the national resources be economized on and the COVID-19 epidemic be conquered early.

79. **Wang SX, Wang Y, Lu YB, Li JY, Song YJ, Nyamgerelt M, Wang XX. Diagnosis and treatment of novel coronavirus pneumonia based on the theory of traditional Chinese medicine. J Integr Med. 2020 Apr 15:S2095-4964(20)30037-6. doi: 10.1016/j.joim.2020.04.001.**

Abstract

Since the outbreak of novel coronavirus pneumonia (coronavirus disease 2019, COVID-19) in December 2019, it has rapidly spread to 187 countries, causing serious harm to the health of people and a huge social burden. However, currently, drugs specifically approved for clinical use are not available, except for vaccines against COVID-19 that are being evaluated. Traditional Chinese medicine (TCM) is capable of performing syndrome differentiation and treatment according to the clinical manifestations of patients, and has a better ability of epidemic prevention and control. The authors comprehensively analyzed the etiology and pathogenesis of COVID-19 based on the theory of TCM, and discussed its syndrome differentiation, treatment and prevention measures so as to provide strategies and reference for the prevention and treatment with TCM.

1. Introduction

Since December 2019, the novel coronavirus pneumonia (coronavirus disease 2019, COVID-19) outbreak in the world and has now infected a total of 348,678 people in 187 countries (March 21, 2020) [1]. China has listed COVID-19 as a class B infectious disease and has undertaken preventive and control measures according to class A infectious diseases [2]. With the spread of the epidemic, presently, patients infected with the novel coronavirus, as well as those with asymptomatic infection, are the main sources of infection; thus, COVID-19 is an infectious disease with a medium to slightly high contagious capacity [3]. The incubation period of the disease is generally 7 days, and the longest is 14 days; however, some cases have reported an incubation period of 24 days as well [4]. Common clinical manifestations include fever, dry cough and fatigue. Computerized tomography of the chest can show multiple ground glass shadows of the pulmonary lobes, indicative of conditions such as respiratory distress syndrome, shock and sepsis, which can even lead to death [5]. Thus far, although China has announced six trials and strategies for the diagnosis and treatment of the disease, the focus remains mainly on symptomatic treatment, new drugs specifically approved for the infection are not available. Clinical trials for new drugs such as remdesivir and coolidge are underway; however, the clinical promotion and short-term use of these drugs is unlikely. Therefore, given the particularities of this disease, it is of critical clinical value to formulate a set of treatment plans using integrated traditional Chinese medicine (TCM) and Western medicine, based on the concept of prevention and treatment. To utilize the advantages of integrated traditional Chinese and Western medicine in the prevention and treatment of COVID-19, this paper discussed the etiology and pathogenesis of COVID-19 from the viewpoint of integrated TCM and Western medicine, the treatment measures for different susceptible groups, and the effect along with matters needing attention, so as to provide a reference for the prevention and treatment of COVID-19.

2. Etiology

Coronavirus is a type of a ribonucleic acid virus with an envelope structure, characterized by rod-like protuberances on the surface and can infect multiple host species, causing a variety of diseases [6]. The COVID-19 pathogen is a novel coronavirus (2019-nCoV), which can infect humans and is significantly clustered with the bat-like severe acute respiratory syndrome coronavirus (SARS-CoV) sequence isolated in 2015, with a nucleotide similarity of 88%. Based on these results, it can be concluded that 2019-nCoV is similar to SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) [7]. It is a zoonotic pathogen that can cause severe respiratory diseases in humans and can be transmitted from person to person [8]. Therefore, the International Committee on Taxonomy of Viruses announced that the official classification of the novel coronavirus is SARS-CoV-2.

There is no virus theory in TCM, and according to the clinical characteristics of COVID-19, the disease belongs to the category of "plague" [9]. As the *General Treatise on the Etiology and Symptomology* says [10], "if a person feels grumpy and gets sick, he will be easily infected by evil Qi, which may even exterminate the family, and extend to outsiders." The common symptoms in the early stage of COVID-19 in mildly infected patients include fever, fatigue and dry cough, which could be easily confused with other common exogenous diseases. However, according to Wu You-ke's *The Treatise on Epidemic Febrile Diseases* [11], which mentions "the febrile disease is non-wind, non-cold, non-heat and non-damp; it is a different feeling between heaven and earth," the cause of this disease is very clear, differing from the other six exotic evils, and presenting characteristics of a strong and contagious epidemic virus.

Wuhan, the center of the outbreak pathogen in China, is located in the east of Jianghan Plain of China. The vertical and horizontal water areas of the city's rivers account for one-fourth of the total city area, demonstrating the natural environmental basis of "damp evil" [12]. At the initial stage of this epidemic, most patients presented symptoms of fever; however, there were also patients with no fever or low fever, and some patients presented gastrointestinal symptoms, such as indigestion and loose stool, as well as chest tightness and fatigue, coinciding with the characteristics of "damp evil" in TCM. Hence, this disease is an epidemic, with the primary nature of "damp" and "toxic," and thus can be termed "damp toxin disease." Furthermore, in 2019, Wuhan experienced a warm winter climate, with incoming heat instead of cold. From the perspective of TCM, this kind of climate change is prone to generate damp-heat in the human body, which persists and is difficult to eliminate from Sanjiao (one of the six fu-organs in TCM theory). Some patients have a recurrent fever, bitter mouth, insomnia, upset, chest tightness and shortness of breath, fatigue, yellow or greasy tongue coating, and slippery pulse, which are manifestations of the evil underlying Shaoyang meridian according to the six-meridian syndrome differentiation theory [13]. Notably, the most severe cases developed dyspnea one week later, which even progressed to acute respiratory distress syndrome, septic shock, intractable metabolic acidosis, and coagulation dysfunction, leading to death [14]. According to TCM, the toxins from these patients not only hurt the Qi but also injure the nutrient blood and result in blood stasis; the pericardium transmission channel is also reversed, resulting in mental changes. Dampness stagnates for a prolonged period and turns into heat; heat toxin accumulates for a long time, resulting in stasis; stasis and heat combine mutually and then generate syncope, which consumes Qi and Yin and leads to a deficiency. According to the principle of "three factors and measures," there exist various etiological characteristics in different regions, as well as climatic factors. Fan et al. [15] observed and analyzed the TCM pathological characteristics of "dryness" in Hunan cases, and concluded that dryness and dampness are mainly due to "child disease to mother" in the course of the disease. In the theory of TCM, dryness evil can easily injure the lung. Lung and spleen belong to "gold" and "soil" in the five-element theory of TCM. Dryness turns into dampness. Dampness easily traps spleen. Wet soil is the mother of dryness "gold." Furthermore, excessive lung dryness injures the spleen "soil"; hence, both lung dryness and spleen dampness are significant. In contrast, Yu et al. [16] summed up the data and concluded that the diseases in Guangdong Province and Gansu Province were caused by warm-heat toxin or epidemic toxin, which was closely related to their regional environment. Guangdong Province is hot and humid all year round, and the epidemic Qi can easily mix with evils of damp and heat to attack the lung; however, the overall 2019 winter temperature in Gansu Province was higher than that experienced in previous years. Additionally, Gansu is located in the northwest region of China, which is mostly dry and warm, and susceptible to the evil of dryness that can cause lung disease. TCM runs through the whole idea of disease differentiation all the time, and the unified relationship between man and nature has been called "correspondence between man and nature" in ancient books, which presents considerable advantage in the treatment of COVID-19 in different populations and regions.

3. Pathogenesis

Compared with other diseases, the evil Qi of the "damp toxin epidemic" is relatively fixed, and hence, the etiology and pathogenesis are relatively fixed, with obvious stages of disease progression in clinical settings. Understanding the consistency of the stages also allows for clinical diagnosis and evidence-based treatment. After careful assessment of the four pieces of diagnostic evidence available regarding the confirmed cases, it

was observed that this epidemic is mainly caused by a dampness pathogen, with the disease location of mainly Sangjiao, and the core pathogenesis is “dampness, heat, poison, blood stasis and deficiency,” which runs through the whole process of dampness and intoxication and closure of the lung. The *Treatise on Epidemic Febrile Diseases* [11] ascertains that typhoid fever does not infect people, but epidemic diseases can infect people. The evil of typhoid fever enters from the orifices, while the evil of epidemic disease enters only from the mouth and nose; it has been observed that during an epidemic, the evil comes from the mouth and nose, remains in the membrane, and unconsciously lurks in the human body [17]. COVID-19 is located in the lung, where evil Qi first invades the body from the mouth and nose, repressing the lung Qi. In turn, the lung fails to disperse and descend, with symptoms of dry cough. The “damp evil” is heavy and viscous, blocking the Qi mechanism and causing chest tightness, body heat, and muscle soreness. “Damp evil” traps the spleen, easily injuring Yang Qi. The spleen dominates and transports water and food through the body. When the Qi fails to rise, the spleen cannot be nourished, and symptoms begin to appear, including fatigue, gastric congestion and loose stool. The spleen and stomach act as the exterior and interior of each other; hence, the spleen’s dysfunction affects the stomach’s absorption. The dampness accompanying the epidemic toxin into heat is manifested as high fever, occasional yellow phlegm, obvious asthma due to the dampness toxin, and sticky stool due to the stagnation of dampness. Wang Qingren stated in *Correcting Mistakes in Medicine* that “the plague toxin burns its blood inside, and if the blood is burned and refined, the blood will coagulate [18].” During this period, the heat toxin burns the body fluid, and dampness suppresses the Qi mechanism, resulting in the stasis of blood, manifested in the form of increased dyspnea and additional mental problems occurring during the reverse transmission of the pericardium. In the later stage, the body is damaged by fluid consumption, and is deficient in both Qi and Yin. During this period of treatment, attention should be paid to whether there is any residual evil in the body.

Given the differences in etiology and pathogenesis attributed to climatic factors in different regions, Fan et al. [19] observed and stated that owing to climatic factors, dryness has been present in the lung for a prolonged period. On encountering the epidemic toxin, dampness and cold will collide with each other, and obstruct the lung and chest, injuring vital Qi, leading to the stagnation of the Qi mechanism and abnormal rise and fall of evil Qi, finally leading to a deficiency of vital energy. Although there exist differing opinions, combined with the clinical manifestations of the published and confirmed cases, the main etiology and pathogenesis can be attributed to “dampness, heat, toxin, blood stasis and deficiency.” Furthermore, the pathogenesis may differ among individuals. In the case of adults without underlying diseases, because of being in the prime of life, the presence of vital Qi, as well as the balance and coordination of movement and transformation of Qi, blood, Yin and Yang, is sufficient to resist external evil, demonstrating no susceptibility or mild susceptibility, with a short disease course and a good prognosis. In children, for the viscera are not well developed, presenting delicate viscera, unfilled shape and Qi, and immature Yin and Yang, they fall sick easily and quickly [20]. However, in the overall growing phase, they are made up of “pure Yang”, are robust, and will recover easily. Patients with underlying diseases, such as diabetes, hypertension, heart disease, chronic tracheitis, tumors, or other diseases, usually suffer from a deficiency of vital Qi; if they experience the external evil, the disease is often characterized by severe illness, rapid progression, and rapid transmission, resulting in critical illness and even death. Pregnant women have a special physiological state, with a relative deficiency of Yin-blood and relative hyperactivity of Yang Qi. The growth of the fetus blocks the rise and fall mechanism of the Qi in pregnant women, which may lead to poor operation of Qi and blood. Therefore, the elderly, the infirm, children and pregnant women require close monitoring.

4. Treatment

Currently, there are no drugs specifically approved for COVID-19. In the treatment plan announced by the National Health Commission of the People’s Republic of China [21], Western medicine offers mainly symptomatic treatment support, including early oxygen therapy and the use of corticosteroids. The treatment plan comprises antiviral therapies, including interferon-α, lopinavir, ritonavir and ribavirin; however, the curative effect remains unsatisfactory.

Referring to the experience of TCM in the treatment of patients with SARS in 2003, Chinese medicine is now fully involved in the treatment of COVID-19
patients [21], [22], [23], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [34], [35], [36], [37], [38], [39], utilizing the advantages of TCM syndrome differentiation and treatment, improving the cure rate, and reducing mortality (Table 1).

Table 1
Specific measures of syndrome differentiation and treatment by disease stages.

Stage	Symptoms and signs	Therapeutic principle	Prescription	Reference
Initial stage	Fever is the main symptom of the disease, with most hiding fever not accompanied by a cold. Other symptoms include sleepiness and discomfort, dry cough and less phlegm, thirst without the desire to drink, chest tightness and palpitation, occasional muscle aches, nasal congestion and runny nose, and loose stools. No sweat, no sthenic heat or irritable heat sensation, and no sore throat. The tongue is dark red, or its edge is red, the moss is thin and white, and the pulse has no fixed point.	Eliminating the filth with aromatics, promoting Qi mechanism, separating and dissipating damp-heat, removing blood stasis, and freeing the network vessels	Modified Huopu Xialing Decoction and Sanren Decoction	[13,19,39]
Progressive stage	The main characteristics of the progressive stage include high fever, asthma and shortness of breath, difficulty in movement, aggravation of fatigue and tiredness, aggravation of cough, persistent dry cough or less phlegm, white or yellow, sticky cough, thirst without the desire to drink, cyanosis of lips and nails, or with delirium, increased palpitation, dizziness, indigestion, abdominal distention, loose stools or constipation, difficult urination, dark red tongue, greasy fur, and smooth pulse.	Eliminating dampness and dispersing the lung with aromatics, clearing Ying heat, cooling blood, and detoxifying	Lei's aromatic turbid-resolving method and Jiedu Huoxue Decoction combined with Shengjiang Powder	[29,31–33,35,39]
Extreme stage	In the advanced stage, without proper treatment, the disease is further aggravated, with high fever, dyspnea, cyanosis of lips, dark complexion, extreme fatigue, restlessness, syncope, hot hands and feet or cold hands and feet, oliguria, dark red tongue, turbid or yellow greasy fur, and rapid and thinning pulse. It should be noted that some patients during this period may have a moderate or low fever or even no obvious fever.	Dispersing Qi of the lung, healing the body's essence Qi and blood, detoxifying, and rescuing the inverse	Shenfu Sini Decoction, Angong Niuhuang Pill, Zhibao Dan, Zixue Dan, and Suhe Xiang Pill	[22–25,28,39]
Recovery stage	The recovery period is mainly characterized by high fever that has subsided, mental improvement, low fever, fatigue, poor appetite, sticky stool, and other symptoms. Computed tomography shows absorption or fibrosis. The disease pathogenesis demonstrates that the damp toxin is eliminated, but the residual evil is still present, with deficient lung and spleen Qi.	Clearing the residual evil, promoting lung circulation, activating spleen and strengthening healthy Qi	Modified Xue's Wuye Lugen Decoction	[14,16,20,21]

Table 2
List of medicine's sources, and Chinese, Latin and English names.

Chinese name (pin yin)	Medicine source	Latin name	English name (common name)
Xiangfu	*Cyperus rotundus* L.	Cyperi Rhizoma	Nutgrass galingale rhizome
Xiaoshi	Minerals in aragonite group and calcite group	Nitrum	Niter
Xingren	*Prunus armeniaca* L. var. *ansu* Maxim.	Armeniacae Semen Amarum.	Bitter apricot seed
Xionghuang	Arsenic sulfide mineral	Realgar	Red orpiment
Xuanshen	*Scrophularia ningpoensis* Hemsl.	Scrophulariae Radix	Figwort root
Yiyiren	*Coix lacryma-jobi* L. var. *mayuen* (Roman.) Stapf	Coicis Semen	Semen coicis
Yinbo	Native silver	Argentum	Silver foil
Yujin	*Curcuma wenyujin* Y. H. Chen et C. Ling	Curcumae Radix	Aromatic turmeric root tuber
Zexie	*Alisma orientale* (Sam.) Juzep.	Alismatis Rhizoma	Rhizoma alismatis
Zhenzhu	*Pteria martensii* (Dunker)	Margarita	Pearl
Zhiqiao	*Citrus aurantium* L.	Aurantii Fructus	Immature trifoliate orange fruit
Zhizi	*Gardenia jasminoides* Ellis	Gardeniae Fructus	Common gardenia fruit
Zhusha	*Cinnabaris*	Cinnabaris	Cinnabar

Table 3
Specific prevention measures for different groups of people.

Crowd type	Features	Therapeutic principle	Prescription
Contacts	Close contacts or healthy people returning from the epidemic area	Invigorate Qi for consolidating the exterior of the body, remove heat, and detoxify.	Yupingfeng Powder
Children	Under 14 years of age	Invigorate Qi for consolidating superficies, and remove heat and detoxify.	Yupingfeng Powder or Sijunzi Decoction
People with underlying diseases	High blood sugar	Remove heat and promote fluid retention in the body, and enrich Yin and consolidate superficies.	Yupingfeng Powder
	High blood pressure	Remove heat and nourish Yin, and calm the liver and consolidate superficies.	Yupingfeng Powder
	Coronary heart disease and cardiocerebrovascular diseases	Remove heat, detoxify blood, and promote blood circulation to alleviate blood stasis.	Shengmai Drink
Women during pregnancy	Pregnant women	Nourish Yin and remove heat, regulate Qi flow for soothing the fetus, and strengthen healthy Qi for consolidating superficies.	Yupingfeng Powder
Normal adults	Uninfected adults	Regulate Ying and Wei, invigorate Qi for consolidating superficies, invigorate Qi and nourish Yin, strengthen healthy Qi, remove heat and detoxify, and resolve dampness and exterior symptoms.	Guizhi Decoction, Yupingfeng Powder, Shenzhu Powder, or Huangqi Guizhi Wuwu Decoction

Table 4
Composition and dosage of the prescriptions.

Stage	Prescription	Composition
Prevention	Yupingfeng Powder	Fangfeng 30 g, Huangqi 60 g, Baizhu 60 g
	Sijunzi Decoction	Renshen 9 g, Baizhu 9 g, Fuling 9 g, Gancao 6 g
	Shengmai Drink	Renshen 9 g, Maidong 9 g, Wuweizi 6 g, Danshen 6 g
	Guizhi Decoction	Guizhi 9 g, Shengjiang 9 g, Dazao 9 g, honey-fried Gancao 6 g
	Shenzhu Powder	Cangzhu 1000 g, Chenpi 1000 g, Houpo 1000 g, honey-fried Gancao 360 g, Guanghuoxiang 250 g, Sharen 120 g
	Huangqi Guizhi Wuwu Decoction	Huangqi 15 g, Guizhi 12 g, Baishao 12 g, Shengjiang 25 g, Dazao 4 g
Initial stage	Huopu Xialing Decoction and Sanren Decoction	Guanghuoxiang 6 g, Peilan 6 g, Banxia 9 g, Doukou 6 g, Xingren 9 g, Fuling 9 g, Zexie 6 g, Huashi 18 g, Tongcao 6 g, Danzhuye 6 g, Yiyiren 12 g, Mudanpi 6 g, Chishao 6 g
Progressive stage	Lei's aromatic turbid-resolving method	Guanghuoxiang 3 g, Peilan 3 g, Chenpi 6 g, Banxia 6 g, Dafupi 3 g, Houpo 3 g, Heye 9 g
	Jiedu Huoxue Decoction combined with Shengjiang Powder	Lianqiao 6 g, Gegen 6 g, Chaihu 9 g, Danggui 6 g, Shengdihuang 15 g, Chishao 9 g, Taoren 24 g, Honghua 15 g, Zhiqiao 3 g, Gancao 6 g, Jiangcan (wine fried) 6 g, Chantui 3 g, Jianghuang 9 g, Dahuang (raw) 12 g
Extreme stage	Shenfu Sini Decoction	Honey-fried Gancao 12 g, Fuzi 10 g, Ganjiang 6 g, Renshen 15 g
	Angong Niuhuang Pill	Niuhuang 30 g, Yujin 30 g, Shuiniujiao 30 g, Huangqi 30 g, Huanglian 30 g, Xionghuang 30 g, Zhizi 30 g, Zhusha 30 g, Longnao 7.5 g, Shexiang 7.5 g, Zhenzhu 15 g
	Zhibao Dan	Shuiniujiao 30 g, Daimao (raw scute) 30 g, Hupo 30 g, Zhusha 30 g, Xionghuang 30 g, Niuhuang 0.3 g, Longnao 0.3 g, Shexiang 0.3 g, Anxixiang 4.5 g, Jinbo 50 layers, Yinbo 50 layers
	Zixue Dan	Shigao 1500 g, Hanshuishi 1500 g, Huashi 1500 g, Shuiniujiao 500 g, Lingyangjiao crumbs 500 g, Muxiang 500 g, Chenxiang 500 g, Xuanshen 500 g, Shengma 500 g, Gancao 240 g, Mangxiao 5000 g, Xiaoshi 930 g, Shexiang 38 g, Zhusha 90 g, Jinbo 3000 g, Dingxiang 30 g
	Suhe Xiang Pill	Suhexiang 60 g, Baizhu 60 g, Muxiang 60 g, Shuiniujiao 60 g, Xiangfu 60 g, Zhusha 60 g, Hezi 60 g, Tanxiang 60 g, Anxixiang 60 g, Chenxiang 60 g, Shexiang 60 g, Dingxiang 60 g, Bibo 60 g, Longnao 60 g, Ruxiang 60 g
Recovery stage	Xue's Wuye Lugen Decoction	Huoxiang leaf 6 g, Bohe leaf 1.8 g, fresh lotus leaf 3 g, Pipaye 15 g, Peilan leaf 4.5 g, Lugen 30 g, Dongguazi 15 g

4.1.1. Initial stage

The damp toxin in the Shangjiao membrane should be treated for “damp-heat clearance to cure the lung,” including separating and dissipating the damp-heat, paying critical attention to the smooth release of lung Qi. Furthermore, it is crucial to closely monitor the potential of the toxin to enter the blood in order to resolve the dampness, eliminate the filth, promote the Qi mechanism, resolve blood stasis, and relieve the blood vessel networks, thereby following the principles of the Huopu Xialing Decoction and Sanren Decoction [14]. The combination prescription includes Guanghuoxiang, Peilan, Banxia and Doukou for aromatizing, drying and regulating Qi; Xingren for promoting lung Qi; Fuling, Zexie, Huashi, Tongcao, Danzhuye and Yiyiren for relieving and removing dampness or allowing the removal of dampness; and Mudanpi and Chishao for resolving blood stasis. The combination of these herbs can smoothen the Sanjiao Qi mechanism, dissipate and dissolve the dampness, resolve blood stasis, and dredge collaterals.

4.1.2. Progressive stage

Toxins causing dampness can increase the heat in the body; this dampness and heat can lead to infection of the lung and negatively affect the functioning of the lung. Hence, the treatment methods include elimination of dampness, transportation of aromatics to the lung, removal of Ying heat and subsequent cooling of the

blood, and detoxification. This treatment strategy utilizes Lei's aromatic turbid-resolving method and the Jiedu Huoxue Decoction combined with Shengjiang Powder [29], in which Guanghuoxiang, Peilan and Banxia are fragrant and are used for removing dampness and invigorating the stomach and spleen; Chenpi is used for promoting Qi circulation; Dafupi and Houpo are combined to promote circulation and remove dampness; and Heye can reduce the heat produced in the body and turbidity in the lung. In the Jiedu Huoxue Decoction, Chaihu can dredge the liver and the Qi; Taoren and Honghua can improve blood flow and resolve blood stasis; Chishao, Shengdihuang and Danggui can reduce the heat in the body, thereby cooling the blood and improving its nutrient composition; Lianqiao and Gegen can reduce the heat in the body and enable body detoxification; Gancao can replenish the Qi to stimulate the spleen and be combined with other Chinese medicines, including Jiangcan and Chantui of Shengjiang Powder, to reduce the gas produced and dampness in the body, reduce the heat in the body, and detoxify; Jianghuang can disperse Qi and reduce blood stasis; and Dahuang can reduce heat by causing it to descend. These drugs are used in a combination to improve their effectiveness.

4.1.3. Extreme stage

During the progressive stage, if the disease is not properly treated, the heat and dampness cause the lung to close, with the possibility of internal closure and external prolapse appearing. It is necessary to discuss treatment from the point of Qi and Yin, and treatment should be based on dispersing the Qi of the lung and fixing the body's essence Qi, improving blood flow, enabling detoxification, and rescuing the inverse. Based on these principles, the Shenfu Sini Decoction and three treasures (Angong Niuhuang Pill, Zhibao Dan, and Zixue Dan) or the Suhe Xiang Pill can be utilized [22]. In the Shenfu Sini Decoction, Fuzi warms and strengthens the kidney Yang; Gancao is beneficial to the Qi; Ganjiang can warm the spleen and stomach to dispel cold, and help Yang to dredge the blood vessels; Suhexiang can promote Qi, remove phlegm, and open the orifices to awaken the mind; and Longnao can also awaken the mind. Anxixiang is capable of circulating the Qi and blood and removing the filth; Xiangfu is used to soothe the liver, regulate Qi, and relieve depression; Muxiang soothes Sanjiao; Tanxiang relieves Qi stagnancy in the stomach; Chenxiang can warm the middle energizer and descend Qi, and warm the kidney to control Qi; Ruxiang can improve blood circulation; Dingxiang and Bibo can warm the spleen and stomach, promoting Qi and reducing cold symptoms; Baizhu invigorates the spleen and transports dampness, and it can be used together with various aromatic herbs to remove dampness and turbidity of Sanjiao; Hezi is used to relieve pain, with its astringency preventing all kinds of heat from consuming Qi; Zhusha can enhance heart detoxification and calm the mind; and Shuiniujiao can purge fire and remove toxins. Lastly, Angong Niuhuang Pill or Zixue Dan can be utilized when the heat is removed, and the Suhe Xiang Pill when Yin is closed.

4.1.4. Convalescent stage

This period is dominated by asthenia, with many changes necessitating individual syndrome differentiation and treatment. Some studies have emphasized that Xue's Wuye Lugen Decoction can be used as reference to clear the residual evil and promote lung circulation, activate the spleen, and strengthen healthy Qi [14]. The five leaves in the formula float and bring the evil to the surface. Among them, Huoxiang leaf, Bohe, fresh lotus leaf and Peilan leaf are all fragrant and good at invigorating the spleen. Lugen and Dongguazi can be used for infiltration. The formula is light and nimble and can be used for dispersing, purging, and infiltration.

Additionally, it is necessary to pay attention to the following points. (1) Focus on removing dampness. The process of dampness removal is considered during diagnosis and treatment of the disease. Although the epidemic toxin is mainly characterized by toxic heat, dampness and turbidity, it is difficult to separate the heat and dampness characteristics from the initial stages of the infection to the end. It is also difficult to only get rid of heat without removal of dampness as well. Dampness is Yin evil, and it is unsuitable to use cold and cool drugs during the early stage; warm and tonic herbs in the later stage should also be used cautiously. Furthermore, it is permissible to use a combination of drugs to resolve dampness, dry dampness, and drain dampness during different stages. (2) Promoting blood circulation and resolving blood stasis. The patients should be treated with drugs for promoting blood circulation and resolving blood stasis immediately after

the appearance of lung shadow. During COVID-19, the patients present high fever, sputum mixed with blood, dark purple-colored tongue, and consolidation or ground glass shadows in early lung images. Pathological reports indicated diffuse alveolar damage, hyperemia, hemorrhage, edema, and pulmonary interstitial inflammation, which are all manifestations of blood stasis. Therefore, during the different stages of treatment, it is necessary to combine products promoting blood circulation and eliminating blood stasis, dredging blood vessels, removing blood stasis, reducing alveolar damage, preventing pulmonary interstitial fibrosis, and reducing sequelae, which is conducive to the comprehensive rehabilitation of patients. Additionally, there are no specific Chinese medicine prescriptions for the elderly, pregnant women, children, and patients with underlying diseases. Hence, it is essential to study the signs and symptoms of COVID-19 cases to provide effective treatment.

4.2. Prevention of disease

TCM focuses on the idea of "prevention of disease," advocating prevention before the disease occurs and prevention of change after disease. Therefore, those who have a history of exposure or have been in close contact with epidemic areas should be prioritized with the treatment principles, including stabilizing Qi and strengthening the exterior, removing heat, and detoxifying the body according to the composition of the prescription and climatic factors in different regions, to practice prevention before disease occurrence. In the cases of patients with chronic illness or those susceptible to infection, emphasis should be given to strengthening the vital energy, improving immunity, and using Yupingfeng Powder to invigorate Qi and solidify the exterior [26]. Individuals with underlying diseases can be treated regionally according to their diseases. According to TCM, after conception, the whole body's Yin-blood is gathered in Chong Meridian, Ren Meridian, and the uterus, placing the pregnant women in a special physiological state of a relative deficiency of Yin-blood and relative hyperactivity of Yang Qi. The growing fetus will block the rise and fall of Qi and blood, resulting in poor operation of Qi and blood. "It should be cool before pregnancy and warm after birth" is the general principle of regulation and stabilization in TCM before and after pregnancy. This can be adjusted and treated in advance to prevent the occurrence of disease, and the method of nourishing Yin and clearing heat, regulating the Qi mechanism, and calming the fetus is adopted. Young children are not full of Qi; their Yin and Yang are immature, and their viscera are delicate, especially, the lung, spleen and kidney [26]. Hence, it is recommended to protect the Qi of the spleen and stomach in advance to nourish other organs, strengthen their physique, and resist the evil of epidemic disease, utilizing Sijunzi Decoction or Yupingfeng Powder to invigorate the Qi for consolidating superficies [26]. According to TCM, different people have different physical characteristics, and hence, different treatment principles and medication plans should be generated based on their physical characteristics (Table 3). Based on the existing treatment schemes, the prescription composition and dosage recommended for prevention and treatment of COVID-19 during different stages are introduced in Table 4.

4.3. Evaluation of the curative effect

According to the description of discharge criteria in the *Diagnosis and Treatment Protocol* for *COVID-19* (*Trial Version 7)* [40], the evaluation of COVID-19 should be based on body temperature (more than 3 days after returning to normal), obvious improvement of respiratory symptoms, and pulmonary imaging (obvious alleviation of inflammation). If the above symptoms and signs are relieved and the respiratory pathogen nucleic acid test is negative for two consecutive times (the sampling interval is at least 24 hours), patient can be considered to be cured.

However, we believe that the quantification of lung imaging related to the above efficacy evaluation index is insufficient. Hence, we suggest that the lung imaging of patients with COVID-19 should be comprehensively evaluated, based on the definition of the lung segment and the density criteria of high, medium and low grades, to better evaluate the treatment efficacy.

5. Prospect

The use of TCM adopts the holistic concept, combined with symptoms, signs and other corresponding manifestations, to examine the syndromes and seek the causes, to support the selection of drugs based on syndrome differentiation. Syndrome differentiation and treatment, as one of the characteristics and advantages of TCM, is very important in diagnosis and treatment. For example, in the treatment of COVID-19, the syndrome types differ among individuals in different regions, under different climatic conditions, and for relatively different physiques. Syndrome differentiation should be clearly defined in combination with different factors and clinical syndrome characteristics. Prescriptions are mainly used to improve immunity, balance human Qi, blood, Yin and Yang, and recover organ function. According to the study of COVID-19 treatment herbs [41], [42], Chinese herbal medicines with a higher probability to directly inhibit SARS-CoV-2 have been selected, including Lianqiao, Gancao, Sangbaipi, Jinyinhua, Sangye, Pipaye, and other herbs, commonly used to treat viral pneumonia. Drugs should be selected and utilized according to the corresponding syndrome types. Furthermore, with disease development, the drugs should be given the flexibility to deal with different syndrome types, and the whole process should be adjusted according to the principle of "three factors and measures," undertaking multi-target treatment. TCM also plays a leading role during the recovery period and post-discharge convalescence. Currently, although some patients present a negative nucleic acid test accompanied by alleviated pneumonia and recovered body temperature, they may still demonstrate symptoms, including fatigue, shortness of breath, loss of appetite, and anxiety. Some patients may present pulmonary fibrosis due to poor absorption of pulmonary inflammation, which may affect the quality of life; additionally, some patients may suffer adverse reactions during drug therapy. It is crucial to resolve such related issues when TCM is involved. However, we do not advocate taking medicine without incident, and the preventive prescription is only a corresponding choice for people with an imbalance of Qi, blood, and Yin and Yang. When selecting the prescription, attention should be paid to the syndrome and symptoms, especially in special groups, such as children, pregnant women, and the elderly. Moreover, any special physique should be closely monitored when using medicines, and the improper use of interventions is not recommended; the overuse of cold or hot drugs should be avoided. Current research on Qingfei Paidu Decoction, a combination of prescriptions from the *Treatise on Febrile and Miscellaneous Diseases* written by Zhang Zhongjing in the Han Dynasty, has made progress in the special project documented by the National Administration of Traditional Chinese Medicine of China on screening effective prescriptions for the prevention and treatment of the novel coronavirus pneumonia [43]. As of February 5, four pilot provinces have used the Qingfei Paidu Decoction to treat 214 confirmed cases, with a treatment course of 3 days, demonstrating a total efficacy rate exceeding 90%, among which over 60% of the patients showed significant improvement in symptoms and imaging manifestations, and 30% patients demonstrated stabilized symptoms without aggravation. Concurrently, Lianhua Qingwen Granule [44] is used in clinical treatment based on the principle of clearing away epidemic toxin, dispersing the lung, and expelling heat. Reportedly, it has been confirmed to significantly improve fever, cough, expectoration, and shortness of breath in confirmed cases of COVID-19. According to the feedback of clinical data, the efficacy of TCM in the clinical treatment of COVID-19 is great, and it still needs to be adjusted and improved with the clinical accumulation. In brief, the combination of Chinese and Western medicine can complement each other's advantages, which can improve the comprehension of the core of the disease, and cooperate actively and effectively in clinical settings to enhance overall efficacy.

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Authors' contributions

WSX and LYB wrote the article; WY revised part of the article and made grammatical corrections; WXX provided research ideas; LJY reviewed the article and provided guidance; NM and SYJ collated the data.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Abstract

Viral pneumonia is caused by a spreading of lung infection caused by respiratory viruses. Some virus infections were found to be highly aggressive, leading to lung inflammation and severe damage in respiratory system with high fatality rate. Currently, there is no effective therapeutic drugs in the clinic. The common clinical symptoms of viral pneumonias include fever, rhinitis, runny nose, nonproductive cough, fatigue, myalgias and headaches after the immune system being tricked by driving cytokines and overactivated immune response induced by cytokine storms. Patients with severe symptoms could get persistent high fever, dysfunctional breathing, consciousness disorders and even respiratory failure, post-inflammatory pulmonary fibrosis, multi-organ damages, shock and so on. Most clinical treatments are used to inhibit virus replication, relieve symptoms, inhibit excessive inflammatory response, regulate immune balance and protect organs. Both applied and basic research demonstrate that Chinese patent medicine has certain anti-viral effects, effectively inhibiting viral pneumonia transiting from mild to severe, rapid relieving of patient symptoms because of their multi-component and multi-target integrated roles. This review has summarized the reports on the treatment of viral pneumonia. Based on the pathogenic characteristics of viral pneumonia, this paper summarizes the diverse roles of the marketed Chinese patent medicine, such as their effects in inhibiting the progress of viral replication and overactivated inflammatory response, regulating immune balance, attenuating pulmonary fibrosis and so forth. Our paper summarizes the advantages of Chinese patient medicine in the treatment of viral pneumonia, based on which improvements of clinical therapy are expected to be made soon.

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Abstract

The study aimed to investigate the multi-constituent, multi-target mechanism of Xuanfei Baidu Tang(XFBD) in the treatment of coronavirus disease 2019(COVID-19), through exploring the main ingredients and effective targets of XFBD, as well as analyzing the correlation between XFBD targets and COVID-19. The compounds of each herb in XFBD were collected from TCM-PTD, ETCM, TCMSP and SymMap database. Next, the information of meridian tropisms was collected from Chinese Pharmacopoeia(2015 edition), and the target information of the major constituents of XFBD were obtained from TCM-PTD, ETCM, TCMSP and TargetNet database. Subsequently, the target network model and the major modules were generated by Cytoscape, and the functional enrichment analysis of XFBD targets were completed by DAVID and STRING. As a result, ten of the 13 herbs in XFBD belonged to the lung meridian, and 326 of the 1 224 putative XFBD targets were associated with the disease target of COVID-19, among which 109 targets were enriched in the disease pathways of viral infection and lung injury. The main biological pathways regulated by the key XFBD targets included viral infection, energy metabolism, immunity and inflammation, parasites and bacterial infections. In conclusion, the therapeutic mechanism of XFBD in COVID-19 showed a multi-herb, multi-constituent, multi-target pattern, with lung as the chief targeted organ. By regulating a series of biological pathways closely related to the occurrence and development of diseases, XFBD plays a role in balancing immunity, eliminating inflammation, regulating hepatic and biliary metabolism and recovering energy metabolism balance.

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Abstract

Beginning in December 2019, coronavirus disease 2019 (COVID-19), due to 2019-nCoV infection, emerged in Wuhan and spread rapidly throughout China and even worldwide. Employing combined therapy of modern medicine and traditional Chinese medicine has been proposed, in which Ma Xing Shi Gan Decoction (MXSGD) was recommended as a basic prescription and applied widely in the clinical treatment of COVID-19. We investigated the underlying mechanism of MXSGD in treating COVID-19 utilizing the approaches of integrating network pharmacology. A total of 97 active ingredients of MXSGD were screened out, and 169 targets were predicted. The protein-protein interaction network exhibited hub targets of MXSGD, such as Heat shock protein 90, RAC-alpha serine/threonine-protein kinase, Transcription factor AP-1, Mitogen-activated protein kinase 1, Cellular tumor antigen p53, Vascular endothelial growth factor A, and Tumour necrosis factor. Gene Ontology functional enrichment analysis demonstrated that the biological processes altered within the body after taking MXSGD were closely related to the regulation of such processes as the acute inflammatory response, chemokine production, vascular permeability, response to oxygen radicals, oxidative stress-induced apoptosis, T cell differentiation involved in the immune response, immunoglobulin secretion, and extracellular matrix disassembly. KEGG enrichment analysis indicated that the targets of MXSGD were significantly enriched in inflammation-related pathways, immunomodulation-related pathways, and viral infection-related pathways. The therapeutic mechanisms of MXSGD on COVID-19 may primarily involve the following effects: reducing inflammation, suppressing cytokine storm, protecting the pulmonary alveolar-capillary barrier, alleviating pulmonary edema, regulating the immune response, and decreasing fever.

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Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment

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SUMMARY

Pneumonia associated with the 2019 novel coronavirus (2019-nCoV) is continuously and rapidly circulating at present. No effective antiviral treatment has been verified thus far. We report here the clinical characteristics

and therapeutic procedure for four patients with mild or severe 2019-nCoV pneumonia admitted to Shanghai Public Health Clinical Center. All the patients were given antiviral treatment including lopinavir/ritonavir (Kaletra®), arbidol, and Shufeng Jiedu Capsule (SFJDC, a traditional Chinese medicine) and other necessary support care. After treatment, three patients gained significant improvement in pneumonia associated symptoms, two of whom were confirmed 2019-nCoV negative and discharged, and one of whom was virus negative at the first test. The remaining patient with severe pneumonia had shown signs of improvement by the cutoff date for data collection. Results obtained in the current study may provide clues for treatment of 2019-nCoV pneumonia. The efficacy of antiviral treatment including lopinavir/ritonavir, arbidol, and SFJDC warrants further verification in future study.

Keywords 2019-nCoV, lopinavir, ritonavir, arbidol, Shufeng Jiedu Capsule

1. Introduction

Coronaviruses mainly cause respiratory tract infections and some strains have high infectivity and mortality as well as heavy damage on public health, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (1). A pneumonia associated with the 2019 novel coronavirus (2019- nCoV) emerged in Wuhan, China in December, 2019 and has spread rapidly, with 24,324 confirmed cases in mainland China as of February 4, 2020 (2,3). The most common clinical presentation is fever, fatigue, and dry cough and some patients present with nasal congestion, runny nose, and diarrhea (4). In severe cases, dyspnea usually occurs one week after the disease onset and some patients can rapidly progress to acute respiratory distress syndrome (ARDS), septic shock, refractory metabolic acidosis, and coagulation disorders (4). Thus far, there is no approved or verified effective drugs specific to the virus (5). We report here that four patients with mild or severe 2019-nCoV pneumonia have been cured or have significant improvement in their respiratory symptoms after treatment with combined lopinavir/ritonavir (Kaletra®), arbidol, and Shufeng Jiedu Capsule (SFJDC, a traditional Chinese medicine) on the base of supportive care.

2. Methods

2.1. Patients

For this retrospective study, four patients were recruited from January 21 to January 24, 2020 at Shanghai Public Health Clinical Center, Shanghai, China, which is a designated hospital for 2019-nCoV pneumonia. All patients were diagnosed as having 2019-nCoV pneumonia according to WHO interim guidance. Informed consent to therapeutic regimen was obtained from each patient prior to treatment.

2.2. Data collection

Epidemiological, demographic, clinical, laboratory, management, and outcome data were collected through a review of medical records. Clinical outcomes were followed up until February 4, 2020. Laboratory confirmation of 2019-CoV was done in Shanghai Municipal Center for Disease Control and Prevention. Throat-swab specimens from the upper respiratory tract that were obtained from all patients at admission were maintained in viral-transport medium. 2019- nCoV was confirmed by real-time RT-PCR using the same protocol described previously (6). All patients were given chest computed tomography (CT) or chest radiography.

3. Results and Discussion 3.1. Demographics and baseline characteristics

Four patients with 2019-nCoV are included in this study, two of whom are under the age of 35 and the other two are over the age of 60 (Table 1). All the patients had epidemiologic linkage to areas with community transmission of 2019-nCoV. Among them, two patients (Case 1 and 4) had recent travel history to Wuhan, one patient (case 2) is a student who was ordinarily a resident in Wuhan and went back to

Shanghai for winter holiday, and one patient (Case 3) is the husband of a confirmed 2019-nCoV case. It took 11 and 6 days from disease onset to confirmed diagnosis for case 1 and case 2, while 1 and 2 days for case 3

and case 4. Fatty liver was reported in the case 1. No underlying medical conditions were reported in the other three cases.

3.2. Clinical characteristics and laboratory assessment

On admission, the most common symptoms were fever or history of fever, followed by cough, fatigue, dizziness, nasal congestion, and rhinorrhea (Table 2). Diarrhea was not observed in all patients, on the contrary, two of them were reported to have constipation. Physical examination revealed increased respiratory rate in three patients, one of whom had tachypnea (26/min). Lung auscultation revealed rhonchi in left or right lower lobe in three patients. In all patients, there were marked abnormalities on chest radiography; involvement of both lungs was found by chest computerized tomography (CT) in 2 patients at presentation. Ground-glass opacities and consolidation were the most common radiologic findings. On admission, leucocytes were in the normal range in all the patients (Table 3). One patient (case 4) had neutrophils above the normal range, indicating the existence of concurrent bacterial infection. Lymphocytes were below the normal range in one patient (case 4) and within the normal range in other three patients. Blood gas analysis revealed that oxygen pressure was below the normal range in two patients (7.60 kPa in case 3 and 5.45 kPa in case 4) (Table 3). On the basis of the above results, two patients (case 1 and 2) were diagnosed with mild pneumonia and the other two patients (case 3 and 4) with severe pneumonia

Table 1. Demographics, baseline characteristics, and clinical outcomes of 4 patients admitted to Shanghai Public Health Clinical Center

Items	Case 1	Case 2	Case 3	Case 4
Age	32	19	63	63
Sex	Male	Male	Male	Female
Exposure history	Recent travel to Wuhan	Resident of Wuhan	Close contact with 2019-nCoV patient	Recent travel to Wuhan
Chronic medical illness	Fatty liver	None	None	None
Days from illness onset to diagnosis confirmation	11	6	1	2
Clinical outcome	Discharged	Discharged	Remained in hospital	Remained in hospital

Table 2. Clinical characteristics at presentation and treatment of patients with 2019-nCoV pneumonia

Items	Case 1	Case 2	Case 3	Case 4
Signs and symptoms				
Fever	Yes	Yes	Yes	Yes
Cough		Yes	Yes	Yes
Fatigue	Yes	Yes		
Dizziness	Yes			Yes
Nasal congestion		Yes		
Rhinorrhea		Yes		
Constipation	Yes			Yes
Respiratory rate	22/min	19/min	26/min	22/min
Lung auscultation	Rhonchi (left lower lobe)	No rhonchi	Rhonchi (right lower lobe)	Rhonchi (left lower lobe)
Chest CT findings				
Unilateral pneumonia		Yes	Yes	
Bilateral pneumonia	Yes			Yes
Treatment				
Oxygen therapy	Yes	Yes	Yes	Yes
Mechanical ventilation				Yes
Antibiotic treatment	Yes	Yes	Yes	Yes
Lopinavir/ritonavir/arbidol/SFJDC	Yes	Yes	Yes	Yes
Intravenous immunoglobulin therapy				Yes

Table 3. Clinical laboratory results of patients with 2019-nCoV pneumonia

Variable	Case 1		Case 2		Case 3		Case 4	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Blood, routine								
Leucocytes ($\times 10^9$ per L; normal range 3.5-9.5)	4.23	4.68	6.48	6.58	4.40	5.31	6.84	10.84
Neutrophils (%; normal range 50-70)	57.2	49.1	57.0	47.6	50.0	55.4	93	94
Lymphocytes (%; normal range 20-40)	30.3	37.1	30.6	39.4	24.5	25.0	6.10	3.2
Blood gas analysis								
pH (normal range 7.35-7.45)	7.33	7.33	7.43	7.33	7.40	7.36	7.44	7.33
PCO_2 (kPa, normal range 4.65-6.0)	5.42	6.05	4.55	5.96	5.45	5.59	4.23	5.52
PO_2 (kPa, normal range 10.6-13.3)	22.00	11.90	16.6	13.4	7.60	12.0	5.45	21.9

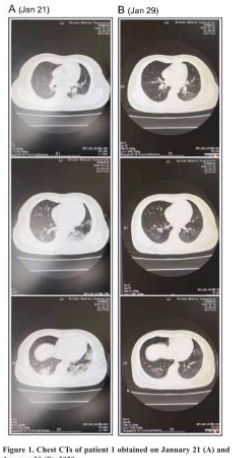

Figure 1. Chest CTs of patient 1 obtained on January 21 (A) and January 29 (B), 2020.

Figure 1. Chest CTs of patient 1 obtained on January 21 (A) and January 29 (B), 2020

3.3. Treatment and clinical outcomes

All patients received antiviral treatment, including lopinavir/ritonavir (Kaletra®, lopinavir 400 mg/ritonavir 100 mg, q12h, po), arbidol (0.2 g, tid, po), and SFJDC (2.08 g, tid, po). The duration of antiviral treatment was 6-15 days. In addition, all patients were all given antibiotic treatment and started on supplemental oxygen, delivered by nasal cannula after admission to hospital (Table 2).

Patient 1 was admitted to hospital on January 21, 2020 and thereafter received the above treatment. On January 27, routine blood analysis revealed that leucocytes and lymphocytes were increased, indicating recovery and restoration of immune function (Table 3). On January 29, chest CT demonstrated bilateral pneumonia with scattered multiple nodules, which was obviously improved compared with that obtained on January 21 (Figure 1). 2019-nCoV was twice negative in throat-swab specimens from the upper respiratory tract. The patient was free of fever, productive cough, dyspnea, short breath, abdominal pain, and diarrhea, and thus discharged on January 29, 2020. Patient 2 was admitted to hospital on January 24, 2020 and then received the above mentioned treatment. On January 28, routine blood analysis showed increased count of leucocytes and lymphocytes (Table 3). Blood gas analysis revealed no obvious abnormity. On January 29, chest CT revealed unilateral pneumonia in the left lobe, which was mildly improved compared with the images obtained on January 24 (Figure 2). Results of two continuous 2019-nCoV tests were negative for throat-swab specimens. Symptoms associated with pneumonia had improved and the patient was discharged on January 30, 2020.

Patient 3 was admitted to hospital on January 24, 2020 and thereafter received the above mentioned treatment. The fever disappeared after one day of treatment. On January 29, chest CT showed progressed pneumonia in the right lobe (Figure 3). The treatment was continuous and the pneumonia appearance improved on February 1 as reflected by the CTimage (Figure 3). On February 3, blood gas analysis demonstrated obviously increased oxygen pressure compared with that at admission. The patient had mild cough with white phlegm, and was free of fever, dyspnea, short breath, abdominal pain, and diarrhea. 2019-nCoV test result was negative for the first time on February 4, 2020. The patient remained in hospital for the second virus test.

Patient 4 was admitted to hospital on January 22, 2020. In addition to the above mentioned treatments, the patient was also given human seroalbumin and γ-immunoglobulin. On January 31, the patient was given an intubated ventilator-assisted breathing therapy because of refractory low blood oxygen pressure. Routine blood analysis on February 1 demonstrated the percentages of neutrophils and lymphocytes were 94% and 3.2%, respectively, which were comparable with those at admission (Table 3).

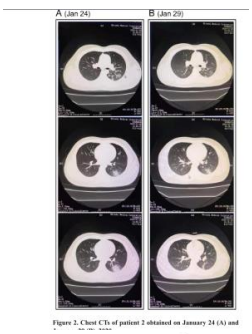

Figure 2. Chest CTs of patient 2 obtained on January 24 (A) and

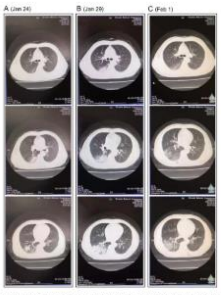

Figure 3. Chest CTs of patient 3 obtained on January 24 (A) and January 29 (B), and February 1 (C), 2020.

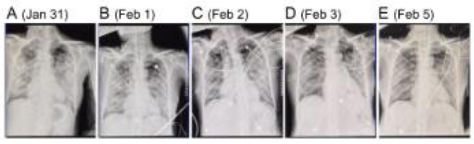

Figure 4. Posteroanterior chest radiographs of patient 4 obtained on January 31 (A), February 1 (B), February 2 (C), February 3 (D), and February 5 (E), 2020.

Chest radiography demonstrated bilateral pneumonia, which improved compared to the image obtained on January 31 (Figure 4). Chest radiograph on February 2 revealed further mild improvement. On February 3, bilateral pneumonia remained but the appearances of left lobe improved and right lobe mildly worsened. On February 5, the appearance of pneumonia improved compared with the last image (Figure 4). The patient was still using ventilators at data cutoff.

We report here the clinical characteristics and therapeutic procedure for four patients with 2019- CoV pneumonia receiving comprehensive therapy. The antivirus treatment regimen includes lopinavir/ritonavir (Kaletra®), arbidol, and SFJDC. By February 4, 2020, two patients were confirmed 2019-nCoV negative and one patient was virus-negative at the first test. Lopinavir/ ritonavir (Kaletra®) is a human immunodeficiency virus (HIV) medicine used in combination with other medicines to treat adults and children over 14 days of age who are infected with HIV-1 (7). It was revealed that lopinavir/ritonavir among SARS-CoV patients was associated with substantial clinical benefit (fewer adverse clinical outcomes) (8). The combination of lopinavir and ritonavir is currently a recommended antivirus regimen in the latest version of Diagnosis and Treatment of Pneumonia Caused by 2019-nCoV (version 5) issued by National Health Commission of the People's Republic of China (4). Arbidol is an antiviral treatment for influenza infection used in Russia and China (9). It was claimed that arbidol was effective against 2019-nCoV at a concentration range of 10-30 μM in vitro (10). A randomized multicenter controlled clinical trial of arbidol in patients with 2019-nCoV (ChiCTR2000029573) has been initiated in China (11). SFJDC is a traditional Chinese medicine for treatment of influenza in China. This drug is also recommended for treating 2019-nCoV infection in the latest version of Diagnosis and Treatment of Pneumonia Caused by 2019-nCoV (version 5) (4).

In conclusion, two mild and two severe 2019- nCoV pneumonia patients were given combined Chinese and Western medicine treatment, three of whom gained significant improvement in pneumonia associated associated symptoms. The remaining patient with severe pneumonia has shown signs of improvement by the cutoff date for data collection. The efficacy of antiviral treatment including lopinavir/ritonavir, arbidol, and SFJDC warrants further verification in future study.

Acknowledgements

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Abstract

Background: There is a growing trend in the use of mobile health (mHealth) technologies in traditional Chinese medicine (TCM) and telemedicine, especially during the coronavirus disease (COVID-19) outbreak. Tongue diagnosis is an important component of TCM diagnosis. However, the procedure of obtaining tongue images has not been standardized and the reliability of tongue diagnosis by smartphone tongue images has yet to be evaluated.

Objective: The first objective of this study was to develop an operating classification scheme for tongue coating diagnosis. The second and main objective of this study was to determine the intra-rater and inter-rater reliability of tongue coating diagnosis using the operating classification scheme.

Methods: An operating classification scheme for tongue coating was developed using a stepwise approach and a quasi-Delphi method. First, tongue images (n=2023) were analyzed by 2 groups of assessors to develop the operating classification scheme for tongue coating diagnosis. Based on clinicians' (n=17) own interpretations as well as their use of the operating classification scheme, the results of tongue diagnosis on a representative tongue image set (n=24) were compared. After gathering consensus for the operating classification scheme, the clinicians were instructed to use the scheme to assess tongue features of their patients under direct visual inspection. At the same time, the clinicians took tongue images of the patients with smartphones and assessed tongue features observed in the smartphone image using the same classification scheme. The intra-rater agreements of these two assessments were calculated to determine which features of tongue coating were better retained by the image. Using the finalized operating classification scheme, clinicians in the study group assessed representative tongue images (n=24) that they had taken, and the intra-rater and inter-rater reliability of their assessments was evaluated.

Results: Intra-rater agreement between direct subject inspection and tongue image inspection was good to very good (Cohen K range 0.69-1.0). Additionally, when comparing the assessment of tongue images on different days, intra-rater reliability was good to very good (K range 0.7-1.0), except for the color of the tongue body (K=0.22) and slippery tongue fur (K=0.1). Inter-rater reliability was moderate for tongue coating (Gwet AC2 range 0.49-0.55), and fair for color and other features of the tongue body (Gwet AC2=0.34).

Conclusions: Taken together, our study has shown that tongue images collected via smartphone contain some reliable features, including tongue coating, that can be used in mHealth analysis. Our findings thus support the use of smartphones in telemedicine for detecting changes in tongue coating.

87. **Wen E. Guía de prevención y tratamiento de Wen Bing del Síndrome de Humedad-Calor de Taiyin Pulmonar (Neumonía por Corona- virus). Classical Chinese Medicine Research. 2020; do:10.12032/CCMR2020004**

Classical Chinese Medicine Research doi : 10.12032/CCMR2020004

Guía de prevención y tratamiento de Wen Bing del Síndrome de Humedad-Calor de Taiyin Pulmonar (Neumonía por Corona- virus)

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Resumen: La Enfermedad Febril por Calor incluye enfermedad Wén Bing (溫病), Wen Bing (瘟病), Yi Bing (疫病) y Li Bing (疠病) en la Medicina Tradicional China (MTC). El Wen Bing del Síndrome de Humedad-Calor de Taiyin Pulmonar (incluye los Yi Bing y Li Bing) abarca múltiples tipos de enfermedades infecciosas pulmonares, tales como: Leptospirosis Hemorrágica Pulmonar, Peste Neumónica, Neumonía por Coronavirus, etc. Existen cuatro fases en el desarrollo de la Neumonía por Coronavirus: fase temprana (grado leve, preponderancia de la Humedad sobre el Calor), fase intermedia (grado moderado, igual gravedad de Humedad y Calor), fase crítica (grado severo) y fase secuelar. Este artículo tiene como objetivo brindar herramientas de manejo clínico en el diagnóstico, tratamiento, pronóstico, prevención y cuidados posteriores a la Neumonía por Coronavirus. Palabras claves: Humedad-Calor, Wen Bing, Coronavirus, Neumonía.

Declaración de intereses: El autor declara no poseer ningún conflicto de interés. Cita bibliográfica: Xiong-Zhi, Wu. Guía de prevención y tratamiento de Wen Bing del Síndrome de Humedad-Calor de Taiyin Pulmonar (Neumonía por Coronavirus). Classical Chinese Medicine Research.
https://www.tmrjournals.com/ccmr/CN/10.12032/CCMR2020004. Editor Ejecutivo: Yang-Tao Li, Ying-Chun Sun. Enviado: 23 de febrero de 2020, Aceptado: 23 de febrero de 2020, En línea: 23 de febrero de 2020

Introducción

Wén Bing (溫病) es la Enfermedad Febril por Calor. Tiene como eje la respuesta inflamatoria que es causada en la mayoría de las veces por enfer- medades infecto-contagiosas de la Medicina Moderna. Wen Bing (瘟病) es un subtipo de la enfermedad anteriormente mencionada. Posee capacidad infecto-contagiosa, y puede dar muerte a grupos de personas con disminución de la inmunidad tales como ancianos, lactantes e infantes. Yi Bing (疫病) se refiere a Wen Bing pero con marcada letalidad y puede provocar la muerte incluso adultos jóvenes sanos. Li Bing (疠病) se refiere a Yi Bing con mayor infectividad y letalidad aún.

Sin importar si el estado inmune de los contactos cercanos sea alto o bajo, son propensos de contraer la enfermedad. Los que debutan en la fase crítica, tiene alta mortalidad a corto plazo, en cuestión de minutos, horas o días. Por estas razones, esto ha causado la extinción de familias completas y hasta pueblos enteros en la antigüedad. El Wen Bing que trata esta guía engloba al Wen Bing, Yi Bing y Li Bing.

Sugiero al Estado que publique documentos relacionados al mismo, así como elaborar un nomenclador de guía de conceptos de Wen Bing, Yi Bing y Li Bing desde MTC. (Consultar el nomenclador de las de enfermedades infecto-con- tagiosas, categoría A, B y C de la medicina moderna). Wén Bing se divide en dos categorías: Enfermedad por Calor intenso y Enfermedad por Humedad-Calor. La Enfermedad por Calor intenso se carac- teriza anatomo-patológicamente por una inflamación de tipo alterativa, donde predomina el daño tisular y la necrosis celular. En cambio, la inflama- ción en la Enfermedad por Humedad-Calor es de índole exudativa donde pre- valece el edema y la exudación tisular que evoluciona en la mayoría de los casos hacia la fibrosis. La Enfermedad Taiyin incluye a las enfermedades del Taiyin Bazo (tracto digestivo) y del Taiyin Pulmón (vías respiratorias). El Wen Bing del Síndrome de Humedad-Calor de Taiyin Pulmonar (incluye los Yi Bing y Li Bing) abarca múltiples tipos de enfermedades infecciosas pulmonares, tales como: Leptos- pirosis Hemorrágica Pulmonar, Peste Neumónica y Neumonía por Coronavirus, etc. Esta guía trata principalmente la Neumonía por Coronavirus, explicando y debatiendo detalladamente a cerca de la prevención y el tratamiento del Wen Bing del Síndrome de Humedad-Calor de Taiyin Pulmonar.

En esta enfermedad es necesario tomar en cuenta las características de la localización de la afección por la Humedad patógena: ① Si la Humedad-Calor no reside en el Bazo-Estómago, reside en Hígado-Vesícula Biliar. Esto se debe a que el Bazo es el origen de la Humedad. Los que sufren de Insuficiencia de Bazo, les es más fácil sufrir la Humedad por afección interna y externa. Shaoyang-Sanjiao es la ruta de humores que par- ticipa en el metabolismo del agua y de los humores, a su vez, “El Qi del Fuego trata las enfermedades en Shaoyang”, esto facilita la génesis de Calor por Represión de Humedad o la afección directa del Calor Patógeno. Por estas razones, en el tratamiento de dicha enfermedad hace hincapié en el Taiyin y Shaoyang. Clínicamente, es frecuente la lesión hepática; la fiebre se acom- paña de astenia (el Hígado es la base de la resistencia a la

fatiga) y, el rechazo a las grasas, síntomas característicos de la lesión en Shaoyang. Otros síntomas a tomar en cuenta son: sensación de gusto amargo en la boca, molestias en la garganta, ojo rojo, pulso de cuerda e hinchazón de los bordes de la lengua. ② La Humedad Patógena impregna Sanjiao, difundiéndose hacia arriba y hacia abajo; hacia arriba se presenta con tos; hacia el medio con diarrea, náuseas, vómitos y distensión abdominal; hacia abajo, dificultad para orinar e injuria renal. ③ La Humedad-Calor puede presentar tanto manifestaciones externas como internas. Por afuera, es frecuente el rush cutáneo y por adentro, daño miocárdico que en casos severos lleva al shock y al coma. ④ Si hay Exceso, es Yangming; si hay Insuficiencia, es Taiyin. Por ende, Yangming predomina Calor sobre Humedad, y Taiyin viceversa.

Claves en las indicaciones médicas: ① El calor no se libera mediante diaforesis. Luego de que el paciente transpira, baja la fiebre y sienta mejoría clínica, con o sin tratamiento, no significa que la enfermedad se haya aliviado. Sin embargo, muchos pacientes presentan fiebre alternante con intervalos de mejoría que se caracterizan por la incapacidad de recuperar la normalidad del pulso (pulso tranquilo), de la temperatura corporal (frialdad) y de la saburra lingual. ② Los que sufren Calor Ondulante en horario fijo (Ej: muchos lo hacen por la tarde), pueden recibir una dosis de medicación treinta minutos a una hora previo al próximo evento (Ej: Los que sufren de Calor intenso luego de las 13:00 horas, adicionar una dosis extra de Yin Chai Xiao Du Dan, ya que el Calor se encuentra en Yangming; por otro lado, los que lo hacen antes de las 13:00 horas o inclusive antes de las 11:00 horas, hay más posibilidad de rece- tar Chai Ling Tang, puesto que el Calor se encuentra casi siempre en Taiyang. ③ A los más graves se les puede indicar una dosis cada 4 horas, mientras que a los críticos, cada 3 horas. Tener especial cuidado con el Bazo y el Estó- mago. ④ El curso de la enfermedad en los pacientes leves es aproximadamente de 14 días. No obstante, pese a la mejoría clínica íntegra luego de unos días de tratamiento, se recomienda completar los 14 días de tratamiento.

1. Fase temprana

1.1Clasificación de fases y tipos

Esta fase se encuentra más frecuente en:

① Fase temprana de la enfermedad: Tener en cuenta que la fase temprana y la fase intermedia no dependen de manera determinante al tiempo de evolución de la enfermedad. Muchos pacientes permanecen en esta fase (los leves), otros debutan en fase intermedia (los moderados), y algunos fa- llecen de manera rápida (los críticos).

② Paciente con marcada Insuficiencia del Bazo, que presenta mayor Humedad que Calor: Al momento de la examinación, se debe prestar mucha atención en la parte media y basal de la lengua ya que si se encuentra pálida, indica Insuficiencia del Bazo o del Yang. Cuando la fiebre no es muy alta o después del descenso de la temperatura con medicación, se puede explorar los dedos de los pies para valorar si hay Insuficiencia del Yang, debido a que los que sufren Insuficiencia del Yang severo, los dedos de sus pies permanecen fríos aun cuando estén cursando una fiebre. Las improntas dentales en losbordes de la lengua indican también Insuficiencia del Bazo, y hay que dife- renciarlo de la hinchazón lingual lateral (fenómeno de represión de Qi del hígado). Cuando ésta última es difícil de apreciar, inspeccionar las improntas dentales en la cara interna de las mejillas.

③ Pacientes leves: Hay que prestar especial atención porque a veces existe una disociación de la clínica con el curso de la enfermedad. Es decir, que sea leve o no, no depende meramente de la presentación clínica, sino que es necesario una evaluación completa semiológica de lengua y pulso, con o sin métodos diagnósticos complementarios contemporáneos como radiografía de tórax o tomografía computada.

La fase temprana muchas veces es diagnosticada de manera errónea como Síndrome Frío-Humedad debido a las siguientes causas:

① La Insuficiencia del Bazo produce Humedad interna, y por afección interna externa, se torna más fácil contraer Patógeno Externo. Estos pacientes presentan a menudo lengua pálida con improntas dentales.

② Mientras más Humedad haya, más saburra blanca se produce, y a veces oculta toda la lengua roja; otras veces se ignora el enrojecimiento del dorso lingual generado por represión del Calor. ③ En el comienzo de la transformación en Calor, se puede observar una capa fina de saburra amarilla por encima de la blanca muchas veces desaper- cibida. Es imprescindible interrogar sobre la materia fecal. La saburra ama- rrilla de los estreñidos desaparecerá una vez que se evacué; por otro lado, la saburra amarilla es producida por la respuesta inflamatoria y confirma la transformación en Calor.

④ La saburra de los inmunosuprimidos no se convertirá en amarilla a pesar de la transformación en Calor.

⑤ La respuesta inflamatoria necesita entre horas a uno o dos días para que los leucocitos se conviertan en piocitos. Por eso, los pacientes con Enfer- medad Calor intenso muestran saburra blanca y seca en la fase temprana. Se puede precisar la saburra granulada en la inspección detallada pese a que la Humedad pueda encubrir la Sequedad.

⑥ Otro punto para recalcar del Síndrome Frío-Húmedad es la presenciadel pulso moderado en ausencia de fiebre, a diferencia de un pulso basal le- vemente más rápido en el Síndrome Humedad-Calor. Por estas razones, no se debe diagnosticar como Frío-Húmedad.

⑦ Algunos pacientes aparentan lengua pálida con saburra blanca y grasienta. Empero, una vez que reciben medicación del Calor, se vuelven con el pulso taquisfígmico y saburra amarillenta, coincidente con el mecanismo de expansión de Humedad-Calor del Wen Bing de Humedad-Calor. En cambio, esta transformación no ocurre en el Síndrome Frío-Humedad, un ejemplo de lo último es Li Zhong Tang para el Síndrome Frío-Humedad por Cólera

⑧ Debido a la presencia de una noxa clara y determinada en Wen Bing, no se sostiene la hipótesis de que una parte de esta enfermedad sea producida por Humedad-Calor y otra por Frío-Humedad. La Humedad es un patógeno Yin y el Calor, un patógeno Yang. Contemplando la variabilidad de la consti- tución física, sea más frío o más calor, se produce diferentes tipos de mani- festaciones clínicas. Sin embargo, esto no influye en el diagnóstico del Sín- drome Humedad-Calor de esta enfermedad. Por lo tanto, solo hay que ajustar las fórmulas según la variabilidad de la constitución física y el nivel de Hume- dad o Calor.

Otros tantos presentan tos seca o con escasa flema, que pueden llevar a un error diagnóstico de Insuficiencia del Yin. La escasa flema se debe a difícil expectoración por su localización en el intersticio alvéolo pulmonar, diferente de la bronquitis y de la bronquiolitis. Por este motivo, no se puede diagnosti- car como Insuficiencia del Yin o ausencia de la Humedad por la escasa flema.

1.2 Tratamiento

1.2.1 Elaboración de la fórmula

Fórmula de Wu Men, Chai Ling Tang modificada. Ingredientes: Chai Hu 24g, Huang Qin 9g, Gui Zhi 6-9g, Bai Zhu 9g, Fu Ling 10g, Zhu Ling 10g, Ze Xie 9g, Sheng Gan Cao 6g, Shi Shang Bo 30g, Lian Qiao 30g.

1.2.2 Explicación de la fórmula

En el ¨Shang Han Lun¨ está documentado el uso de la fórmula Wu Ling San para el tratamiento de vómito, diarrea, tos y fiebre por afección externa; y Xiao Chai Hu Tang para el Calor por afección externa. Sanjiao es la ruta de los humores, Xiao Chai Hu Tang también se indica para la tonificación de los movimientos de los líquidos corporales, así como se dice: ¨Si el Jiao superior (Shangjiao) se desbloquea, los líquidos y los humores bajan, entonces, el Qi del estómago se armoniza y el Calor se libera por sudoración lluviosa.¨ [3] Shi Shang Bo se usa para la infección pulmonar y Sheng Gan Cao para la desinto- xicación ya que el ácido glicirretínico (ácido de Gan Cao) tiene propiedades antiinflamatorias similares a los glucocorticoides. En ¨Jing Yue Quan Shu¨ (Obras completas de Jing Yue) está registrado que al Wu Ling San hay que agregarle Qiang Huo para la afección externa por Frío-Humedad; mientras que en mi experiencia es adicionar Lian Qiao cuando la afección externa

fuera por Humedad-Calor. Lian Qiao tiene funciones antieméticas (como Gan Lu Xiao Du Dan), refresca la sangre (como Qing Ying Tang), depura el corazón (como Qing Gong Tang), depura el hígado (como Ma Huang Lian Qiao Chi Xiao Dou Tang) además de liberar la superficie. Puesto que la fisiopatología de Shaoyang es lucha entre lo recto y lo perverso, los que sufren de modo crítico el Síndrome Humedad-Calor evoluciona casi siempre con falla hepática fulmi- nante. Asimismo, es frecuente que Wen Bing afecta directamente a la capa nutricia (營) y a la capa sanguínea (血) por lo que se ve en la fase temprana una lengua espiculada. Contemplando todos los factores mencionados ante- riormente, hay que dar más dosis de Lian Qiao. Hay que tener mucho cuidado con el uso de medicamentos que refuerzan la Energía Recta, como Huan Qi o Ren Shen, ya que éstos pueden exacerbar la respuesta inflamatoria. Por esta razón, se quita Ren Shen del Xiao Chai Hu Tang. Para los individuos con complexión débil, se puede aumentar la dosis de Sheng Gan Cao hasta 9-15g e incluso añadir Tai Zhi Shen 15-50g. Cabe destacar, hay que controlar la tensión de lucha entre el Qi recto y el Qi per- verso. Se necesita Ren Shen y Huan Qi para eliminar definitivamente la causa patogénica de la infección citolítica en individuos con Insuficiencia del Bazo. Sin embargo, no es ésta la fisiopatología de esta enfermedad. Cuando existe una alteración inmunológica, el tratamiento alopático son los antiinflamato- rios anticitoquinas. Empero, el uso de Ren Shen y Huan Qi en MTC refuerza la respuesta inmune, por lo cual se debe indicar con mucha precaución. No se recomienda su uso si no fueran expertos tanto en Wén Bing Xue de MTC como en Infectología de medicina alopática. Se recomienda sumar Zhi Ban Xia 9g y una cucharada de jugo de Jengibre para enfermos con marcada hiperemesis y diarrea. El jugo de Jengibre facilita la transformación de la Humedad y no genera Calor, por lo que su uso es relativamente mayor que Sheng Jiang en Wén Bing. Por este motivo, el Jugo de Jengibre sustituye a Sheng Jiang en Xiao Chai Hu Tang. Se recomienda incorporar Dang Gui 9g en pacientes con evidente Insuficiencia de sangre o en las mujeres durante el período menstrual. En los pacientes con Humedad intensa, se descarta Da Zhao de Xiao Chai Hu Tang por su nutrición grasienta, y se prefiere al Dang Gui que tiene acción antiinflama- toria específica en capa sanguínea (血分) además de vigorizar y nutrir la sangre.

Se recomienda asociar Ge Gen 30g (método Ge Gen Qin Lian Tang) a los pacientes con diarrea abundante o presentan tez colorada destacable. Se recomienda agregar Cao Guo 3-6g (método Da Yuan Yin) si presenta saburra blanca, gruesa y símil polvo. Cao Guo es medicamento especial para saburra blanca y gruesa (engrosamiento de papilas filiformes). Agregar Hou Pu en caso de distensión abdominal o Bing Lang (debe tener materia fecal sólida) para los más severos.

1.3 Evolución La evolución clínica habitual es la transformación en Calor por Represión de la Humedad, que se asemeja al concepto del síndrome de respuesta infla- matoria sistémica (SIRS) o de sobreinfección bacteriana en la medicina occi- dental moderna. A continuación se detallan algunos métodos para determinar si la Humedad Patógena se ha transformado en Calor: El primero es ver si sobre la saburra blanca existe otra capa amarilla. El segundo es examinar la presencia o no de enrojecimiento lingual o gloquidios en el dorso de la lengua. El tercero es palpar el pulso. El pulso impetuoso (躁脈 Zao Mai) indica transformación en Calor, pero éste no solo se limita a la frecuencia de pulsos por minuto. Los principiantes pueden contar la frecuencia teniendo en cuenta lo siguiente: la frecuencia basal del pulso moderado de Insuficiencia del Bazo es de 70 por minuto, mientras que la basal de pulso lento de Insuficiencia del Yang es de 60 por minuto. Entonces, palpar el pulso es una manera de evaluar la temperatura corporal ya que por cada aumento de 1℃, la frecuencia de pulso aumenta 10 por minuto. De esta manera, se puede estimar el nivel de transformación en Calor o si existe Calor interno según la relación entre la temperatura corporal y la frecuencia de pulso. El cuarto es verificar si hay sobreinfección bacteriana. Clínicamente es común encontrar dolor de garganta, inflamación o supuración amigdalina o aumento de leucocitos a predominio de neutrófilos absolutos en el análisis de sangre. Como esta enfermedad genera linfopenia, el aumento porcentual re- lativo de neutrófilos no implica infección bacteriana. Se recomienda quitar Gui Zhi o bajar su dosis a 3-6g o reemplazarla por Rou Gui cuando es difícil determinar si hay transformación en Calor o no. La fórmula de la fase intermedia es más segura mientras que la de fase temprana libera más rápido el Calor. Es decir, al no poder discernir clara- mente la presencia de transformación en Calor, indicar directamente la fór- mula de fase intermedia, especialmente para los principiantes de la MTC.

Al indicar fórmula de fase intermedia en pacientes de fase temprana, que mu- chas veces sufren de Insuficiencia del Bazo constitucional, es importante adi- cionar Chen Pi y/o Cang Zhu para cuidado del Bazo y Estómago. Hay dos formas de manejo en los pacientes que predomina el Calor: el primero es indicar directamente la fórmula de la fase intermedia y; el se- gundo es incorporar Shi Gao, Hua Shi y Han Shui Shi (Método Gan Lu Yin) sobre la fórmula de la fase temprana así como sustituir Rou Gui 3g por Gui Zhi en la decocción o Rou Gui 1g vía oral.

2. Fase intermedia

2.1 Clasificación de fases y tipos

Existen tres tipos:

① Los individuos con Calor Interno constitucional, debutan directamente en fase intermedia cuando contraen esta enfermedad.

② Los pacientes con predominio de Humedad en síndrome Humedad-Calor son más propensos a desarrollar la Transformación en Calor por Represión de Humedad.

③ Pacientes tipo moderado. Hay que prestar especial atención porque amenudo existe una disociación de la mejoría clínica con la progresión de la enfermedad. Es decir, que sea moderado o no, no depende meramente de la presentación clínica, sino que es necesario una evaluación semiológica com- pleta de lengua y pulso, con o sin métodos diagnósticos complementarios con- temporáneos como radiografía de tórax o tomografía computada.

Se debe prestar atención al pulso en casos de Wen Bin sin Calor:

① La presencia de Calor. Se revela con el pulso impetuoso. En caso de no dominar la semiología de pulso, contar la frecuencia diariamente. Ante la ausencia de Calor con aumento de pulsos por minuto, se debe prestar atención a la aparición de fiebre alta súbita, semejando al estado previo del SIRS. En el texto original de ¨Shang Han Lun¨ menciona el término fiebre alta súbita y se diagnostica con el pulso.

② El nivel de Humedad. Se revela de 3 formas: Primero, si el pulso es fino. Segundo, si el pulso es moderado. Prestar atención a Yi Bing del Taiyin Bazo, por ejemplo la fiebre tifoidea intestinal, que se ve poco en Yi Bing del Taiyin Pulmón Tercero, si el pulso es áspero y débil. Una manera para los principiantes de MTC es ver si pueden sentir claramente bajo sus dedos cada pulsación. De lo contrario puede estar relacionado con la Humedad. (El pulso áspero y débil no solo aparece en enfermedades de Humedad).

Fíjese que la Humedad es un patógeno Yin perverso mientas que el Calor, un patógeno Yang perverso. La mayoría de las manifestaciones clínicas de la Humedad y del Calor son contrapuestas:

① El pulso del Calor es rebosante, grande y rápido; mientras que el de la Humedad es fino, débil y moderado (rebosante-débil, grande-fino, moderado-rápido), totalmente opuestos. A pesar de que los pulsos se enmascaran entre sí, la presencia de saburra gruesa grasienta y amarilla o el dorso de la lengua enrojecida indican gravedad igual de Humedad y Calor.

② Los aspectos de la lengua también se enmascaran entre sí. Cuando hay Humedad intensa, se ve lengua pálida e Insuficiencia del Bazo, mientras que en el Calor intenso, lengua roja vinosa. La clave para diferenciar del Calor está en la punta de la lengua y en la saburra. Si hay Calor oculto, ver dorso de la lengua.

③ La insuficiencia del Yin también se enmascara con la Humedad. El pulso de Humedad-Calor es fino pero no hueco (芤 Kou), ya que la presencia de éste indica Insuficiencia del Yin o de sangre. Cuando sufren de síndrome Humedad-Calor, en los individuos con insuficiencia de sangre constitucional, se produce una disminución rápida de glóbulos rojos, como ocurre en los casos de glomerulonefritis crónica. Tener en cuenta la observación de las grietas finas linguales.

④ Los gloquidios también pueden ser enmascarados por la Insuficiencia del Bazo, particularmente en la punta de la lengua. Hay que prestar atención que los abundantes gloquidios no enrojecidos indican déficit de Qi Recto, grupo de pacientes que fallecen fácilmente ante infección citotóxica por dis- minución de su inmunidad. Sin embargo, ante una infección viral no citotó- xica (sí citolítica) se desencadeuna un daño inmunitario inflamatorio, que por la respuesta inmune deficiente, raras veces genera muerte súbita. Empero, facilita la persistencia de la enfermedad, que puede llevar a la muerte por sobreinfección bacteriana o al desarrollo de la fibrosis una vez superada la fase aguda. Otros pueden cronificarse dependiendo de la naturaleza propia de la enfermedad.

2.2 Tratamiento

2.2.1 Elaboración de la fórmula

Fórmula Yin Chai Xiao Du Dan modificado. Ingredientes: Chai Hu 24g, Huang Qin 9g, Jin Yin Hua 30g, Lian Qiao 30g, Chang Pu 9g, Yu Jin 9g, Huo Xiang (agregar tarde) 9g, Pei Lan 9g, Yin Chen 30g, Bai Dou Kou (agregar tarde) 6g, Dang Gui 9g, Dan Pi 9g, Yi Yi Ren 60g, Shi Shang Bo 30g, Shen Gan Cao 6g.

2.2.2 Explicación de la fórmula Chai Hu más Huang Qin se usa en el Calor por afección externa. Como Sanjiao es la ruta de los humores, Xiao Chai Hu Tang también se indica para la tonificación de los movimientos de los líquidos corporales, así como dice: “Si el Jiao superior se desbloquea, los líquidos y los humores bajan y entonces, el Qi del estómago se armoniza y el Calor se libera por sudoración lluviosa”. Se combina Jin Yin Hua con Lian Quio para liberar el patógeno externo. La asociación de Chang Pu con Yu Jin previene el deterioro hacia la fase crítica como la aparición de coma y convulsiones, conceptualmente entendido por la MTC de la siguiente manera: “Una vez que el Calor superficial se interioriza, los meridianos colaterales profundos se bloquean”. La suma de Huo Xiang y Pei Lan transforman la Humedad por sus propiedades aromáticas. Cabe destacar que Pei Lan es un medicamento específico para la inhibición de secre- ción de mucoproteínas, que tradicionalmente se indica para tratar boca pas- tosa y boca dulce en la MTC. Se puede usar hasta 20-30g de Pei Lan para disminuir la secreción de grandes cantidades de mucoproteínas en el pulmón, evitando las obstrucciones por flema. Yin Chen más Bai Dou Kou liberan la Humedad-Calor de Sanjiao. Dang Gui más Dan Pi protege la capa sanguínea. En caso de constipación, agregar Zi Cao. Yi Yi Ren elimina la Humedad-Calor externa y es antiviral. Shi Shang Bo es específico para la infección pulmonar. Sheng Gan Cao se usa para la desintoxicación ya que el ácido glicirretínico posee propiedades antiinflamatorias similares a los glucocorticoides. Chai Hu es bueno para eliminar el Calor. Jin Yin Hua tiene buen efecto contra el dolor de garganta. Se recomienda añadir Pei Lan si hay síntomas gastrointestinales marcadas. Las propiedades específicas del Yin Chai Xiao Du Dan consiste en lo siguiente: Chai Hu más Huan Qin elimina Calor; Jin Yin Hua más Lian Qiao ex- pulsa el patógeno a través de la superficie y Pei Lan más Huo Xiang tonifica el estómago. Se puede sustituir Jin Yin Hua por su rocío puesto que posee mejor propiedad para liberar calor y evitar el bloqueo interno.

2.2.3 Modificaciones

Debe prestarse especial atención en pacientes con Insuficiencia del Yin, y evaluar la eliminación de Chai Hu o en el peor de los casos sustituirlo por Sheng Di Huang 15g en pacientes que cursan con fiebre persistente por varios días, ya que éste puede secuestrar Yin de Hígado. La Insuficiencia del Yin aparece a menudo en pacientes con fiebre, vómito o diarrea persistente. Para el diagnóstico precoz de la Insuficiencia del Yin por Síndrome Humedad-Calor hay que precisar si en la lengua existe pequeñas grietas no constitucionales (explicado en la clase de inspección de lengua las grietas constitucionales), que confirma la Insuficiencia.

Hay 4 formas de abordaje para estos casos. Primero, se aconseja quitar Chai Hu y agregar Sheng Di Huang para alimentar el Yin, de lo contrario, la Humedad-Calor difícilmente reduce. Segundo, agregar Xuan Shen que potencia el cultivo del Yin. Se recomienda asociar Xuan Shen con Dang Gui 30g como antiinflamatorio (Método Si Miao Yong An Tang). Esto está contraindicado en pacientes en shock porque Xuan Shen y Dang Gui son vasodilatadores y bajan la presión arterial. Se ex- cepcionan su uso en casos con soporte vasopresor y

monitoreo estricto conti- nuo, por lo que se desaconseja su empleo en principiantes. Tercero, sumar Lu Gen 30-50g. Este medicamento contiene coixenolida que facilita la eliminación de Humedad y actúa como antiviral; también con- tiene asparagina que potencia el cultivo del Yin y es antitusígena; y vitamina B que regenera la saburra; y antipirética. Por último, agregar Zhi Mu cuando el Síndrome Humedad-Calor deteriora el Yin y se acompaña de Calor. La fiebre persistente agota la corteza adrenal o altera el ritmo circadiano de la secreción corticoidea. Zhi Mu protege la corteza adrenal (Métodos Bai Hu Tang y Da Yuan Yin) previniendo el deterioro del Yin por el Síndrome Humedad-Calor. Por lo que se recomienda su uso en estos pacientes. Se recomienda incorporar Dan Zhu Ye 30g más Lu Gen 30g cuando la Humedad es más intensa.

Se recomienda incorporar Da Qing Ye 30g cuando el Calor es más intenso y Chen Pi 6-9g como protector gástrico. Si a esta fórmula se le añade Da Qing Ye, puede aparecer dolor abdominal, diarrea, falta de apetito en pacientes con Insuficiencia del Bazo, por lo que no se recomienda dosis altas. Solo Da Qing Ye 15g combinando con Chen Pi 9g o Cang Zhu 9g. Aunque altas dosis de Yi Yi Ren posee efectos antivirales marcados, pero enfría el estómago, entonces, se recomienda agregar Chen Pi.

2.3 Evolución

La primera evolución es hacia la fase crítica de tipo severo. La segunda es la mejoría post tratamiento. La tercera es la aparición de Insuficiencia del Bazo más Humedad intensa luego de la mejoría posterior a la transformación en Calor por Represión de Humedad. Clínicamente se manifiesta el cambio de saburra a blanca, y la lengua a pálida. En este momento de desaparición de calor, hay que indicar la fórmula de fase temprana, sustituyendo Gui Zhi por Rou Gui 3g en decocciones o Rou Gui 1g vía oral y agregar hierbas que dispersan calor tales como Hua Shi, Han Shui Shi, etc. Si vuelve el Calor, reiniciar la fórmula de fase intermedia, y así viceversa. Ante la inexistencia de Insuficiencia del Bazo y de lengua pálida, no se recomienda abordar de esta manera. La cuarta evolución consiste en la persistencia de Humedad-Calor. Debido a que la Humedad es un patógeno Yin perverso, no se disuelve si no es con Calor. Pacientes con marcada Insuficiencia del Bazo que no muestra clara respuesta de transformación de Humedad luego de recibir esta fórmula, se recomienda rotar a fórmula de fase temprana, sustituyendo Gui Zhi por Rou Gui 3g en decocciones o Rou Gui 1g vía oral y agregar hierbas que dispersan calor tales como Hua Shi, Han Shui Shi, etc y monitorizar estrictamente la respuesta inflamatoria.

3.Fase Huai Bing Huai

Huai Bing se asemeja a la fase crítica de la medicina contemporánea. Esta enfermedad tiene dos tipos de presentaciones: el primero, de tipo fulmi- nante, donde el paciente debuta en fase crítica y se observa principalmente cuando existe el Calor Tóxico Candente. Este grupo de paciente evoluciona de manera rápida y tórpida hacia la muerte en poco tiempo, inclusive en mi- nutos cuando haya bloqueo interno del pericardio; el segundo es de tipo co- mún, donde las fases iniciales tienen un período prolongado hasta que el pa- ciente alcance el punto crítico y desarrolle velozmente la transformación en Calor con pronto deterioro de la enfermedad. Este estado crítico requiere una atención urgente y aparece muchas veces por haber ignorado las fases inicia- les demasiado estables. Clínicamente en Huai Bing (fase crítica) se observan insuficiencia cardíaca, insuficiencia respiratoria e insuficiencia renal, con disfunción hepática, gastrointestinal y trastorno de la coagulación que requiere reanimación ur- gente. Frecuentemente se encuentra una disociación entre el alivio sintomático y el curso de la enfermedad en Yi Bing. Para determinar el punto crítico, hay que examinar si la saburra lingual pertenece al Calor intenso de Yangming. En casos no fulminantes el punto crítico generalmente está alrededor del décimo día, mientras que si no hubo deterioro luego de 14 días, comparativamente están fuera de peligro.

Las formas de diagnosticar Huai Bing son: Primero, examinar si existe gran cantidad de gloquidios en la lengua ya que esto indica la profundización de la enfermedad a la capa sanguínea y ha ge- nerado cierto grado de hemorragia. Segundo, examinar la presencia o no de pulso cuerda filosa, que indica convulsión debido a la Agitación Interna del Viento. La saburra lingual tam- bién aporta en el diagnóstico: es blanca, de capa gruesa

y símil polvo en Hu- medad intensa, mientras que es amarilla, de capa gruesa y seca, o exfoliada en Calor intenso. Ambos indican la persistencia de la enfermedad con proba- bilidad de deterioro hacia la fase crítica. La textura oscura y púrpura de la lengua señala hipercoagulabilidad causada por la infección si no fuese por éstasis sanguínea constitucional. En este caso, la progresión de la enfermedad suele ser más lenta. En la aparición rápida de lengua oscura y púrpura en Wen Bing de Taiyin Pulmonar se debe descartar la baja saturación de oxígeno, indicador de gravedad en enferme- dades pulmonares, del éstasis sanguínea causada por Calor ferviente. Este último puede generar hemorragias por lo que se recomienda agregar Qian Cao 30g, Zi Cao 9g, Guan Zhong 15g, o directamente la fórmula Shen Xi Dan de ¨Wen Re Jing Wei. ¨ En comparación, la dificultad respiratoria más la semiología de lengua y pulso son determinantes más específicos que los síntomas gastrointestinales, la tos, el esputo y la fiebre para definir el avance de la enfermedad. Ante la aparición de dificultad respiratoria, en medios accesibles, hay que monitorear la saturación de oxígeno y efectuar rápidamente radiografía de tórax o tomo- grafía computada para esclarecer el estado pulmonar. En caso de falla respi- ratoria derivar de modo urgente al servicio de emergencias. Se recomienda agregar Ma Huang 9g con acción anti-choque, y la dosis puede alcanzar hasta 30-40g según la tensión arterial; así como las fórmulas Ma Xing Shi Gan Tang, Ma Xing Yi Gan Tang y Ma Huang Lian Qiao Chi Xiao Dou Tang, éste último con buen efecto protector de daño hepático post infección viral. La efedrina tiene acción símil adrenalina. Ma Huang Sheng Ma Tang es una fórmula estándar de MTC para tratar infecciones graves. Dicha fórmula trata las infecciones severas y a los 6 meridianos en simultáneo. Para su indicación hay que modificarla según signo sintomatología. Se recomienda la posibilidad de aplicación de inyectables anti insuficiencia cardíaca como Ren Shen, Fu Zi, Yu Zhu, Shen Fu o Sheng Ma Por otro lado, pese a que Ban Bian Lian tiene efecto específico contra la falla respiratoria de origen central, no se recomienda su uso en esta enfermedad por sus resul- tados desalentadores especialmente en pacientes con alteración del sensorio y convulsiones. El ácido glicirretínico es un símil glucocorticoides. En caso de no contar con otra medicación antiinflamatoria, sustituir los glucocorticoides por altas dosis de Gan Cao 30g que tiene efecto antiinflamatorio potente. No obstante, añadir medicación evacuadora de agua como Chen Pi y Fu Ling para antagonizar los efectos adversos de Gan Cao. Chang Pu y Yu Jin son buenas opciones para prevenir la fase crítica ya que evita el coma y las convulsiones, precedidas por la opresión precordial de la fase temprana así como lo explica “Una vez que el Calor superficial se interio- riza, los meridianos colaterales profundos se bloquean.” Una vez instalado el coma y las convulsiones, el tratamiento urgente tradicional de MTC son los “Tres Tesoros.” El los pacientes que sufren daño del Yin por el síndrome Humedad-Calor se recomienda indicar fórmula Jia Wei Bai He Di Huang Tang (Bai He, Sheng Di, Zhu Ye, Shi Gao y Dan Pi) más Hua Shi y Gan Cao con efecto analéptico marcado. Se recomienda agregar Jiang Nan Quan Bo 30g de inmediato sin otras evaluaciones en el marco de derrame pericárdico o derrame pleural secunda- rio a la extravasación de líquido. Chan Tui es un estimulante de la síntesis de IFN y posee efecto antiviral potente como por ejemplo en la fórmula Sheng Jiang San. Se puede usar altas dosis de 30-40g, pero hay que tener extrema precaución en casos severos. No se recomienda indicarlo en contexto de respuesta inflamatoria exagerada ni tampoco se recomienda su uso si no fueran expertos tanto en Wén Bing Xue de MTC como en Infectología de medicina alopática.

4. Fase secuelar

La secuela principal de esta enfermedad es el desarrollo de la fibrosis pulmonar. En este contexto se indica Yu Ke Tang (Xiang Fu, Xuan Fu Hua, Yi Yi Ren, Tao Ren, Dang Gui, Wu Gong, San Qi, Bai Jie Zi) de la fórmula de Wu Men. Contemplando los efectos adversos de cada medicina, se puede adicionar Shan Ci Gu, Shang Lu, Zao Jiao Ci según signo-sintomatología. Los que sufren de enfisema pulmonar deben tomar Tai Yi Xi Sui Gao durante 3 años sucesivos. Para conocer su uso específico, consulte el libro Wu Men Yan Fang (Fórmulas eficaces de la familia Wu).

5. Prevención y medidas higiénico-dietéticas

5.1 Prevención Optimizar la ventilación ambiental; evitar la concurrencia a lugares de concentración multitudinaria; prevenir el contacto con pacientes infectados; y aún más importante, evitar el cansancio y el

estrés. Es fundamental el la- vado de manos con frecuencia y el uso de barbijos. Es necesario considerar la alta infectividad de Yi Bing y Li Bing, así como los antiguos chinos lo denominaban “enfermedades de fácil contagio”. Bajo estas condiciones, la inmunocompetencia de los individuos sanos es insufi- ciente para no contraer esta enfermedad. Entonces, tampoco encuadra el concepto de “Si conserva Qi Recto en el interior, el Qi Patógeno perverso no puede invadir” en esta patología de alta infectividad, por ende, es sustancial evitar el contacto cercano. La MTC sugiere alimentar la energía en postura sentada (Qigong y meditación sīmā-bandha), prender incienso (incluye el uso de sachet aromática) y evitar el miasma, medicamentos como Xiao Jin Dan de Neijing. Según este mismo protocolo, se puede alternar la indicación de fórmulas de fase tem- prana e intermedia según la constitución física de cada paciente y el nivel de exposición. También se puede digitopuntar la planta del pie (centro del aca- punto Yon Quan, K1, 81 veces) y el arco del pie (en el centro acupunto de Ran Gu, K2, 81 veces, o simplemente de 5 a 10 minutos). Además, hay que tener mucha cautela el contacto con pacientes conva- lecientes hasta los 14 días posteriores ya que permanecen con infectividad activa. Por el momento, tampoco se puede descartar la posibilidad de con- vertirse en portadores crónicos en algunos casos esporádicos. La clave reside en el pulso: a los que aún conservan el pulso impetuoso a la palpación media y profunda, pese a la mejoría clínica e imagenológica, llámese curado, tienen mayor probabilidad de transformarse en portador crónico.

5.2 Medidas higiénico-dietéticas

① Dieta: caldo de arroz y gran cantidad de verduras. Evitar comidas grasientas, dulzonas y bebidas frías. Es importante mantener un buen ritmo eva- cuatorio.

② Evitar agotamiento físico, mental y sexual. Se recomienda abstinencia sexual hasta 14 días posteriores a la convalecencia.

6. Instructivo de la guía

6.1 Puntos claves para el diagnóstico y tratamiento:

El punto clave para diferenciar y tratar las enfermedades Wen Bing reside en dilucidar el mecanismo fisiopatológico principal. Cuantos más tipos de sín- dromes se describa por esta enfermedad más lejos estamos de la esencia de Wen Bing. Con respecto a esto, lo mejor es lograr una hierba por enfermedad, o si no fuera posible, una fórmula. Aún no poder conseguirlo, hay que definir por lo menos el síndrome central según el mecanismo fisiopatológico princi- pal.

Aunque clínicamente diferentes factores originarán múltiples tipos de síndromes paralelos o transformantes capaces de influir en el pronóstico, no obstante, no reflejan la esencia propia de la enfermedad. Es considerable mejorar el síndrome, compuesto por signos y síntomas, mientras que es im- prescindible el tratamiento dirigido desde la MTC a la noxa causante de Wen Bing y Li Bing y al mecanismo fisiopatológico principal. Debido a que la noxa de cada enfermedad infecto-contagiosa es única, su mecanismo fisiopatológico central es relativamente homogénea, por lo que miles de variaciones no se alejan del eje.

6.2 Pronóstico Es frecuente la disociación entre la mejoría clínica sintomatológica y el avance de la enfermedad en Wen Bing e Yi Bing severos. Esto quiere decir que la evolución sindromática y el curso de la enfermedad son disicrónicos. Con- cretamente, el cambio de la semiología lingual y del pulso es crucial y, a veces superiores, que los síntomas para la determinación del pronóstico.

6.3 Integración Medicina Tradicional China - Medicina Alopática Se recomienda la integración de la Medicina Tradicional China con la Medicina Alopática para la prevención y el tratamiento de Wen Bing e Yi Bing. Es necesario que la MTC se fusione con la Infectología y la Terapia Intensiva y además, con las áreas de vacunación, aislamiento, desifección, prevención, tratamiento de soporte y Emergentología de la medicina contemporánea.

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SUMMARY

Pneumonia associated with the 2019 novel coronavirus (2019-nCoV) is continuously and rapidly circulating at present. No effective antiviral treatment has been verified thus far. We report here the clinical characteristics and therapeutic procedure for four patients with mild or severe 2019-nCoV pneumonia admitted to Shanghai Public Health Clinical Center. All the patients were given antiviral treatment including lopinavir/ritonavir (Kaletra®), arbidol, and Shufeng Jiedu Capsule (SFJDC, a traditional Chinese medicine) and other necessary support care. After treatment, three patients gained significant improvement in pneumonia associated symptoms, two of whom were confirmed 2019-nCoV negative and discharged, and one of whom was virus negative at the first test. The remaining patient with severe pneumonia had shown signs of improvement by the cutoff date for data collection. Results obtained in the current study may provide clues for treatment of 2019-nCoV pneumonia. The efficacy of antiviral treatment including lopinavir/ritonavir, arbidol, and SFJDC warrants further verification in future study.

Introduction Coronaviruses mainly cause respiratory tract infections and some strains have high infectivity and mortality as well as heavy damage on public health, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (1). A pneumonia associated with the 2019 novel coronavirus (2019-nCoV) emerged in Wuhan, China in December, 2019 and has spread rapidly, with 24,324 confirmed cases in mainland China as of February 4, 2020 (2,3). The most common clinical presentation is fever, fatigue, and dry cough and some patients present with nasal congestion, runny nose, and diarrhea (4). In severe cases, dyspnea usually occurs one week after the disease onset and some patients can rapidly progress to acute respiratory distress syndrome (ARDS), septic shock, refractory metabolic acidosis, and coagulation disorders (4). Thus far, there is no approved or verified effective drugs specific to the virus (5). We report here that four patients with mild or severe 2019-nCoV pneumonia have been cured or have significant improvement in their respiratory symptoms after treatment with combined lopinavir/ritonavir (Kaletra®), arbidol, and Shufeng Jiedu Capsule (SFJDC, a traditional Chinese medicine) on the base of supportive care. 2. Methods 2.1. Patients For this retrospective study, four patients were recruited from January 21 to January 24, 2020 at Shanghai Public Health Clinical Center, Shanghai, China, which is a designated hospital for 2019-nCoV pneumonia. All patients were diagnosed as having 2019-nCoV pneumonia according to WHO interim guidance. Informed consent to therapeutic regimen was obtained from each patient prior to treatment. 2.2. Data collection Epidemiological, demographic, clinical, laboratory, management, and outcome data were collected through a review of medical records. Clinical outcomes were followed up until February 4, 2020. Laboratory confirmation of 2019-CoV was done in Shanghai Municipal Center for Disease Control and Prevention. Throat-swab specimens from the upper respiratory tract that were obtained from all patients at admission were maintained in viral-transport medium. 2019- nCoV was confirmed by real-time RT-PCR using the same protocol described previously (6). All patients were given chest computed tomography (CT) or chest radiography.

Table 1. Demographics, baseline characteristics, and clinical outcomes of 4 patients admitted to Shanghai Public Health Clinical Center

Items	Case 1	Case 2	Case 3	Case 4
Age	32	19	63	63
Sex	Male	Male	Male	Female
Exposure history	Recent travel to Wuhan	Resident of Wuhan	Close contact with 2019-nCoV patient	Recent travel to Wuhan
Chronic medical illness	Fatty liver	None	None	None
Days from illness onset to diagnosis confirmation	11	6	1	2
Clinical outcome	Discharged	Discharged	Remained in hospital	Remained in hospital

Table 2. Clinical characteristics at presentation and treatment of patients with 2019-nCoV pneumonia

Items	Case 1	Case 2	Case 3	Case 4
Signs and symptoms				
Fever	Yes	Yes	Yes	Yes
Cough		Yes	Yes	Yes
Fatigue	Yes	Yes		
Dizziness	Yes			Yes
Nasal congestion		Yes		
Rhinorrhea		Yes		
Constipation	Yes			Yes
Respiratory rate	22/min	19/min	26/min	22/min
Lung auscultation	Rhonchi (left lower lobe)	No rhonchi	Rhonchi (right lower lobe)	Rhonchi (left lower lobe)
Chest CT findings				
Unilateral pneumonia		Yes	Yes	
Bilateral pneumonia	Yes			Yes
Treatment				
Oxygen therapy	Yes	Yes	Yes	Yes
Mechanical ventilation				Yes
Antibiotic treatment	Yes	Yes	Yes	Yes
Lopinavir/ritonavir/arbidol/SFJDC	Yes	Yes	Yes	Yes
Intravenous immunoglobulin therapy				Yes

3. Results and Discussion 3.1. Demographics and baseline characteristics Four patients with 2019-nCoV are included in this study, two of whom are under the age of 35 and the other two are over the age of 60 (Table 1). All the patients had epidemiologic linkage to areas with community transmission of 2019-nCoV. Among them, two patients (Case 1 and 4) had recent travel history to Wuhan, one patient (case 2) is a student who was ordinarily a resident in Wuhan and went back to Shanghai for winter holiday, and one patient (Case 3) is the husband of a confirmed 2019-nCoV case. It took 11 and 6 days from disease onset to confirmed diagnosis for case 1 and case 2, while 1 and 2 days for case 3 and case 4. Fatty liver was reported in the case 1. No underlying medical conditions were reported in the other three cases. 3.2. Clinical characteristics and laboratory assessment On admission, the most common symptoms were fever or history of fever, followed by cough, fatigue, dizziness, nasal congestion, and rhinorrhea (Table 2). Diarrhea was not observed in all patients, on the contrary, two of them were reported to have constipation. Physical examination revealed increased respiratory rate in three patients, one of whom had tachypnea (26/min). Lung auscultation revealed rhonchi in left or right lower lobe in three patients. In all patients, there were marked abnormalities on chest radiography; involvement of both lungs was found by chest computerized tomography (CT) in 2 patients at presentation. Ground-glass opacities and consolidation were the most common radiologic findings. On admission, leucocytes were in the normal range in all the patients (Table 3). One patient (case 4) had neutrophils above the normal range, indicating the existence of concurrent bacterial infection. Lymphocytes were below the normal range in one patient (case 4) and within the normal range in other three patients. Blood gas analysis revealed that oxygen pressure was below the normal range in two patients (7.60 kPa in case 3 and 5.45 kPa in case 4) (Table 3). On the basis of the above results, two patients (case 1 and 2) were diagnosed with mild pneumonia and the other two patients (case 3 and 4) with severe pneumonia. 3.3. Treatment and clinical outcomes All patients received antiviral treatment, including lopinavir/ritonavir (Kaletra® , lopinavir 400 mg/ritonavir 100 mg, q12h, po), arbidol (0.2 g, tid, po), and SFJDC (2.08 g, tid, po). The duration of antiviral treatment was 6-15 days. In addition, all patients were all given antibiotic treatment and started on supplemental oxygen, delivered by nasal cannula after admission to hospital (Table 2). Patient 1 was admitted to hospital on January 21, 2020 and thereafter received the above treatment. On January 27, routine blood analysis revealed that leucocytes and lymphocytes were increased, indicating recovery and restoration of immune function (Table 3). On January 29, chest CT demonstrated bilateral pneumonia with scattered multiple nodules, which was obviously improved compared with that obtained on January 21 (Figure 1). 2019-nCoV was twice negative in throat-swab specimens from the upper respiratory tract. The patient was free of fever, productive cough, dyspnea, short breath, abdominal pain, and diarrhea, and thus discharged on January 29, 2020. Patient 2 was admitted to hospital on January 24, 2020 and then received the above mentioned treatment. On January 28, routine blood analysis showed increased count of leucocytes and lymphocytes (Table 3). Blood gas analysis revealed no obvious abnormity. On January 29, chest CT revealed unilateral pneumonia in the left lobe, which was mildly improved compared with the images obtained on

January 24 (Figure 2). Results of two continuous 2019-nCoV tests were negative for throat-swab specimens. Symptoms associated with pneumonia had improved and the patient was discharged on January 30, 2020. Patient 3 was admitted to hospital on January 24, 2020 and thereafter received the above mentioned treatment. The fever disappeared after one day of treatment. On January 29, chest CT showed progressed pneumonia in the right lobe (Figure 3). The treatment was continuous and the pneumonia appearance improved on February 1 as reflected by the CT image (Figure 3). On February 3, blood gas analysis demonstrated obviously increased oxygen pressure compared with that at admission. The patient had mild cough with white phlegm, and was free of fever, dyspnea, short breath, abdominal pain, and diarrhea. 2019-nCoV test result was negative for the first time on February 4, 2020. The patient remained in hospital for the second virus test. Patient 4 was admitted to hospital on January 22, 2020. In addition to the above mentioned treatments, the patient was also given human seroalbumin and γ-immunoglobulin. On January 31, the patient was given an intubated ventilator-assisted breathing therapy because of refractory low blood oxygen pressure. Routine blood analysis on February 1 demonstrated the percentages of neutrophils and lymphocytes were 94% and 3.2%, respectively, which were comparable with those at admission (Table 3). Chest radiograph on this day demonstrated bilateral pneumonia, which improved compared to the image obtained on January 31 (Figure 4). Chest radiograph on February 2 revealed further mild improvement. On February 3, bilateral pneumonia remained but the appearances of left lobe improved and right lobe mildly worsened. On February 5, the appearance of pneumonia improved compared with the last image (Figure 4). The patient was still using ventilators at data cutoff. We report here the clinical characteristics and therapeutic procedure for four patients with 2019- CoV pneumonia receiving comprehensive therapy. The antivirus treatment regimen includes lopinavir/ritonavir (Kaletra®), arbidol, and SFJDC. By February 4, 2020, two patients were confirmed 2019-nCoV negative and one patient was virus-negative at the first test. Lopinavir/ ritonavir (Kaletra®) is a human immunodeficiency virus (HIV) medicine used in combination with other medicines to treat adults and children over 14 days of age who are infected with HIV-1 (7). It was revealed that lopinavir/ritonavir among SARS-CoV patients was associated with substantial clinical benefit (fewer adverse clinical outcomes) (8). The combination of lopinavir and ritonavir is currently a recommended antivirus regimen in the latest version of Diagnosis and Treatment of Pneumonia Caused by 2019-nCoV (version 5) issued by National Health Commission of the People's Republic of China (4). Arbidol is an antiviral treatment for influenza infection used in Russia and China (9). It was claimed that arbidol was effective against 2019-nCoV at a concentration range of 10-30 μM in vitro (10). A randomized multicenter controlled clinical trial of arbidol in patients with 2019-nCoV (ChiCTR2000029573) has been initiated in China (11). SFJDC is a traditional Chinese medicine for treatment of influenza in China. This drug is also recommended for treating 2019-nCoV infection in the latest version of Diagnosis and Treatment of Pneumonia Caused by 2019-nCoV (version 5) (4). In conclusion, two mild and two severe 2019- nCoV pneumonia patients were given combined Chinese and Western medicine treatment, three of whom gained significant improvement in pneumonia associated symptoms. The remaining patient with severe pneumonia has shown signs of improvement by the cutoff date for data collection. The efficacy of antiviral treatment including lopinavir/ritonavir, arbidol, and SFJDC warrants further verification in future study. Acknowledgements The study is supported by Shanghai Municipal Key Clinical Specialty (shslczdzk05101) and Shanghai Key Clinical Laboratory of Internal Medicine of Traditional Chinese Medicine (14DZ2273200).

Table 3. Clinical laboratory results of patients with 2019-nCoV pneumonia

Variable	Case 1		Case 2		Case 3		Case 4	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Blood, routine								
Leucocytes ($\times 10^9$ per L; normal range 3.5-9.5)	4.23	4.68	6.48	6.58	4.40	5.31	6.84	10.84
Neutrophils (%; normal range 50-70)	57.2	49.1	57.0	47.6	50.0	55.4	93	94
Lymphocytes (%; normal range 20-40)	30.3	37.1	30.6	39.4	24.5	25.0	6.10	3.2
Blood gas analysis								
pH (normal range 7.35-7.45)	7.33	7.33	7.43	7.33	7.40	7.36	7.44	7.33
PCO_2 (kPa, normal range 4.65-6.0)	5.42	6.05	4.55	5.96	5.45	5.59	4.23	5.52
PO_2 (kPa, normal range 10.6-13.3)	22.00	11.90	16.6	13.4	7.60	12.0	5.45	21.9

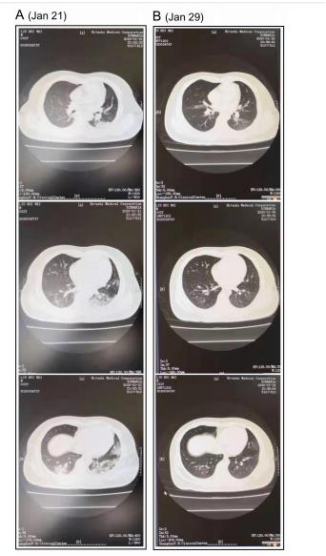
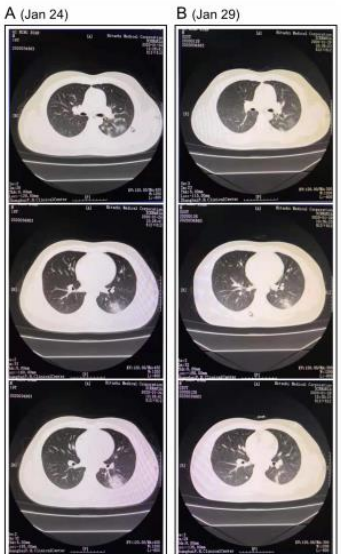


Figure 1. Chest CTs of patient 1 obtained on January 21 (A) and January 29 (B), 2020.



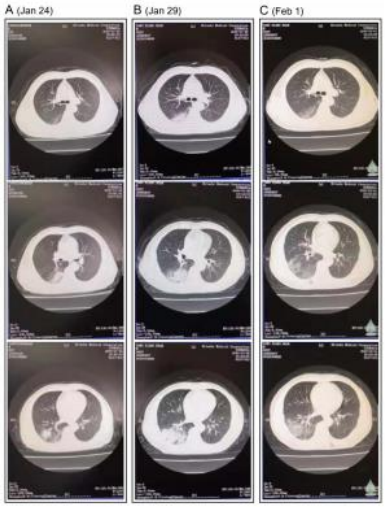


Figure 3. Chest CTs of patient 3 obtained on January 24 (A) and January 29 (B), and February 1 (C), 2020.

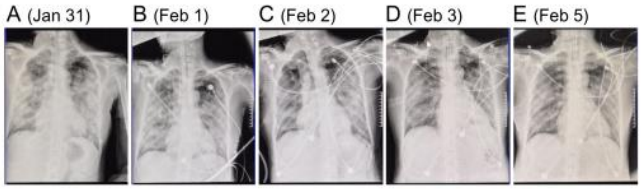


Figure 4. Posteroanterior chest radiographs of patient 4 obtained on January 31 (A), February 1 (B), February 2 (C), February 3 (D), and February 5 (E), 2020.

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Abstract

The Corona Virus Disease 2019 (COVID-19) is an acute respiratory infectious disease. At present, COVID-19 has no specific therapeutic drugs, and the main clinical treatment is symptomatic treatment and control of complications. On March 5, 2020, the National Health Commission of the People's Republic of China issued the *Guidelines for the Diagnosis and Treatment of Novel Coronavirus* (*2019-nCoV*) *Infection* (*Trial Version 7*), which integrated traditional Chinese medicine (TCM) into the treatment of COVID-19. The purpose of this study is to summarize recent studies on the clinic application, pharmacological action, chemical substances and mechanism of Qingwen Baidu Decoction (QBD) on the treatment of various diseases. The results suggested that QBD has multiple pharmacological effects such as anti-inflammation, antiviral, antibacterial, immunomodulatory, antipyretic and so on. It has been used in the treatment of sepsis, epidemic hemorrhagic fever, epidemic cerebrospinal meningitis, infantile pneumonia, sepsis-related encephalopathy, epidemic encephalitis B and other diseases. In addition, this study attempts to explore the possible mechanism of QBD in the prevention and treatment of COVID-19. Through the analysis of the chemical substances, pharmacological action and mechanism of QBD, this paper will provide a reference theoretical basis for the prevention and treatment of COVID-19 by QBD.

Background

Novel Coronavirus Pneumonia (NCP) is an acute infectious pneumonia whose pathogen is novel coronavirus, 2019-nCoV, which has not been previously found in humans. On February 11, 2020, the World Health Organization (WHO) named it as 2019 coronavirus disease, and its English name was Corona Virus Disease 2019 (COVID-19). Since December 2019, a number of pneumonia patients with COVID-19 infection have been found in Wuhan, Hubei Province, China. With the spread of the epidemic, such cases have also been found in other parts of China and worldwide. At present, most of the reported cases have a history of residence or travel in Wuhan, and no case of travel history in Wuhan has been found in some areas. NCP is officially declared a public health emergencies of international concern (PHEIC) on January 30, 2020 by WHO. NCP has been included in the Class B infectious diseases stipulated in the *Law of the People's Republic of China on Prevention and Control of Infectious Diseases*. Simultaneously, prevention and control measures for class A infectious diseases are taken and implemented. In China, as of 10 AM on May 3, 2020, a total of 84,393 cases have been confirmed, 78,939 cases have been cured and 4643 cases have died, with a mortality rate of 5.50%. NCP has spread all over the world and posed a major threat to human health. There is no vaccine or specific effective drugs for COVID-19 infected pneumonia at present. On March 5, 2020, the National Health Commission of the People's Republic of China issued the *Guidelines for the Diagnosis and Treatment of Novel Coronavirus* (*2019-nCoV*) *Infection* (*Trial Version 7*), which integrated traditional Chinese medicine (TCM) into the treatment of COVID-19. The therapeutic schedule is mainly isolation treatment and symptomatic support treatment.

Traditional Chinese medicine is a great treasure house of China, and it has unique advantages in the treatment of viral infectious diseases. COVID-19, which belongs to the category of "pestilence" and "epidemic disease" in TCM, has the pathogenesis characteristics of "dampness, poison, blood stasis and closure", and presents the syndrome of "dampness poisoning heat and poisoning lung collaterals" in the progressive stage of pneumonia [1]. The theory of syndrome differentiation characterized by defensive energy nutrients and blood shows its unique advantages in guiding the clinical prevention and treatment of viral infectious diseases with unknown characteristics and strong infectivity. It provides an effective treatment scheme for infectious diseases such as SARS, H1N1, H7N9, and has been internationally

recognized [2,3,4]. Viral infectious disease is called epidemic disease in TCM. It is an acute febrile disease caused by pathogenic *qi* disease invading the human body through mouth, nose or muscle surface. It belongs to the category of febrile disease. TCM emphasizes the overall concept, syndrome differentiation and treatment, which can not only be treated, but also can be prevented, especially in the improvement of body symptoms, which has unique advantages. In the novel coronavirus campaign, TCM is playing a new therapeutic role in different stages of NCP, that is, “supporting the healthy and eliminating the evil, and keeping the same strain”, “syndrome differentiation and treatment to change strain”, restraining “cytokine storm”, and controlling the serious development of the disease, reducing the sequelae caused by hormones and other drugs, decreasing the fatality rate of patients, and so on. The strategy of “TCM is added to the main battlefield of anti-epidemic” is implemented, and the clinical curative effect of the existing Chinese medicine preparation or decoction is observed. Currently, the conventional treatment plus TCM plan has been integrated and published, forming a unified, highly directive and practical “integrative medicine for the prevention and treatment of NCP”.

At present, COVID-19 serves as the pathogenic mechanism of NCP has not been fully revealed. Inhibiting virus proliferation in host cells and reducing host system response is considered to be an important way to alleviate viral infectious diseases. As a β-coronavirus, COVID-19 has more than 85% homology with SARS-like coronavirus (bat-SL-CoVZC45). Angiotensin converting enzyme 2 (ACE2) and coronavirus 3CL Mpro on host epithelial cells affected by its S-protein are considered to be the core targets for inhibiting virus proliferation [5]. Simultaneously, cytokine storm induced by virus is the main cause of inflammation, septic shock and multiple organ failure [6].

In this research, we summarized the pharmacological action, mechanism of action and clinical application of Qingwen Baidu Decoction (QBD), and analyzed its characteristics and advantages of clinical application. Thus, this study will provide theoretical basis and practical reference for its clinical application in anti-NCP and other diseases.

Brief introduction of QBD

Qingwen Baidu Decoction, which originated from *A View of Epidemic Febrile Diseases with Rashes* (*Yi Zhen Yi De named in Chinese*) in the Qing Dynasty [7], was created by Shiyu Yu, a famous febrile pathologist in the Qing Dynasty. The original prescription of QBD was made from Baihu Decoction, Antidotal Decoction of Coptis and Decoction of Rhinoceros Horn and Rehmannia, including Gypsum Fibrosum, Rehmanniae Radix, Rhinoceros nicornis LR.simus Burchell, Gardeniae Fructus, Platycodonis Radix, Coptidis Rhizoma, Scutellariae Radix, Anemarrhenae Rhizoma, Paeoniae Radix Rubra, Scrophulariae Radix, Forsythiae Fructus, Lophatheri Herba, Glycyrrhizae Radix et Rhizoma, and Moutan Cortex, which has the effect of clearing heat and cooling blood, alleviating heat-producing and disintoxicating. It is one of the most representative anti-epidemic agents in TCM [8, 9]. Clinical evidence suggests that QBD can be used in the treatment of a variety of viral infectious diseases, which can relieve symptoms with a good clinical effect. Pharmacological studies show that the antiviral effect of QBD is mainly associated with Gardeniae Fructus, Coptidis Rhizoma, Scutellariae Radix, and Scrophulariae Radix, which have obvious inhibitory effect on many kinds of influenza viruses via repressing virus replication and preventing virus from entering cells. At the same time, Rehmanniae Radix and Anemarrhenae Rhizoma can significantly regulate cellular and humoral immune function, enhance the activity of immune system and reduce the expression of inflammatory factors [10].

Clinical research progress of QBD against virus infection related diseases

The pharmacological effect of QBD is exerted through multi-pathway, multi-target and multi-action mechanism, and its modern clinical research is mainly used in the follow aspects.

Sepsis

Clinically, patients with high fever accompanied by extreme thirst, headache, dysphoria, delirium, red tongue and scorched lips, it is syndrome of flaring heat in *qifen* and *xuefen*, which is difficult to treat. It is common in pneumonia, septicemia, sepsis, infectious mononucleosis and so on in modern medicine. Among them, sepsis is a life-threatening organ dysfunction syndrome caused by the body's dysfunctional response to infection [11,12,13]. QBD has effects on relieving sepsis fever, inhibiting early inflammatory reaction, improving blood coagulation dysfunction, and reducing organ function damage, also effectively improving patients' clinical symptoms, ameliorating patients' prognosis and so on [14,15,16]. Nowadays, extensive studies have shown that QBD combined with conventional therapy in the treatment of sepsis can effectively ameliorate the clinical symptoms of patients, reduce or inhibit systemic inflammatory reaction, protect the function of various organs and systems, and improve the prognosis of patients. Conventional therapy combined with QBD in the treatment of sepsis (syndrome of flaring heat in *qifen* and *xuefen*) has effects on reducing the serum immunological indexes such as IgG, IgA, IgM, C3, CRP and TNF-α, reducing Acute Physiology and Chronic Health Evaluation II (APACHE II) score, as well as improving the effective rate of clinical treatment with good clinical effect, which is worth popularizing in clinical [17, 18].

A prospective randomized controlled study was used to observe and evaluate the clinical effect of QBD on blood coagulation indexes and TCM clinical symptom scores in patients with sepsis with blood coagulation dysfunction. The results indicate that QBD can increase the total clinical effective rate of patients with sepsis coagulation dysfunction from 74.2% of the basic treatment to 93.9%. Also, QBD can also significantly decrease the TCM clinical symptom score, APACHE II score and sequential (sepsis-related) organ failure assessment (SOFA). These results indicate that QBD combined with routine treatment can significantly improve the clinical symptoms and blood coagulation function indexes of sepsis patients, ameliorate patient's health status and protect internal organs [19]. Xi et al. [20] used QBD plus routine treatment to investigate its effect on patients with sepsis with pathogen invading lung defense syndrome. The APACHE II score, C-reactive protein (CRP), white blood cell (WBC) count, TCM syndrome score, blood glucose and antipyretic time were significantly improved in the integrated group, and there was no obvious manifestation of organ failure. It shows that QBD can significantly improve the clinical symptoms and shorten the course of disease. Lin et al. [21] established sepsis model in rats by Cecal ligation and puncture (CLP). It was found that the mechanism of QBD in treating sepsis was related to regulating the expression of genes related to IL-17 signal pathway in rat spleen. Yi et al. [22] established sepsis model by intravenous injection of colibacillus endotoxin into rabbit ear vein. It was found that QBD reduced systemic inflammation in septic rabbits by regulating TLR4/NF-κB signalling pathway. Wang et al. [23] found that the physiological and pathological conditions of septic acute lung injury (ALI) in QBD rats were significantly better than those of the model group (including pathological injury, pathological score, respiratory function, mental state and mortality, etc.), and the expressions of JAK2, p-JAK2, STAT3, p-STAT3, IKKα and NF-κBp65 protein in lung tissue of rats in QBD group were significantly down-regulated. It is suggested that QBD can exert its protective effect by inhibiting the continuous activation of JAK2/STAT3 and IKKα/NF-κB pathway, and its down-regulating effect is significantly related to the improvement of physiological and pathological conditions in rats with ALI. In addition, it can effectively inhibit the lung tissue inflammatory factors (TNF-α, IL-1β, IL-6) and its mediated JAK2/STAT3 signal pathway in septic ALI rats. In addition, QBD could improve the negative feedback regulation mechanism of this pathway, and have a protective effect on ALI model rats [24]. The effects of QBD on lung pathology and the expression of nuclear factor-κBp65 (NF-κBp65) in rats with ALI induced by lipopolysaccharide (LPS) were observed to explore the therapeutic effect and intervention mechanism of QBD on ALI. The results showed that QBD could reduce the degree of lung injury induced by LPS in ALI rats, the accumulation, infiltration and exudation of inflammatory cells in the lungs to a certain extent, playing a definite role in lung protection. QBD can effectively reduce the expression of NF-κBp65 in the lung tissue of ALI rats induced by LPS. It could reduce the "cascade" reaction of inflammation by inhibiting the activation of NF-κB and the production of inflammatory cytokines, as well as inhibiting the inflammatory reaction. The use of QBD in the early stage of ALI induced by LPS can effectively reduce the injury course of lung tissue and the inflammatory reaction, as well as preventing the occurrence and development of inflammation [25].

Epidemic hemorrhagic fever

Li et al. [26] investigated the potential mechanism of QBD in treating the fever period of hemorrhagic fever with renal syndrome (HFRS). The results showed that QBD combined with conventional therapy was significantly superior to routine therapy in the treatment of HFRS and the former shows its effects on the regulation of cell immune function and the improvement of the HFRS symptoms. By detecting the level of CD^{4+} and CD^{8+} cells, CD^{4+}/CD^{8+} ratio and the concentration of TNF-α and IL-10 in lymphocyte, the authors found that the prescription could significantly change the clinical symptoms, and the integrated methods was better than that conventional therapy used alone. According to the different stages of epidemic hemorrhagic fever patients. According to the different stages of epidemic hemorrhagic fever patients, Hao et al. [27] combined conventional therapy with the application of QBD for treatment, the results show that the therapeutic effect of integrated strategy is significant with no adverse reactions.

Epidemic cerebrospinal meningitis

Qin et al. [28] treated four cases of meningococcal meningitis mainly by oral QBD supplemented by antibiotics. All of them were cured and the course of treatment was shortened. Sun et al. [29] treated 62 patients with epidemic cerebrospinal meningitis with QBD. The results showed that 58 cases were cured, 3 cases were significantly improved and 1 case was ineffective.

Infantile pneumonia

On the basis of routine treatment, QBD plus subtraction retention enema increased the cure rate of infantile pneumonia from 81.4 to 97.62%, which significantly improved the clinical effect of the patients [30].

Sepsis-associated encephalopathy (SAE)

Through the intervention of QBD, the relationship between TLR4, cytokines and inflammatory mediators in SAE rats induced by CLP was observed. The results showed that QBD could inhibit the number of WBC, the percentage of neutrophils (NE), TNF-α, IL-6, leukotriene B4 (LTB4) and the content of TLR4 positive cells in brain tissue of rats with sepsis-related encephalopathy. QBD can reduce the excessive inflammatory response and regulate the immune response in sepsis-related encephalopathy rats through its anti-inflammatory factors (TNF-α, IL-6 and TLB4) and regulating the expression of TLR4-mediated inflammatory signal pathway, indicating that QBD can improve the neurological function and inflammatory response of sepsis-related encephalopathy rats [31].

Epidemic encephalitis B

Hong [32] found that QBD plus Rhei Radix et Rhizoma combined with conventional therapy had obvious advantages over conventional therapy used alone in the treatment of epidemic encephalitis B in children. Yang et al. [33] treated 16 cases of epidemic encephalitis B with oral QBD. The results showed that the effect of QBD was good and the total effective rate was 87.5%.

Pharmacological research progress of QBD on anti-virus and infection related inflammation

QBD has a wide range of biological activities. Studies have shown that it has the effects of anti-inflammation, anti-virus, antibacterial, protecting liver and gallbladder, regulating immunity, anti-cardiovascular disease and so on. Modern pharmacological studies show that QBD has multiple pharmacological effects, such as antipyretic, reducing blood viscosity, antagonizing platelet aggregation, antibacterial, anti-viral, anti-inflammatory, sedative, analgesic, liver protection, cardiotonic, detoxification, diuresis and so on.

Anti-inflammatory effect

By dynamically observing the effect of QBD on the expression of serum cytokines TNF-α, IL-8 and IL-10 in rats with ALI induced by non-invasive instillation of endotoxin (LPS, 2 mg/kg) solution through larynx, He et al. [34] found that QBD could regulate the imbalance of pro-inflammatory and anti-inflammatory factors during ALI, alleviate pulmonary inflammatory injury, and thus protect lung tissue. Wang et al. [35, 36] also found that QBD can effectively regulate the expression levels of inflammatory cytokines IL-1β and anti-inflammatory cytokines IL-13 in blood of rats with ALI induced by LPS, promote the dynamic balance of inflammatory and anti-inflammatory cytokines, reduce the total number of white blood cells in bronchoalveolar lavage fluid, improve alveolar injury, inflammatory cell infiltration and erythrocyte exudation, etc., thereby reducing inflammatory cell infiltration in the lung. It can repair and protect the injured lung tissue. Oral or nasal feeding with QBD on the basis of conventional treatment can reduce the expression levels of TNF-α, IL-1, IL-6 and IL-10 in patients with septic acute renal injury, inhibiting excessive inflammation and delaying renal injury [37].

Wang et al. [35] observed the effect of QBD on the level of serum inflammatory factors in rats with ALI, and found that it can effectively regulate the expression of inflammatory cytokines IL-1β and IL-13 to achieve a dynamic balance between inflammatory and anti-inflammatory cytokines, repair and protect the injured lung tissue. Wu et al. [38] observed the anti-inflammatory effects of paeonol and geniposide by increasing celiac capillary permeability and foot swelling test in mice. The results showed that both paeonol and geniposide had obvious anti-inflammatory effects. These results indicate that the anti-inflammatory effect of QBD is closely related to the anti-inflammatory effect of Moutan Cortex and Gardeniae Fructus in the prescription.

Antiviral effect

Shi et al. [39] used Gardeniae Fructus extract for detecting the expression of VP16 mRNA and IFN-γ in the brain of mice infected with herpes simplex virus type I in vivo. They found that Gardeniae Fructus extract could inhibit the replication of herpes simplex virus in the brain of mice. Therefore, it can be known that QBD has significant antiviral effect, which is related to the large doses of Forsythiae Fructus, Scutellariae Radix, and Gardeniae Fructus and so on. By observing the efficacy of QBD in the treatment of H1N1 influenza virus pneumonia, the results showed that the total effective rate of QBD treatment group (96.0%) was significantly higher than that of the control group (70.0%). And the serum levels of TNF-α, IL-6, IL-8 and CRP in the QBD were lower than those in the control group, while the level of IL-10 was higher than that in the control group. It was effective in the treatment of H1N1 influenza A complicated with respiratory distress syndrome. It shows that QBD can reduce the inflammatory reaction and improve the symptoms of patients, which is of great significance in the prevention and treatment of influenza A H1N1 viral pneumonia. TCM not only has the effect of multi-link anti-influenza A virus, but also can adjust the immune state of the body, improve the antiviral ability, enhance the stability of the tissue itself, reduce the excessive inflammatory reaction of the body, and protect the cell tissue. QBD plays a significant role in reducing fever, reducing temperature, relieving cough and removing phlegm, improving symptoms and other effects, and its curative effect is easy for patients to accept, which reflects the advantages of TCM compound prescription in the treatment of multi-pathway, multi-link and multi-target, and is of great significance to effectively control the epidemic situation of influenza A H1N1 [15, 40].

The clinical observation of QBD combined with ganciclovir in the treatment of infectious mononucleosis (IM) caused by epstein–barr virus infection showed that the total effective rate of the combined group was 93.48%, significantly higher than that of the control group (80.00%), and the effect was more obvious [41]. The curative effect was more obvious and the time of fever, pharyngitis and enlargement of liver and spleen lymph nodes in the QBD group was significantly shortened. The time for the total number of WBC, abnormal lymphocytes and liver function to return to normal was significantly shortened, indicating that QBD is effective in the treatment of infectious mononucleosis in children [42].

To study the clinical efficacy of colon infusion of QBD in the treatment of severe entero virus 71 (EV71) infection. The control group was treated with routine therapy, and the experimental group was treated with routine therapy combined with colon drip of QBD. The number of advanced cases in the experimental group was significantly reduced, and the time of fever regression and hospitalization in the experimental group was significantly shorter than that in the control group. The results show that QBD colonic drip is effective in the treatment of EV71 infection. It is worth popularizing for early antipyretic and preventing disease progression [43].

Shen et al. [44] infected mice by adenovirus ADV3 and influenza virus FM1. Then, mice were treated with different concentrations of Scutellariae Radix and Forsythiae Fructus water extract. It was found that both of them could significantly reduce the mortality of mice infected with the two viruses, indicating that their antiviral effect was significant. The effect of Gardeniae Fructus should not be ignored though served as an adjuvant in this prescription.

In addition, QBD has the effects of antipyretic, anti-inflammatory, antibacterial and antiviral. It can shorten the antipyretic time in the treatment of SARS, and no adverse reactions are found, so it can be used as an antiviral adjuvant in clinic [45].

Antimicrobial effect

Yu [46] used Coptidis Rhizoma to treat the skin model of bacterial and fungal infection in Japanese white rabbits, and the results showed that it had a strong antibacterial effect. Zhu et al. [47] found that Coptidis Rhizoma and its different processed products had obvious bacteriostatic effect. Han et al. [48] used the different extracts of Anemarrhenae Rhizoma to test the bacteriostasis of *Staphylococcus aureus*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Escherichia coli* in vitro. The results showed that sarsasapogenin in the extract had the strongest antibacterial activity. The Coptidis Rhizoma also has significant antibacterial activity in vitro. Liu et al. [49] used QBD water extract to inhibit *Escherichia coli* and *Klebsiella pneumoniae* in vitro. The results showed that it had inhibitory effect on two kinds of enzyme-producing bacteria. Therefore, it is speculated that the antibacterial effect of QBD may be exerted through the combination of the above drugs.

Immunomodulatory effect

Using QBD to treat rats with summer heat syndrome of febrile disease, the levels of IL-2, IL-6 and IL-18 in the large dose QBD group and the IL-10 level in the low dose QBD group were higher than those in the LPS control group, and the levels of IL-2 and IL-18 in the high dose QBD were higher than those in the low dose QBD group. QBD can increase the levels of IL-2, IL-6, IL-10 and IL-18 in the rat model of heat–heat syndrome of febrile disease, which may have the effect of immune enhancement [50]. Zhang et al. [51] observed the changes of IgG, IgA, IgM, CRP, TNF-α and C3 in peripheral venous blood of patients with sepsis before and after treatment with QBD. The results showed that QBD could inhibit the excessive immune response of patients and reduce its damage to the body. Moutan Cortex is reused in QBD, and its main active ingredient paeonol has the effect of regulating immunity. Adding QBD on the basis of routine treatment of western medicine can improve the clinical curative effect of patients with sepsis from 75.00 to 84.00%, reduce the symptom score of TCM and the levels of IgG, IgA, IgM, C3, CRP and TNF-α, inhibit the excessive immune response of septic patients and reduce the damage of excessive immune response to the body [52].

Zhang et al. [53] through intraperitoneal injection of 5% chicken erythrocyte suspension and intragastric administration of paeonol of different concentrations. Finally, they found that paeonol could enhance the specific humoral and cellular immune function of mice. Fu et al. [54] treated 18 patients with sepsis by QBD, and detected the peripheral blood prothrombin time, activated partial thromboplastin time, thrombin time and platelet count before and after treatment. The results showed that the prescription can improve the blood coagulation function of patients with sepsis and play a protective role in patients with sepsis.

Antipyretic effect

Wang et al. [55] observed the clinical efficacy of QBD in the treatment of high fever, and found that the total effective rate of QBD in the treatment of high fever (92.3%) was significantly higher than that in the control group of Angong Niuhuang Pill (57.69%). The clinical effect was satisfactory. Wang et al. [56] studied the changes of body temperature in the fever model of rats and mice induced by dry yeast, endotoxin and 2,4-dinitrophenol, and found that Yuanshen can reduce the fever temperature of rats and mice within 4–8 h after the fever. It is inferred that the antipyretic effect of QBD may be played by Gypsum Fibrosum, Scutellariae Radix, Forsythiae Fructus and so on. Li et al. [57] induced fever in rats by subcutaneous injection of yeast solution, intragastric administration of different doses of baicalin solvent, detected body temperature in different periods of time, and found that its antipyretic effect was significant. Yu et al. [58] induced systemic inflammatory response syndrome in rabbit by injecting LPS into ear vein. After intragastric administration of different doses of Gypsum Fibrosum, the anal temperature was measured and it was found that QBD had obvious antipyretic effect. Hu et al. [59] used different extracts of Fructus Forsythiae shell and seed to observe the neutralization effect on fever of rabbit, ear swelling of mouse and endotoxin. The results showed that the antipyretic effect of the extracts was obvious.

Material basis of anti-virus and infection-related inflammation of QBD

In QBD, the sovereign drug is Gypsum Fibrosum, and its main active ingredient is hydrous calcium sulfate; Rehmanniae Radix is a minister drug, which mainly contains rehmannia glycoside, catalpol and so on; The adjuvants are Coptidis Rhizoma, Scutellariae Radix, Anemarrhenae Rhizoma, Scrophulariae Radix, Gardeniae Fructus etc. Coptidis Rhizoma mainly contains berberine. Scutellariae Radix contains a lot of baicalin. the main active ingredients of Anemarrhenae Rhizoma are sarsasapogenin. Scrophulariae Radix contains paeonol. Gardeniae Fructu mainly contains geniposide and geniposide. Paeoniae Radix Rubra mainly contains paeoniflorin, etc. The main components of Forsythiae Fructus are phillyrin and forsythoside, Rhinoceros nicornis LR.simus Burchell mainly contains cholesterol and so on. Pentacyclic triterpenoid glycosides are the main active components of Platycodonis Radix, and the effective components of Moutan Cortex are paeonol and paeoniflorin. The conductant drug of this prescription are fresh Lophatheri Herba and Glycyrrhizae Radix et Rhizoma, which contain flavonoids, glycyrrhizin and glycyrrhetinic acid, respectively [60].

Gypsum Fibrosum and Rhinoceros nicornis LR.simus Burchell powder are commonly used and effective antipyretic in TCM. Scutellariae Radix, Forsythiae Fructus, Scrophulariae Radix and Gardeniae Fructus can not only assist the main drug in heat-clearing and detoxification, but also have direct antiviral effects confirmed by modern pharmacological studies. TCM believes that “superheat consuming qi” and “consumption of yin caused by febrile diseases”. Thus, both heat-clearing and detoxification and replenishing qi and nourishing yin should be taken into accounts in treatment. Astragali Radix, Radix et Rhizoma, Anemarrhenae Rhizoma, and Rehmanniae Radix were added to the original prescription, while Astragali Radix can achieve indirect antiviral effect by regulating the immune function of the body [45].

Through the analysis of the compatibility of disassembled prescriptions and the parallel comparison of multiple indexes, Ding et al. [61] selected the effective ingredient of QBD to clear away heat and purge fire, and made it clear that the effective component of QBD in interfering with endotoxic ALI in rats was crocin-1.

The pharmacological effect of QBD in animal and clinical study

Studies have shown that LPS or endotoxin were mainly used to establish ALI model of rats. Moreover, endotoxin of *Escherichia coli* was used to establish sepsis models in New Zealand rabbit. These studies mainly investigated the influence of QBD on ALI, sepsis or SAE model in terms of lung histopathology, W/D ratio, WBC level, PaO2 level, PaCO2 level, and other indicators. The specific details of disease types, animal types, experimental model, does of QBD, medical effects in QBD group and targets are shown in Table 1.

Notably, the relevant research of QBD is mainly focused on clinical research. In clinical, the therapeutic effects of QBD on sepsis, viral pneumonia caused by influenza A virus subtype H1N1, SARS, hyperthermia and other diseases were discussed by collecting clinical cases. In general, QBD combined with conventional therapy can significantly improve the clinical treatment efficiency of these diseases. The reported indicators in QBD group are shown in Table 2.

Effect of QBD on lung histopathology

The effect of QBD on lung histopathology was mainly reported in animal experiments. He et al. [34] found that the alveoli space of LPS induced-ALI rats became narrow with reduced swelling of capillary endothelial cells, and the bleeding and exudation of edema fluid were significantly improved in QBD group. Wang et al. [25] found that QBD can reduce the damage degree of ALI rats' lung tissue, and can reduce the aggregation, infiltration and exudation of inflammatory cells in the lung tissue, so as to play a more obvious role in protecting the lung tissue. QBD can effectively decrease the relative protein expression of NF-κB p65 in ALI rats induced by LPS. By inhibiting the activation of NF-κB and the production of inflammatory cytokines, the inflammatory response of rat lung tissue can be reduced. Early application of QBD can effectively reduce the degree of lung injury and the inflammatory response of alveoli. Zhang et al. [62] found that QBD reduced the degree of congestion and edema of pulmonary interstitium and alveoli, and decreased the infiltration of inflammatory cells. Wang et al. [24] used endotoxin to establish sepsis ALI. The results indicated that pathological type II alveolar cell injury score of observation data significantly reduced in QBD group. In addition, QBD could significantly alleviate the alveolar pathological damage in endotoxin-induced ALI in rats. The results showed that the alveolar structure of QBD group was relatively complete, and the consolidation of alveoli, infiltration of inflammatory cells and edema in alveoli were alleviated in varying degrees.

Pharmacological effects in QBD group

ALI is a syndrome caused by severe infection, trauma and shock, which is characterized by diffuse high permeability pulmonary edema and parenchymal cell injury. It is a serious complication in the early stage of sepsis. In essence, the disease is an out of control systemic self-destructive inflammatory response. Previous studies have shown that QBD can reduce WBC, PMNs, W/D ratio, PaO2 and PaCO2 levels of LPS-induced ALI [24, 25, 31, 34,35,36, 61,62,63]. It was found that the pharmacodynamics of QBD was mainly related to the regulation of IL-8, IL-10 and TNF-α levels [34]. Pathway study showed that QBD could significantly reduce the over activation of NF-κB p65, JAK2/STAT3 and p38 MAPK signaling pathway [25, 63]. Simultaneously, QBD can decrease the degree of lung inflammation and injury, and the over release of inflammatory factors in ALI rats.

The pharmacological effect of QBD in clinical trials

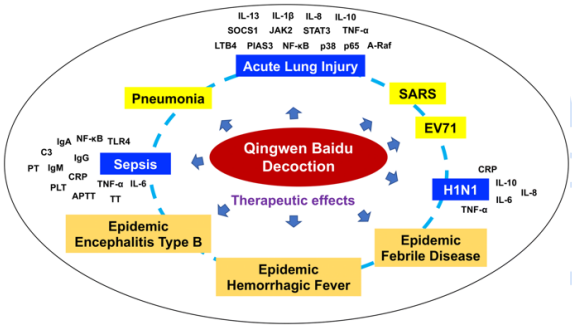
QBD can be used in the treatment of sepsis, influenza A H1N1 virus pneumonia, SARS, pneumonia, and erythroderma psoriasis and so on, among which the treatment of sepsis is the most reported (Table 2). Most of the collected clinical cases were treated with conventional therapy combined with QBD. The course of treatment was generally about 7 days, which could significantly improve the clinical efficiency. For the treatment of sepsis, the influence of QBD on the APACHE II score, TCM symptom score, serum immunology index, serum PT, APTT, TT and PLT values, mortality and safety of the patients was focused. As sepsis patients have obvious immune dysfunction, QBD can play a role in treating sepsis by regulating the immunological index, and the clinical effect is better than that of the conventional treatment group. Relevant detection indicators of other diseases are shown in Table 2.

NCP is mainly characterized by pneumonia, fever, cough, fatigue, and gradually dyspnea. A few patients have gastrointestinal symptoms such as nausea, vomiting and diarrhea. Currently, novel coronavirus infection has not been confirmed effective specific antiviral drugs. From the outbreak of SARS in 2003 and MERS in 2018 to the recent outbreak of COVID-19, clinicians and scientists have provided several potentially effective

antiviral drugs for clinical use, including remdesivir, lopinavir/ritonavir, convalescent plasma and monoclonal antibodies. However, the specific efficacy and safety of these drugs in patients with COVID-19 still need further clinical trials.

Modern pharmacological studies have found that QBD has significant pharmacological activities in antiviral, regulating immune function, inhibiting inflammation, improving vomiting and diarrhea. From the theory of TCM to modern clinical and pharmacological studies, it has been shown that QBD combined with modern medical conventional treatment has therapeutic effect on viral infection related diseases, and its mechanism includes inhibition of virus, infection related inflammation and improvement of disease-related symptoms. QBD has therapeutic effect on a variety of virus (influenza virus H1N1, SARS virus, EV 71, etc.) infection [40, 43, 45]. It is a clinical goal for the body to remove the virus from the body's immune system. Pharmacological studies show that QBD and its components have significant anti-inflammatory effects, which can inhibit inflammatory exudation, reduce the levels of a variety of pro-inflammatory cytokines, increase the level of anti-inflammatory cytokines IL-10, and regulate the inflammatory related NF-κB/p65, JAK2/STAT3, TLR4 pathways. It is suggested that NCP can alleviate inflammation in QBD, protect organs from inflammation and slow down the progress of inflammation. In addition, QBD could improve the symptoms of fever and diarrhea associated with viral infection, suggesting that QBD may also improve the symptoms of new crown pneumonia. The pharmacological effect and multi-target mechanism of QBD in the treatment of various diseases are shown in Fig. 1.

Fig. 1



Pharmacological effect and multi-target mechanism of QBD in the treatment of various diseases

Possible mechanism of QBD in prevention and treatment of COVID-19

The inflammation caused by virus infection can promote the clearance of invading virus, and return to normal under negative feedback regulation after virus clearance. However, excessive inflammatory reaction can lead to more serious damage to the body than the virus infection itself, and in some cases even lead to the imbalance of immune regulation, the lack of negative feedback and the continuous amplification of positive feedback, and the abnormal increase of a variety of pro-inflammatory cytokines, resulting in a storm of cytokines, which eventually leads to organ damage, functional failure and even death. COVID-19 patients also experienced a cytokine storm and related acute respiratory distress syndrome (ARDS). In the absence of a specific drug for COVID-19, the clinical goal is to support the treatment and control of inflammation and let the body immune system clear the virus.

The pathogenesis of COVID-19 is not clear. It may be related to the damage of immunity and respiratory epithelial cells caused by COVID-19, and the susceptibility of the body, but regardless of the pathogenesis. It is reported that there is overexpression of inflammatory factors in patients with advanced COVID-19, which leads to cytokine storm [70]. Cytokine storm is considered to be an important factor in the development of viral pneumonia. The roles of cytokines in immune network in promoting disease progression, inducing local inflammation, eliminating infection, regulating cellular and molecular immune response, and regulating tissue repair are very complex [71]. When the immune system is out of balance and cytokines are overexpressed, there will be a cytokine storm, which will cause serious damage to the body, such as diffuse

alveolar injury, hyaline membrane formation, fibrin exudation, etc. At the same time, accelerated lung injury, the emergence of more serious pulmonary capillary injury and damage to the immune function of the body, cytokines in the circulatory system will lead to systemic cytokine storm, further lead to systemic organ dysfunction, accompanied by the overexpression of inflammatory factors such as IFN, TNF, IL and so on. Regulating the immune balance in the state of COVID19 and inhibiting the occurrence of cytokine storm will be an important way and mechanism to block the deterioration of the disease.

Overall, QBD has been shown to have a therapeutic effect on exogenous diseases related to viral infection from the theory of TCM to modern clinical and pharmacological studies. QBD and its basic prescription exert the effect of “detoxifying and cooling blood”, and play a significant therapeutic effect on a variety of viral infections (EB virus, H1N1 influenza virus, SARS virus, EV71, etc.). It may be related to the systematic regulation of inhibition of virus proliferation in the host and excessive expression of host inflammatory response, and is expected to be used in the treatment of severe pneumonia in COVID-19. The specific mechanism of its curative effect may be closely related to the inhibition of COVID-19 invasion into the host for proliferation and the improvement of cytokine storm. The replication cycle of animal virus infection has similarity. With the joint efforts of colleagues in the pharmaceutical industry, it has been found that a number of drugs developed for other viruses can inhibit COVID-19 in vitro [72, 73]. Based on the pharmacological effects of QBD, such as antipyretic, antibacterial, antiviral, anti-inflammatory, regulating immunity and so on, it is commonly used in clinical treatment of infectious diseases, such as sepsis, epidemic cerebrospinal meningitis, epidemic hemorrhagic fever, epidemic encephalitis B and so on, with remarkable curative effect, not easy to relapse and little side effect. As far as the material basis of the prescription is concerned, the future research should not be limited to the application of the whole prescription, but should be flexibly applied. The relevant components and contents can be added or decreased according to the disease, and the combination of TCM and western medicine should be adhered.

QBD has a dual regulatory effect on immune function, through the regulation of immunity to achieve the purpose of anti-infection and anti-inflammation [74]. The combination of heat-clearing and detoxifying drugs and drugs for promoting blood circulation and removing blood stasis can enhance the non-specific anti-infective effect of the former [75]. Modern experimental studies have found that QBD has some pharmacological effects, such as heat-clearing and detoxification, anti-platelet aggregation, reducing blood viscosity, anti-inflammation, analgesia, sedation, antibacterial, antiviral, liver protection, cardiotonic, diuretic and so on [76]. All the heat-clearing and detoxifying drugs such as Anemarrhenae Rhizoma, Gypsum Fibrosum, Scrophulariae Radix, Forsythiae Fructus, Coptidis Rhizoma have certain antiviral ability, which can remove the virus or inhibit its replication, and reduce the inflammatory damage of the virus. Forsythiae Fructus can stimulate mononuclear macrophage system, enhance phagocytosis and promote antibody production; Scrophulariae Radix can prolong the existence time of antibody; Scutellariae Radix can enhance leukocyte phagocytosis, promote lymphocyte transformation and improve immune function. Drugs for promoting blood circulation and removing blood stasis such as Paeoniae Radix Rubra and Moutan Cortex can not only inhibit cellular immunity and possibly inhibit the extensive toxic effect of T cells, so as to reduce multiple organ damage, but also promote non-specific immunity, antivirus, regulate immunity and so on [77,78,79,80,81,82]. Moutan Cortex cool blood and dissipate blood stasis, rash, clear camp diathermy to nourish Yin. Scutellariae Radix, Glycyrrhizae Radix et Rhizoma, Gardeniae Fructus have effect on protecting liver function and forsythia. Forsythiae Fructus, Scutellariae Radix, and Lophatheri Herba can promote the excretion of endotoxin and virus. All kinds of medicines are compatible, clearing heat and detoxification, activating blood circulation and removing blood stasis, replenishing qi and nourishing yin, and by regulating immunity and other effects of antivirus, antipyretic, detoxification and liver protection, so as to achieve the purpose of treatment. It is suggested that QBD can reduce the inflammatory reaction of COVID-19 patients and may have a protective effect on organ damage caused by inflammation. Although there is no complete data on the treatment of COVID-19 by QBD, it is believed that in the near future, the TCM and modern medicine can achieve positive clinical treatment effect and reduce the suffering of patients, improve prognosis and rehabilitation.

In this fight against NCP, TCM has shown its unique advantages in reducing mortality and improving cure rate [83, 84]. Notably, TCM also pays attention to three elements in the treatment of diseases: first, the climate environment. Now, people are paying attention to the relationship between the climate humidity and temperature on the epidemic situation, which is a common problem. Second, we should attach importance to special disease and specific prescription and syndrome differentiation and treatment. Third, we should attach importance to people's constitution. Although QBD can be used in the treatment of a variety of viral infectious diseases and alleviates the symptoms of the disease with good clinical efficacy, from the perspective of drug properties, QBD belongs to "cold medicine". If the patient's normal constitution is partial to *Yang* deficiency, it is not suitable to take more and use for a long time to avoid damaging the organism.

Conclusions

Currently, the number of convalescent patients of COVID-19 is increasing, especially in clinical practice, by "strengthening the integration of traditional Chinese and modern medicine", the course of disease is shortened and the cure rate is improved. Modern pharmacology and clinical research also confirmed that the treatment of QBD in COVID-19 has the theory of TCM and modern research results as the theoretical basis, which is suitable for the treatment of patients with COVID-19. It is speculated that its main role is to reduce the level of inflammation in patients. Simultaneously, it plays a role in inhibiting the replication and infection of virus, inhibiting the concurrent bacterial infection, and improving the immunity of the body. However, there are few researches on the mechanism of QBD's action on human coronavirus. Through the study on the mechanism of QBD's multi-channel anti coronavirus action, we hope to provide new ideas for the clinical treatment of COVID-19, which improve the cure rate of patients infected with COVID-19, reduce their mortality, and further explore the pharmacological effect and phase of QBD. Thus, the series researches will provide scientific basis for the study and development of new drugs.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations

NCP: Novel Coronavirus Pneumonia

WHO: World Health Organization

COVID-19: Corona Virus Disease 2019

PHEIC: Public health emergencies of international concern

TCM: Traditional Chinese medicine

ACE2: Angiotensin converting enzyme 2

QBD: Qingwen Baidu Decoction

CRP: C-reactive protein

WBC: White blood cell

CLP: Cecal ligation and puncture

ALI: Acute lung injury

LPS: Lipopolysaccharide

HFRS: Hemorrhagic fever with renal syndrome

W/D: Wet to dry ratio

SAE: Sepsis-associated encephalopathy

NG: Neutrophil granulocyte

LTB4: Leukotriene B4

EVLW: Extravascular lung water

IM: Infectious mononucleosis

EV71: Entero virus 71

ARDS: Acute respiratory distress syndrome

APACHE: Acute physiology and chronic health evaluation

SOFA:Sequential organ failure assessment

PLT: Platelet count

PT: Prothrombin time

APTT: Activated partial thromboplastin time

TT: Thrombin time

Fib: Fibrinogen

D-D: D-Dimer

AKI: Acute kidney injury

BUN: Blood urea nitrogen

NR: Not report

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ABSTRACT

Currently, coronavirus disease 2019 (COVID-19), which can lead to severe respiratory failure and death, is now aglobal pandemic with no specific anti-viral drugs or vaccines. However, It is worth noting that traditionalChinese medicine (TCM), especially Chinese herbal medicine (CHM), has been widely applied in mainland Chinasince outbreak, bringing new hope for the prevention and control of COVID-19. A comprehensive literaturesearching was conducted in 7 electronic databases from their inception up to June 21, 2020 to evaluate theefficacy and safety of CHM for COVID-19. Eighteen randomized controlled trials (RCTs) involving 2275 patientswere enrolled. Most of CHMs were originated from classical Chinese herbal formulas. Liquoric Root (Gancao,Radix Glycyrrhizae), Baical Skullcap Root (Huangqin, Radix Scutellariae Baicalensis), Pinellia Rhizome (Banxia,Rhizoma Pinelliae Tematae), Forsythia Fruit (Lianqiao, Fructus Forsythiae Suspensae), and Bitter Apricot Seed(Kuxingren, Semen Armeniacae Amarum) were most frequently used Chinese herbs. The most commonly useddosage formulation was decoction. Our meta-analyses found that comparing CHM group and conventionalwestern medicine group, CHM group has improvements in several clinical parameters including lung CT, clinicalcure rate, ranging from mild to critical cases, length of hospital stay, total score of clinical symptoms, feverreduction time, symptom score of fever, number of cough reduction cases, symptom score of cough, number offatigue reduction cases, symptom score of fatigue, disappearing time of fatigue, TCM syndrome, viral nucleicacid testing, and inflammatory biomarkers (C-reactive protein). Besides, no severe adverse effects was identifiedby CHM. CHM, especially classical Chinese herbal formulas, could be used as potential candidates for COVID-19in this battle.1. IntroductionSince December 2019, coronavirus disease 2019 (COVID-19) hasbroken out inWuhan, China [1]. The main symptoms include fever, drycough, and fatigue, while some patients with myalgia and diarrhea. Itcan lead to severe respiratory failure, acute respiratory distress syn-drome, septic shock, and even death. Currently, COVID-19 outbreak ismoving rapidly. As of June 21, 2020, a total of 8,949,953 confirmedcases of COVID-19 has been reported in China and 200 other countries,with 4,760,539 (53.19 %) cured cases and 467,347 (5.22 %) deaths.Unfortunately, the number of confirmed cases continues to rise due torapid spread. World Health Organization (WHO) has defined it as aglobal pandemic. Worst of all, except for conventional western medi-cine (CWM) including antiviral drugs, antibacterial drugs, antitussive, expectorant and antiasthmatic drugs, and symptomatic and supportivetherapy, no specific anti-viral drugs or vaccines has been discovered forthis virus. More efforts should be made to understand the pathophy-siology and improve clinical efficacy of this new disease. Thus, it has become a major global public health problem.In China, the pandemic is under control due to government strongmeasure, public surveillance, and utilization of both CWM and tradi-tional Chinese medicine (TCM). TCM, especially Chinese herbal medi-cine (CHM), has been used extensively in the treatment of several acuteepidemic infectious diseases including severe acute respiratory syn-drome (SARS), influenza A H1N1, avian influenza, malaria, etc [2,3].According to the research reports of World Health Organization, com-pared to CWM group, TCM group has achieved remarkable therapeuticeffect with 3 days for average fever reduction time, 10 days for averagehospital stay, low medical costs, and no death, sequelae, transfer, andinfection of nurses and doctors during the SARS epidemic in 2003 [4].Among the 564 patients with COVID-19 admitted toJiangxia FangcangTCM Hospital, 482 were cured, and the rest 82 complicated with basicdiseases were transferred to designated hospitals. During the treatment,no patients turned from mild to critical cases, and no nurses and

doctorswere infected by COVID-19. TCM has played an indispensable role andTCM therapeutic schedule was included in the guideline on diagnosisand treatment of COVID-19 [5].Currently, a large number of published clinical studies includingcase reports, case series, and randomized controlled trials (RCTs)showed that CHM could improve clinical symptom and and lung CTimage, shorten fever reduction time and average length of hospital stay,and reduce the conversion rate from mild to severe, bringing new hopefor clinical treatment and new drug discovery in treating COVID-19[6,7]. Although 4 systematic reviews [8–11] regardingLianhua Qingwengranules and other CHM for COVID-19 have been published in advancerespectively, serious methodological shortcomings were also identified.Non-RCTs were enrolled in the review by Dr Qi et al., which should beexcluded actually [8]. For the other systematic review, more databasesand RCTs should be updated in order to reduce potential bias [9–11].Therefore, in this study, a systematic review of RCTs was performed toevaluate the current clinical evidence on CHM for the treatment ofCOVID-19.2. MethodsThis study was conducted and reported according to the guidelinesof Preferred Reporting Items for Systematic Reviews and Meta-Analyses(PRISMA) [12].2.1. Eligibility criteria2.1.1. Types of studiesRCTs which have evaluated the efficacy of CHM for COVID-19 wereincluded in this study.2.1.2. Types of participantsPatients diagnosed as COVID-19 could be enrolled in this review. Inorder to ensure including all relevant studies, no restriction on gender,age and nationality was preseted.2.1.3. Types of interventionsCHM, which include Chinese natural herb and its processed pro-ducts that originated from botanical drug, mineral, animal, chemicaland biological products sources, should be used under the guidance ofTCM theory. The dosage forms of CHM contain decoction, tablet, pill,powder, pellet, granule, capsule, cream formula, oral liquid, plaster,and injection [13].Patients in the treatment group should be treated by CHM or com-bination of CHM and CWM. Patients in the control group should betreated by CWM or combination of CHM placebo and CWM. CWM inthe treatment and control group must be identical in name, usage,dosage, etc. No restrictions on dosage forms, type, quantity, or treat-ment course of CHM was preseted.RCTs will be excluded if the following conditions are met: (a clinical experiences, theoretical discussion, reviews, commentaries,editorials, case reports, case series, and experimental studies; (b)non−COVID-19 patients; (c) other TCM therapeutic methods beyondCHM, including acupuncture, moxibustion, cupping, massage,qigong,Tai Chi,baduanjin, and music therapy, were applied in either treatmentor control group; (d) no detailed information regarding clinical efficacycould be extracted; and (e) duplicate publications reporting the sameresults.2.1.4. Types of outcome measuresThe primary outcome measure was defined as lung CT. The sec-ondary outcome measures were death, clinical cure rate, ranging be-tween mild and critical cases, length of hospital stay, clinical symptoms(total score of clinical symptoms, fever, cough, fatigue), TCM syn-drome, viral nucleic acid testing, and inflammatory biomarkers in-cluding white blood cell (WBC), neutrophils (NEU), lymphocyte (LYM),and C-reactive protein (CRP).2.2. Literature searchRelevant literatures assessing the efficacy and safety of CHM forCOVID-19 were searched in 7 electronic databases including CochraneCentral Register of Controlled Trials, EMBASE, PubMed, ChineseNational Knowledge Infrastructure (CNKI), VIP Information Database(VIP), Chinese Biomedical Literature Database (CBM), and WanfangDatabase from inception up to June 21, 2020. The following groupedkeywords were used as search strategy and modified according to dif-ferent databases: (“coronavirus disease 2019” OR “COVID-19” OR“SARS-CoV-2” OR “novel coronavirus pneumonia” OR “novel cor-onavirus” OR “xin xing guan zhuang bing du fei yan” OR “xin guan fei yan”OR “xin xing guan zhuang bing du”) AND (“Chinese herbal medicine” OR“traditional Chinese medicine” OR “classical Chinese herbal formulas”OR “Chinese herb” OR “Chinese herb therapy” OR “herbal medicine”OR “herb therapy” OR “herbal remedy” OR “zhong yi yao” OR “zhongyao”) AND (“clinical trial” OR “clinical study” OR “randomized con-trolled trial”OR “randomised controlled trial” OR “lin chuang yan jiu”OR “lin chuang shi yan”). In order to reduce bias, we also retrieved theongoing registered clinical trials and unpublished papers on CHM forCOVID-19. We also manually retrieved relevant articles and clinicalstudies to obtain as much literature as we can. No language and statusrestriction was set in this review.2.3. Study selection and data extractionThe selection of studies and data extraction were performed in-dependently by two reviewers (Xiong XJ and Wang PQ) according tothe preseted inclusion and exclusion criteria. Detailed information ofenrolled study was listed as below: (a) basic characteristics of includedstudies: title of study, authors’ name, publication date, sample size,diagnostic

criteria, methodological quality, therapeutic schedule intreatment and control groups, components and dosage of CHM, with-draws, and course of treatment; (b) basic characteristics of includedpatients: age, gender, ratio of mild to severe cases, baseline data of bodytemperature, heart rate, respiration, blood pressure, previous medicalhistory, and laboratory examination; (c) both primary and secondaryoutcome measures; and (d) adverse effects. If detailed information onoutcome measure was lacking, the first or correspondence author oforiginal study was contacted by email, fax, and telephone, which wererecorded in the article. If no response was obtained from the authors,data was recalculated from the graphs using digital ruler software.Otherwise, it was excluded. If disagreements on data extraction wereidentified, a third party (Xing YW and William CC) was consulted.2.4. Assessment of methodological qualityMethodological quality of the included trials was also assessed by reviewers (Xiong XJ and Su KL) independently. According to CochraneCollaboration's tool [14], 7 fields of risk of bias (ROB) were evaluatedas below: adequate sequence generation, allocation concealment,blinding of participants and personnel, blinding of outcome assessment,incomplete outcome data, selective reporting, and other bias. Each fieldwas assessed to be "yes" (low ROB), "no" (high ROB), or "unclear"(unclear ROB).2.5. Data analysisReview Manager software (RevMan, Version 5.3, Copenhagen: TheNordic Cochrane Centre, The Cochrane Collaboration, 2014) was uti-lized to conduct the data analysis of dichotomous and continuousoutcome measures, which were extracted from the original studies.Weighted mean difference (WMD) was utilized for data measurement ofcontinuous outcomes, while risk ratio (RR) for dichotomous outcomes.All of them were expressed with a 95 % confidence interval (CI). Whenno statistical heterogeneity was identified (heterogeneity test,$P \geq 0.10$, or$I2 \leq 50$ %), fixed-effects model was selected, otherwiserandom-effects model was applied. Funnel plot was also used to eval-uate the publication bias. It was regarded as significant difference when$P < 0.05$.3. Results3.1. Study selectionThe flow chart of literature identification and screening is describedin Fig. 1. In total, 5182 related literatures were derived from the above7 electronic databases. After removing duplicate publications, 4393studies were remained. And then, we excluded 4325 studies as they arenot RCT, specially, these include reviews, commentaries, editorials,case reports, case series, experimental researches, data mining articles,and irrelevant to COVID-19 after scanning titles and abstracts. Fur-thermore, after reading the rest 68 full papers, we further excluded 50literatures as follows: participants did not meet the inclusion criteria(n= 31); duplicate publications (n=1); no control group (n =15);intervention included other medical therapies (n =2); no clinical datafor extraction (n =1). Ultimately, 18 eligible RCTs were included[15–32].3.2. Study characteristicsBasic characteristics of enrolled studies and subjects was listed inTable 1. Among the 18 included trials, 5 were multi-centered trials[21,24,27,30,32] and the rest 13 were single-centered trials. All of the18 studies were conducted in mainland China in 2020. One paper wasonline published in advance with English language [32], and the restwere in Chinese [15–31]. There were altogether 2275 patients enrolledin this review, with the sample size ranged from 20 to 517. All theincluded trials evaluated the effects of CHM combined with CWMcompared to CWM alone. The name, usage, dosage of western medicineused in CHM group should be the same as used in CWM group. There isno trial utilized CHM placebo. Treatment duration varied from 5 to 15days. Primary outcome measure was reported in 13 studies[15–19,21,22,24,26,28,30–32]. Death was reported in 4 trials[17,18,21,31]. Clinical cure rate was reported in 7 trials[18,19,22,24,29,31,32]. Ranging between mild and critical cases was reported in 12 trials [15–18,20,21,24,25,28,30–32]. Length of hospitalstay was reported in 2 trials [17,18].

All the included trials reported clinical symptoms. TCM syndrome was evalated in 5 studies[18,20,28,29,31]. Viral nucleic acid testing was reported in 4 trials[15,21,23,32]. Inflammatory biomarkers were reported in 8 trials, in-cluding WBC, NEU, LYM, and CRP [15,16,18,19,21,22,29,31]. Advers effects were ported in 10 trials (16,18–20,22,23,25,31,32).

Fig. 1.Flow diagram of study selection and identification.

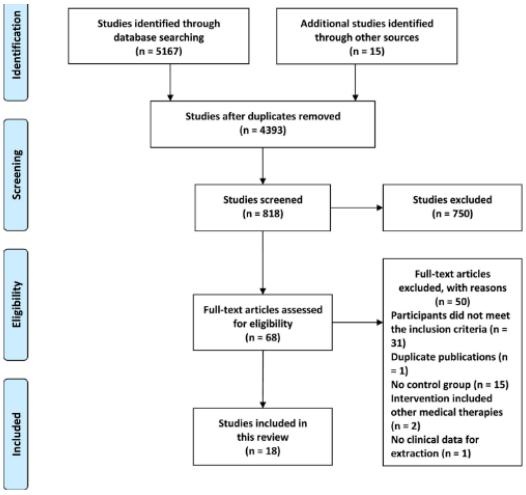

Fig. 1. Flow diagram of study selection and identification.

Table 1
Brief characteristics of included trials and subjects.

Reference	Sample size (Case/Control/Total)	TCC (M/F)	Age (yrs)	Diagnosis standard	Intervention	Control	Treatment duration	Adverse effects reporting	Outcome measures
Xiaoqing H et al. 2020 [18]	68/68	T: 39 (28/ 29)C: 18 (9/9)	T: 60.43 ± 13.84 C: 61.16 ± 13.98	COVID-19	CHM 11 class(es) + C	CHM including an herbal drug (Qingwenbai / rhinoviri, shibid, ribavirin, lopinavir, arbidol, darunavir, umifenovir, antibacterial drugs (moxifloxacin, azithromycin, cefoperazone, ceftriaxone, and cefoperazone-sulbactam), and symptomatic and supportive therapy	10 days	Y	Lung CT, merging between mild and critical cases, clinical symptoms, virus nucleic acid testing, and inflammatory biomarkers.
Xiang XJ et al. 2020 [19]	100/100	T: 81 (56/ 25)C: 19 (12/ 7)	T: 54.7 ± 12.3 C: 50.8 ± 13.8	COVID-19	Qingfei Touxie Jianbeng recipe + C class(es) + C	CHM including an herbal drug (Lianhua qingwen granules, Jinhua qinggan granules, and Xuebijing injection), and symptomatic and supportive therapy	10 days	Y	Lung CT, merging between mild and critical cases, clinical symptoms, and inflammatory biomarkers.
Bai J et al. 2020 [17]	65/67	T: 49 (26/ 23)C: 18 (11/ 7)	T: 47.04 ± 14.44 C: 46.72 ± 17.40	COVID-19	CHM + C	Arbidol drugs (umifenovir, lopinavir, ritonavir, arbidol, darunavir, umifenovir-X, hydroxychloroquine), antibacterial drugs, glucocorticoid drugs, immunomodulatory drugs, gamma globulin, and symptomatic and supportive therapy	6 days	N	Lung CT, clerk, merging between mild and critical cases, length of hospital stay, clinical symptoms.
Xia WG et al. 2020 [18]	30/30	T: 34 (17/ 17) C: 18 (9/ 9)	T: 54.18 ± 13.38 C: 53.60 ± 13.70	COVID-19	CHM + C	CHM including an herbal drug (Arbidol, ribavirin, lopinavir, ritonavir, oseltamivir, arbidol, darunavir, umifenovir, antibacterial drugs (moxifloxacin, azithromycin, cefoperazone, ceftriaxone, and cefoperazone-sulbactam), and symptomatic and supportive therapy (gamma globulin, and methylprednisolone)	10 days	Y	Lung CT, clerk, clinical cure rate, merging between mild and critical cases, length of hospital stay, clinical symptoms, TCM syndrome, virus nucleic acid testing, and inflammatory biomarkers.
Yu XB et al. 2020 [20]	75/75	T: 37 (19/ 18) C: 38 (19/ 19) N: 75	T: 48.39 ± 7.28 C: 44.68 ± 7.48	COVID-19	Tieqi Yu Qiwen granules (3 class(es) + C	Arbidol drugs (abidol tablets) + C	18 days	Y	Lung CT, clinical cure rate, clinical response, and inflammatory biomarkers.
Duan C et al. 2020 [21]	129/132	T: 89 (56/ 33) C: 40 (22/ 18) N: 261	T: 51.89 ± 7.28 C: 50.98 ± 13.17	COVID-19	Jinhua Qinggan granules (CFA + C	CHM including an herbal drug and antibacterial drugs	5 days	Y	Peripheral blood cell and critical cases, clinical symptoms, and TCM syndrome.
Yang MB et al. 2020 [22]	84/49	T: 26 (14/ 12) C: 23 (9/ 14) N: 49	T: 63.58 ± 13.97 C: 47.17 ± 16.97	COVID-19	Xiyanning infusion (20 + 20 mL 5% glucose) + C	CHM including an herbal drug (Qingwenbai / rhinoviri, lopinavir, arbidol, hydroxychloroquine sulfate, Shenzhiling)	7 days	N	Lung CT, clerk, merging between mild and critical cases, clinical symptoms, Neutrophil cell count, and inflammatory biomarkers.
Xiao Q et al. 2020 [23]	200/200	T: 200 (94/106) C: 200 (98/ 102) N: 400	T: 60.00 ± 8.70 C: 62.00 ± 7.80	COVID-19	Shufeng Jiedu capsules (3.25 g, 3 times/d) + C	Arbidol drugs (abidol tablets) + C	14 days	Y	Lung CT, clinical cure rate, clinical response, and inflammatory biomarkers.
Ge XG et al. 2020 [24]	70/70	T: 40 (25/ 15) C: 30 (16/14) N: 70	T: 40.65 ± 8.25 C: 38.68 ± 6.43	COVID-19	Shufeng Jiedu capsules (3.25 g, 3 times/d) + C	CHM including an herbal drug (Arbidol, antibacterial drugs (moxifloxacin), oseltamivir, and supportive therapy	10 days	Y	Clinical symptoms and virus nucleic acid testing.
Chang ZX et al. 2020 [25]	102/102	T: 81 (56/ 25) C: 21 (10/ 11) N: 102	T: 38.8 ± 12.9 C: 36.8 ± 11.6	COVID-19	Jianhua Qingwen granules (9 g, 3 times/d) + C	CHM including an herbal drugs, antibacterial drugs and supportive therapy	7 days	N	Lung CT, clinical cure rate, merging between mild and critical cases, and clinical symptoms.
Lu RB et al. 2020 [26]	101/101	T: 49 (28/ 21) C: 38 (18/ 20) N: 87	T: 39.1 ± 16.96 C: 43.2 ± 17.01	COVID-19	Jianhua Qingwen granules (9 g, 3 times/d) + C	CHM including an herbal drugs (Lianhuaqingwen), oseltamivir, antibacterial drugs (moxifloxacin), and supportive therapy	10 days	Y	Peripheral blood cell and critical cases, and clinical symptoms
Wang YL et al. 2020 [27]	20/20	T: 20 (9/ 11) C: 20 (8/ 12) N: 40	T: 39.4 ± 15.03 C: 38.45 ± 5.71	COVID-19	CHM + C	CHM including an herbal drugs (Arbidol, antibacterial drugs, and supportive therapy	7 days	N	Lung CT and clinical symptoms
Pan ST et al. 2020 [27]	42/42	T: 21 (14/ 7) C: 21 (8/ 13) N: 42	T: 57.15 ± 14.06 C: 62.45 ± 13.30	COVID-19	Jianhua Qingwen granules (9 g, 3 times/d) + C	CHM recommended in 6thTCOVID-19	14 days	N	Clinical symptoms

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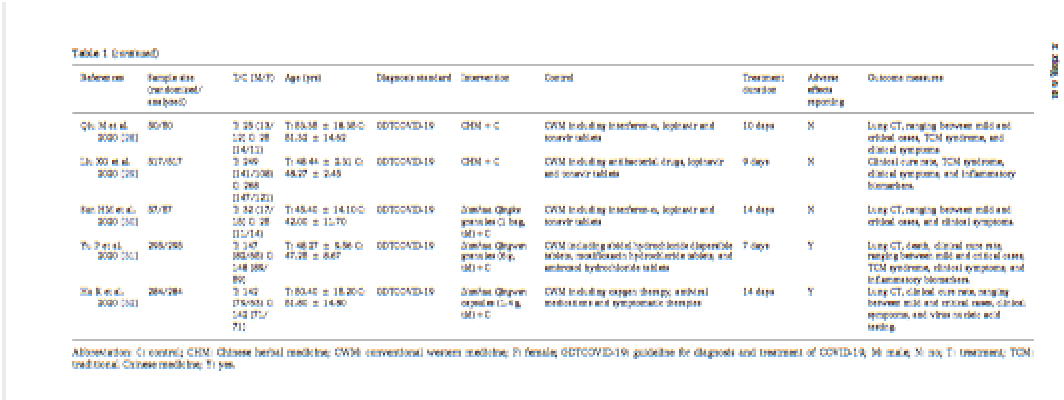

Table 1 (continued)

Reference	Sample size (randomized/analyzed)	T/C (M/F)	Age (yrs)	Diagnosis standard	Intervention	Control	Treatment duration	Adverse effects reporting	Outcome measures
Qiu M et al. 2020 [28]	50/50	T: 25 (13/12) C: 25 (14/11)	T: 53.35 ± 18.35 C: 51.32 ± 14.92	GDTCOVID-19	CHM + C	CWM including interferon-α, lopinavir and ritonavir tablets	10 days	N	Lung CT, ranging between mild and critical cases, TCM syndrome, and clinical symptoms
Liu XQ et al. 2020 [29]	517/517	T: 249 (141/108) C: 268 (147/121)	T: 48.44 ± 2.51 C: 48.27 ± 2.45	GDTCOVID-19	CHM + C	CWM including antibacterial drugs, lopinavir and ritonavir tablets	9 days	N	Clinical cure rate, TCM syndrome, clinical symptoms, and inflammatory biomarkers
Sun HM et al. 2020 [30]	57/57	T: 32 (17/15) C: 25 (11/14)	T: 45.45 ± 14.10 C: 42.00 ± 11.70	GDTCOVID-19	Lianhua Qingke granules (1 bag, tid) + C	CWM including interferon-α, lopinavir and ritonavir tablets	14 days	N	Lung CT, ranging between mild and critical cases, and clinical symptoms
Yu P et al. 2020 [31]	295/295	T: 147 (82/65) C: 148 (89/59)	T: 48.27 ± 9.56 C: 47.25 ± 8.67	GDTCOVID-19	Lianhua Qingwen granules (6 g, tid) + C	CWM including arbidol hydrochloride dispersible tablets, moxifloxacin hydrochloride tablets, and ambroxol hydrochloride tablets	7 days	Y	Lung CT, death, clinical cure rate, ranging between mild and critical cases, TCM syndrome, clinical symptoms, and inflammatory biomarkers
Hu K et al. 2020 [32]	284/284	T: 142 (76/66) C: 142 (71/71)	T: 50.40 ± 15.20 C: 51.80 ± 14.80	GDTCOVID-19	Lianhua Qingwen capsules (1.4 g, tid) + C	CWM including oxygen therapy, antiviral medications and symptomatic therapies	14 days	Y	Lung CT, clinical cure rate, ranging between mild and critical cases, clinical symptoms, and virus nucleic acid testing

Abbreviation: C: control; CHM: Chinese herbal medicine; CWM: conventional western medicine; F: female; GDTCOVID-19: guideline for diagnosis and treatment of COVID-19; M: male; N: no; T: treatment; TCM: traditional Chinese medicine; Y: yes.

3.3. Assessment of methodological quality

As shown inTable 2, the methodological quality of the includedstudies was evaluated based on the criteria in Cochrane handbook.Detailed information on sequence generation of randomization wasreported in 6 trials (6/18, 33.33 %) [16,20,28,30–32]. Specific methodof allocation concealment was not described in this review. One trialreported no application of blinding [30]. One trial only reportedblinding of assessor [32]. Detailed information regarding blinding ofpatient, investigator, and assessor was unclear in the rest 16 trials[15–29,31]. Dropouts were reported in 4 trials (4/18, 22.22 %)[15,16,20,21].3.4. Description of single herb and CHMThirty-one CHM were used in this review, includingMaxing Shigandecoction,Chailing Pingweidecoction,Haoqin Qingdandecoction,HuopuXialingdecoction, ModifiedBuzhong Yiqidecoction, Pneumonia No. 1formula, Powerful Pneumonia No. 1 formula, Pneumonia No. 2 for-mula,Qingfei Touxie Fuzhengrecipe, Damp-toxin obstructing lung for-mula, Toxin blocking lung formula,Qiweidecoction,Toujie Quwengranules,Shufeng Jieducapsules,Lianhua Qingwengranules and cap-sules,Xuanfei Zhisoumixture,Shuanghuanglianoral liquids,Yupingfenggranules,Ganlu Xiaodupills,Huoxiang Zhengqiliquids,Reyanning mix-ture,Jinhua Qinggangranules,Xuebijinginjection,Tanreqinginjection,Shengmaiinjection,Shenfuinjection,Lianhua Qingkegranules, MaxingXuanfei Jiedu decoction, etc. Among them, 13 (13/31, 41.94 %) wereoriginated from classical Chinese herbal formulas, which have beenused for 189–1800 years.The frequency of each Chinese herb in this review was also sum-marized manually. In total, 100 Chinese herbs were included. And thetop 5 ranked Chinese herbs were Liquoric Root (Gancao, RadixGlycyrrhizae) (15/31, 48.39 %), Baical Skullcap Root (Huangqin, RadixScutellariae Baicalensis) (11/31, 35.48 %), Pinellia Rhizome (Banxia,Rhizoma Pinelliae Tematae) (11/31, 35.48 %), Forsythia Fruit(Lianqiao, Fructus Forsythiae Suspensae) (10/31, 32.26 %), and BitterApricot Seed (Kuxingren, Semen Armeniacae Amarum) (10/31, 32.26%).Six dosage formulations of CHM were included, including decoc-tion, granule, capsule, oral liquid, pill, and injection. The most com-monly used dosage formulation was decoction (17/31, 54.84 %), fol-lowed by granule (7/31, 22.58 %), injection (4/31, 12.90 %), oralliquid (2/31, 6.45 %), capsule (2/31, 6.45 %), and pill (1/31, 3.23 %).The decoction of CHM was orally taken 1 dose every day, with about400mL in every dose. The administration of CHM in each trial wasdescribed inTable 1and the compositions were summarized inTable 3.3.5. Efficacy assessment3.5.1. Lung CTThirteen trials assessed the efficacy of CHM on lung CT in this study[15–19,21,22,24,26,28,30–32]. There were 749 patients in CHM groupand 653 in CWM group. A significant improvement in lung CT wasidentified by CHM in this meta-analysis (13 trials, n= 1402;RR= 1.23; 95 % CI: 1.15–1.32;I2=31 %,P< 0.00001;Fig. 2a).3.5.2. DeathThe effect of CHM on death was reported in 4 trials [17,18,21,31].There were 256 patients in CHM group and 207 in CWM group. Meta-analysis showed no significant difference on death between CHM and CWM (4 trials, n= 463; RR =0.34; 95 % CI: 0.05–2.18;I2=0%,P=0.26; Fig. 2b).

Table 2
Methodological quality of included trials according to Cochrane handbook.

References	A	B	C	D	E	F	G	H
Huang H et al 2020 [15]	?	?	?	?	?	+	?	?
DIng XJ et al. 2020 [16]	+	?	?	?	?	+	?	?
Shl J et al. 2020 [17]	?	?	?	?	?	+	?	?
Xla WG et al. 2020 [18]	?	?	?	?	?	+	?	?
Fu XX et al. 2020 [19]	?	?	?	?	?	+	?	?
Duan C et al. 2020 [20]	+	?	?	?	?	+	?	?
Yang MB et al. 2020 [21]	?	?	?	?	?	+	?	?
Xlao Q et al. 2020 [22]	?	?	?	?	?	+	?	?
Qu XK et al. 2020 [23]	?	?	?	?	?	+	?	?
Cheng DZ et al. 2020 [24]	?	?	?	?	?	+	?	?
Lv RB et al. 2020 [25]	?	?	?	?	?	+	?	?
Wang YL et al. 2020 [26]	?	?	?	?	?	+	?	?
Yao KT et al. 2020 [27]	?	?	?	?	?	+	?	?
Qiu M et al. 2020 [28]	+	?	?	?	?	+	?	?
LIu XG et al. 2020 [29]	?	?	?	?	?	+	?	?
Sun HM et al. 2020 [30]	+	?	–	–	–	+	?	?
Yu P et al. 2020 [31]	+	?	?	?	?	+	?	?
Hu K et al. 2020 [32]	+	?	–	–	+	+	?	?

Abbreviation: A: Adequate sequence generation; B: Concealment of allocation; C: Blinding (patient); D: Blinding (investigator); E: Blinding (assessor); F: Incomplete outcome data addressed (ITT analysis); G: Free of selective reporting; H: Other potential threat to validity; +: Low risk; -: High risk;? : Unclear.

3.5.3. Clinical cure rateClinical cure rate was defined as the following 4 discharge criterionin guideline for the diagnosis and treatment of 2019 novel coronavirus(2019-nCoV) infected pneumonia: a) body temperature returned tonormal for more than 3 days, b) respiratory symptoms improved significantly, c) pulmonary imaging showed obvious absorption of in-flammation, and d) two consecutive times of novel coronavirus nucleicacid test negative in respiratory tract (the sampling interval shall be atleast 1 day) [7]. Seven trials evaluated the effects of CHM on clinicalcure rate [18,19,22,24,29,31,32]. There were 760 patients in CHMgroup and 763 in CWM group. CHM exhibited a significant improvement on clinical cure rate (7 trials, n= 1523; RR=1.18; 95 % CI:1.13–1.24;I2=24 %,P< 0.00001;Fig. 2c).3.5.4. Ranging between mild and critical casesTwelve trials evaluated the effects of CHM on ranging between mildand critical cases [15–18,20,21,24,25,28,30–32]. Among them, effectsof CHM on ranging from critical to mild cases were evaluated in 2 trials[16,17]. There were 100 patients in CHM group and 67 in CWM group.Compared with CWM, no significant difference on ranging from criticalto mild cases was identified (RR =1.34; 95 % CI: 0.47–3.80;I2=0%,P= 0.58;Fig. 3a).In addition, effects of CHM on ranging from mild to critical caseswere evaluated in 11 trials [15,17,18,20,21,24,25,28,30–32]. Therewere 703 patients in CHM group and 543 in CWM group. A significantimprovement on ranging from mild to critical cases was observed byCHM (11 trials, n =1246; RR=0.40; 95 % CI: 0.29 to 0.56;I2=0%,P< 0.00001;Fig. 3b).3.5.5. Length of hospital stayTwo trials evaluating length of hospital stay were included forfurther analysis [17,18]. There were 83 patients in CHM group and 36in CWM group. Meta-analysis showed a significant reduction on lengthof hospital stay by CHM (2 trials, n= 119; WMD: -1.99; 95 % CI: -3.28to -0.70;I2=0%,P= 0.002;Fig. 3c).

3.5.6. Clinical symptomsClinical symptoms including fever, dry cough, expectoration, fa-tigue, sore throat, itchy throat, chest tightness, asthma, shortness ofbreath, poor appetite, diarrhea, nausea, vomiting, abdominal disten-tion, and abdominal pain were reported in all the included studies[15–32]. As fever, dry cough, and fatigue were main clinical symptomsof COVID-19, individual symptom score, disappearing time, number ofimproved cases, and total score of clinical symptom were summarized.3.5.7. Total score of clinical symptomsTotal score of clinical symptom was evaluated in 2 studies [15,17].There were 101 patients in CHM group and 32 in CWM group. Meta-analysis revealed a significant improvement on total score of clinicalsymptom (2 trials, n= 133; WMD: -1.84; 95 % CI: -3.10 to -0.58;I2=0%,P= 0.004;Fig. 4a).3.5.8. FeverThe symptom of fever was reported in 15 trials [15–25,27–30,32].Among them, 5 studies reported number of fever reduction cases[15,16,20,27,30], 10 reported fever reduction time[17,18,21,16–25,27,28,32], and 3 reported symptom score of

fever[19,29,31].In the field of number of fever reduction cases, there were 238patients in CHM group and 150 in CWM group. Meta-analysis revealedno significant difference on fever reduction number between CHM andCWM (5 trials, n= 388; RR=1.28; 95 % CI: 0.98–1.67;I2=66 %,P= 0.07;Fig. 4b).In the field of fever reduction time, there were 551 patients in CHMgroup and 466 in CWM group. The aggregated results including 10trials suggested that fever reduction time is significantly improved byCHM (10 trials, n =1017; WMD: -1.36; 95 % CI: -1.80 to -0.93;I2=58%,P< 0.00001;Fig. 4c).In the field of symptom score of fever, there were 433 patients inCHM group and 452 in CWM group. It has been identified that score offever is significantly reduced by CHM (3 trials, n =885; WMD: -0.60;95 % CI: -0.69 to -0.50;I2=61 %,P< 0.00001;Fig. 4d).3.5.9. CoughThe symptom of cough was reported in all the trials, and only 14were enrolled in this review [16,19–25,27–32]. Among them, 6 studiesreported number of cough reduction cases [16,20,24,25,27,30], 4 re-ported symptom score of cough [19,21,29,31], and 6 reported dis-appearing time of cough [22–24,28,30,32].In the field of number of cough reduction cases, there were 240patients in CHM group and 182 in CWM group. Meta-analysis showed asignificant improvement on number of cough reduction cases by CHM(6 trials, n= 422; RR= 1.50; 95 % CI: 1.26–1.78;I2=0%,P<0.00001;Fig. 5a).In the field of symptom score of cough, there were 459 patients inCHM group and 475 in CWM group. The aggregated results suggestedthat cough is significantly improved by CHM (4 trials, n= 934; WMD:-0.78; 95 % CI: -1.32 to -0.24;I2=99 %,P= 0.004;Fig. 5b).In the field of disappearing time of cough, there were 362 patientsin CHM group and 336 in CWM group. No significant difference ondisappearing time of cough between CHM and CWM was identified inthis study (6 trials, n =698; WMD: -1.42; 95 % CI: -2.82 to -0.01;I2=90 %,P= 0.05;Fig. 5c).3.5.10. FatigueThe effect of CHM on fatigue was evaluated in 12 studies[19–25,27,29–32]. Among them, 5 studies reported number of fatigueereduction cases [20,24,25,27,30], 4 reported individual symptom score[19,21,29,31], and 4 reported disappearing time of fatigue [22–24,32].For number of fatigue reduction cases, there were 167 patients inCHM group and 140 in CWM group. A significant improvement onnumber of fatigue reduction cases by CHM was identified in this meta- analysis (5 trials, n= 307; RR =1.73; 95 % CI: 1.39–2.16;I2=0%,P< 0.00001;Fig. 6a).For symptom score of fatigue, there were 459 patients in CHMgroup and 475 in CWM group. Compared to CWM, a significant improvement on symptom score of fatigue was observed by CHM (4 trials,n =934; WMD: -0.70; 95 % CI: -0.98 to -0.42;I2=97 %,P<0.00001;Fig. 6b).For disappearing time of fatigue, there were 301 patients in CHMgroup and 284 in CWM group. Improvement on disappearing time offatigue was also identified in CHM group compared to CWM group (4trials, n= 585; WMD: -1.13; 95 % CI: -2.22 to -0.04;I2=93 %,P=0.04; Fig. 6c).

Table 3
Components of Chinese herbal medicine used in the included studies.

References	Chinese herbal medicine	Components
[illegible]	CHM	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	CHM	[illegible]
[illegible]	CHM	[illegible]

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L CHINA

Table 3 (continued)

References	Chinese herbal medicine	Components
		[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

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Table 3 (continued)

[illegible]	[illegible]	[illegible]
		[illegible]
[illegible]	CHM	[illegible]
		[illegible]
		[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	CHM	[illegible]
[illegible]	CHM	[illegible]
		[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

Abbreviation: CHM: Chinese herbal medicine; ▲: originated from classical Chinese herbal formulas.

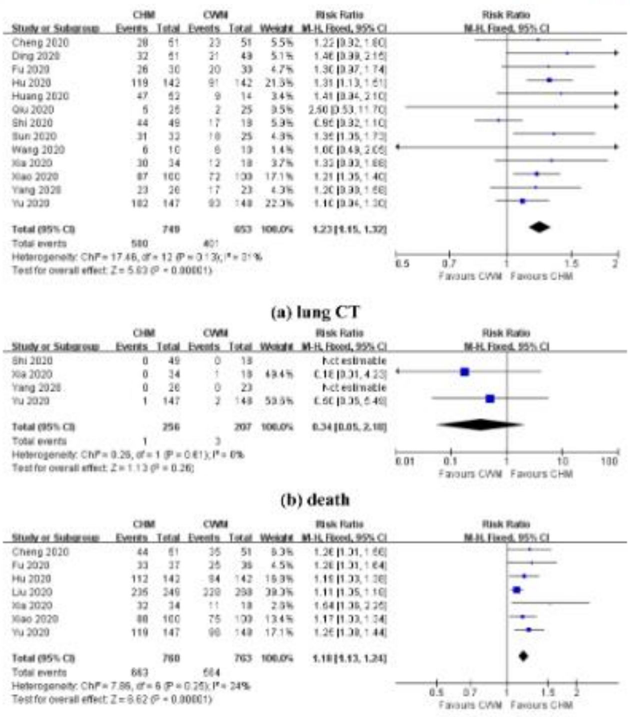


Fig. 2. Forest plot of the effects of CHM for outcomes of (a) lung CT, (b) death, and (c) clinical cure rate.

3.5.11. TCM syndrome

The efficacy of CHM on TCM syndrome was evalated in 5 studies[18,20,28,29,31]. Only 3 trials could be enrolled in this study. Therewere 141 patients in CHM group and 84 in CWM group. Meta-analysisshowed significant improvement by CHM on TCM syndrome (3 trials,n =225; WMD: -3.67; 95 % CI: -6.60 to -0.73;I2=86 %,P= 0.01;Fig. 7a).

3.5.12. Viral nucleic acid testing

The effect of CHM on viral nucleic acid testing was reported in 4trials [15,21,23,32]. There were 260 patients in CHM group and 209 inCWM group. A significant improvement on negative conversion rate ofviral nucleic acid testing was identified by CHM when compared withCWM (4 trials, n= 469; RR= 1.18; 95 % CI: 1.04–1.34;I2=41 %,P= 0.01;Fig. 7b). Additionally, the negative conversion time of viralnucleic acid testing was reported in 1 trial [23], and a shorter time wasidentified in CHM group (9.32 ± 3.03 vs 11.89 ± 3.21).

3.6. Inflammatory biomarkers

4.6.1. WBC

Six trials evaluated the efficacy of CHM on number of WBC[15,18,19,22,29,31]. As enumeration data was used in 1 trial [18], 5trials were included in this study [15,19,22,29,31]. There were 585patients in CHM group and 566 in CWM group. Meta-analysis showedno significant difference between CHM and CWM on the number ofWBC in patients with COVID-19 (5 trials, n =1151; WMD: 0.27; 95 %CI: -0.22 to 0.76;I2=95 %,P= 0.28;Fig. 7c).Fig. 2.Forest plot of the effects of CHM for outcomes of (a) lung CT, (b) death, and (c) clinical cure rate.X. Xiong, et al.Pharmacological Research 160 (2020) 10505610

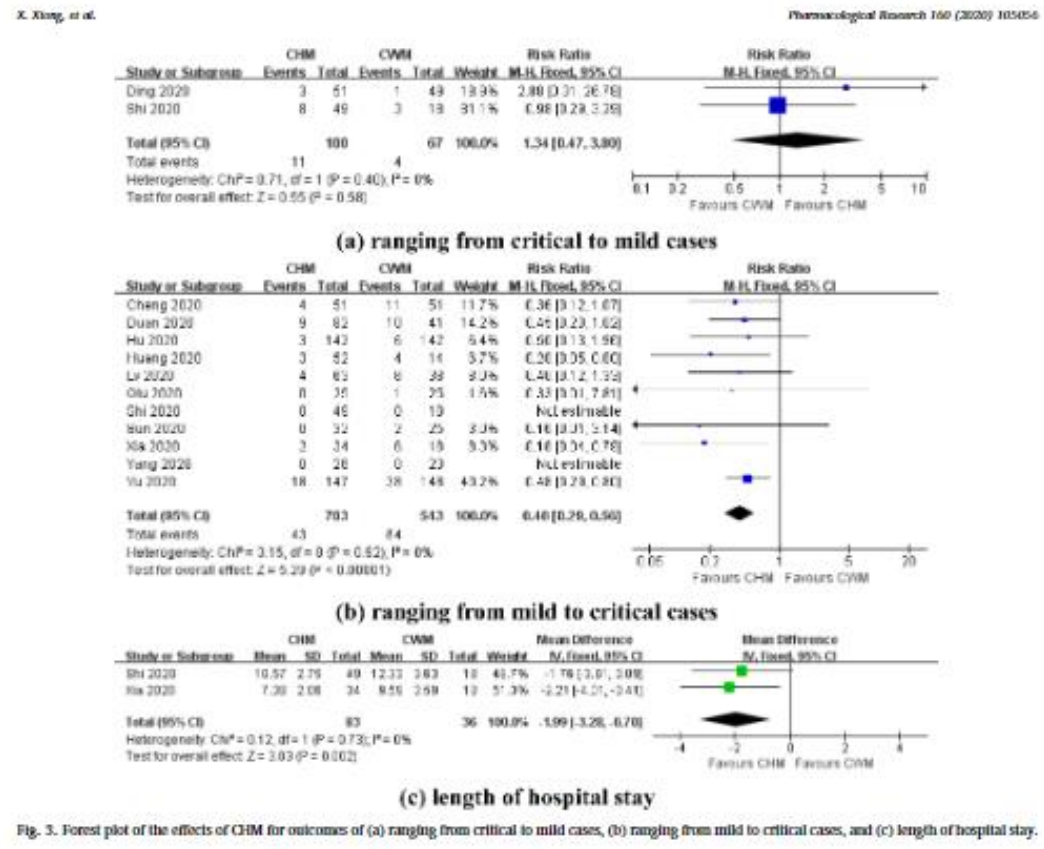


Fig. 3. Forest plot of the effects of CHM for outcomes of (a) ranging from critical to mild cases, (b) ranging from mild to critical cases, and (c) length of hospital stay.

4.6.2. NEU

For the number of NEU, 3 trials [15,18,21] involving 167 patientswere enrolled. As enumeration data and percentage were used in 2trials [15,21], meta-analysis could not be conducted accordingly.Among

them, a significant improvement of NEU was identified in 1 trial[18] (P< 0.05), while negative conclusions were found in the rest 2trials [15,21].

4.6.3. LYM

Effects of CHM on the level of LYM were assessed in 6 trials[15,18,19,21,22,31]. As enumeration data and percentage were re-ported in 2 trials with positive conclusions [19,22], only 4 trials wereincluded in this meta-analysis. Meta-analysis revealed no significantdifference between CHM and CWM on the level of LYM (4 trials,n =483; WMD: 0.24; 95 % CI: -0.04 to 0.51;I2=97 %,P= 0.09;Fig. 7d).

4.6.4. CRP

The levels of CRP at baseline and after intervention were recordedin 7 trials [15,16,18,19,21,29,31]. Although positive conclusion wasidentified in 1 trial [19], it could not be included in the meta-analysisdue to enumeration data. Meta-analysis of the rest 6 trials revealed thatCRP is significantly reduced by CHM (6 trials, n= 1100; WMD: -8.91;95 % CI: -12.56 to -5.27;I2=97 %,P< 0.00001;Fig. 7e).

4.6.5. Adverse effects

In this review, 10 trials reported adverse effects (10/18, 55.56 %)[15,16,18–20,22,23,25,31,32]. Among them, no adverse effect wasidentified in both CHM and CWM groups [18,19,25,31]. Adverse effectsin the rest 6 trials included gastrointestinal reactions (abdominal dis-tention, diarrhea, abdominal pain, nausea, vomiting, belching, acidreflux, poor appetite), headache, dizziness, drowsiness, abnormal liverfunction, renal dysfunction, and drug allergy [15,16,20,22,23,32]. All of the reported adverse effects were released spontaneously in bothCHM and CWM groups. Meta-analysis identified that no significantdifference between CHM and CWM was identified (9 trials, n= 1069;RR= 0.93; 95 % CI: 0.49–1.75;I2=46 %,P= 0.82;Fig. 8).

4.6.6. Publication bias

Publication bias was detected by the funnel plot of lung CT. The asymmetry suggested a mild publication bias in the study (Fig. 9).

4. Discussion

4.1. Summary of evidence

Currently, COVID-19 has become a major public health problem inthe whole world [33,34]. It has been identified that clusters of fatalpneumonia could be caused by 2019 novel coronavirus (2019-nCoV),Fig. 3.Forest plot of the effects of CHM for outcomes of (a) ranging from critical to mild cases, (b) ranging from mild to critical cases, and (c) length of hospital stay.X. Xiong, et al.Pharmacological Research 160 (2020) 10505611

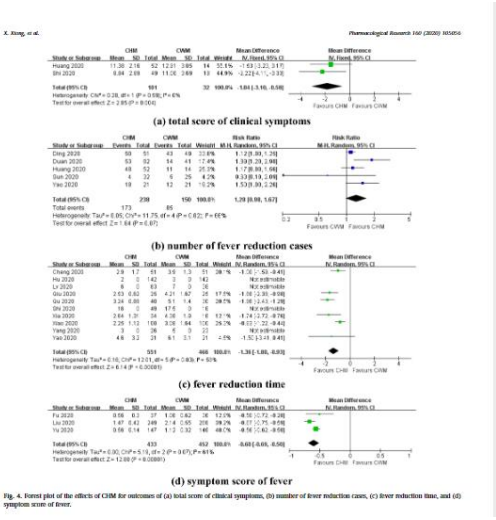


Fig. 4. Forest plot of the effects of CHM for outcomes of (a) total score of clinical symptoms, (b) number of fever reduction cases, (c) fever reduction time, and (d) symptom score of fever.

The clinical manifestation of which is greatly resembling severe acuterespiratory syndrome coronavirus (SARS-CoV) [35]. During the epi-demic, rapid and robust research is important to help guide clinicaltreatment, formulating public health policy, and new drug research anddevelopment. From the beginning of outbreak, CHM has been widelyused in China and evidences of CHM for COVID-19 are emerging gra-dually. To our knowledge, this is the first strictly designed systematicreview and meta-analysis of all the published RCTs to assess the efficacyand safety of CHM for COVID-19 in English.In this study, several research highlights deserved our attention.Firstly, extensive literature searching of relevant clinical trials pub-lished in both Chinese and English databases was performed. In theprevious published systematic reviews, only less than 5 RCTs were in-cluded. However, up to 18 trials were enrolled in our review, theconclusion of which was more persuasive and feasible. Although thenumber of included studies was still small, these data are very valuableand timely in light of no specific drugs and high mortality of COVID-19.Secondly, large number of objective and subjective outcome mea-sures including lung CT, death, clinical cure rate, ranging between mildand critical cases, length of hospital stay, clinical symptoms, TCMsyndrome, viral nucleic acid testing, and inflammatory biomarkerswere utilized to assess the efficacy of CHM comprehensively. This isdifferent from the traditional evaluation of CHM that only focused onsubjective indicators. The whole research findings from 18 trials nvolving 2275 patients showed that lung CT, clinical cure rate, rangingfrom mild to critical cases, length of hospital stay, total score of clinicalsymptoms, fever reduction time, symptom score of fever, number ofcough reduction cases, symptom score of cough, number of fatiguereduction cases, symptom score of fatigue, disappearing time of fatigue,TCM syndrome, viral nucleic acid testing, and inflammatory biomarkers(CRP) were significantly improved by CHM. In my opinion, achieve-ment of clinical efficacy is closely related to traditional medical ex-perience and extensive use of large number of classical Chinese herbalformulas. Although there is no records in TCM, it does not affect theunderstanding of TCM pathogenesis and clinical treatment of COVID-19. As symptoms and signs including tongue coating and pulse are thebasis of diagnosis and treatment in TCM, TCM syndrome and formulaesyndrome rather than disease were focused accordingly [36]. When intreating acute infectious febrile diseases, large quantity of classicalChinese herbal formulas have been formed and accumulated in ancienttimes [37]. It is held that COVID-19 belong to “damp-heat epidemic” or“damp-toxin epidemic” according to TCM theory. The disease can bedivided into 3 stages on the basis of different TCM pathogenesis: illnessin all threeyangchannels in the mild stage, phlegm-heat obstructing thelung in the critical stage, and deficiency of lung and spleenqiin therecovery stage. Therefore, therapeutic principles of dispelling cold,relieving exterior, dissipating phlegm, clearing away heat, invigoratingspleen, and replenishingqiwere widely used. Classical Chinese herbalformulas includingMaxing Shigandecoction,Xiaochaihudecoction, etc.were frequently applied correspondingly. What’s more, all these for-mulas possessed a wide range of pharmacological functions includinganti-inflammatory, antiviral, antipyretic, expectorant, antiasthmatic,antitussive effects [38]. However, more researches on clinical evidenceand molecular mechanism by classical Chinese herbal formulas are alsowarranted.Thirdly, treatment course of CHM was also worthy of attention. Thereported course ranged from 5 to 15 days. Whether CHM can

play a rolein such a short period of time is a widely concerned issue. Patientsdiagnosed as COVID-19 could develop severe pulmonary infection andacute respiratory distress syndrome, which have a high likelihood ofhospitalization in intensive care unit and death. However, TCM oftenheld that “to treat what and when it is not ill”. That is to say, althoughpatient manifested fever in the early stage and mild stage of COVID-19,it belongs to the category of typical exterior syndrome from the per-spective of TCM. If it was intervened in time at the stage of exteriorsyndrome, COVID-19 can be effectively blocked in ranging from mild tocritical cases, thus reducing mortality, length of hospital stay, and feverreduction time.Fourthly, in terms of adverse effects, no severe discomfort and ab-normal liver and kidney function was identified in CHM group. Theresults indicated that CHM maybe relatively safe for COVID-19.However, as adverse effects were not reported in some studies, thesafety of CHM should be observed and reported in more detailed in-formation.

Fig. 5.Forest plot of the effects of CHM for outcomes of (a) number of cough reduction cases, (b) symptom score of cough, and (c) disappearing time of cough.

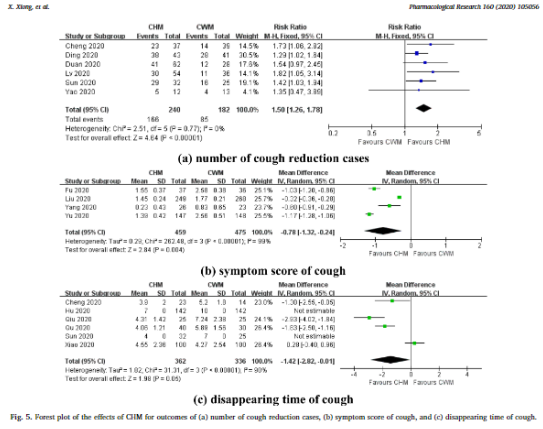


Fig. 5. Forest plot of the effects of CHM for outcomes of (a) number of cough reduction cases, (b) symptom score of cough, and (c) disappearing time of cough.

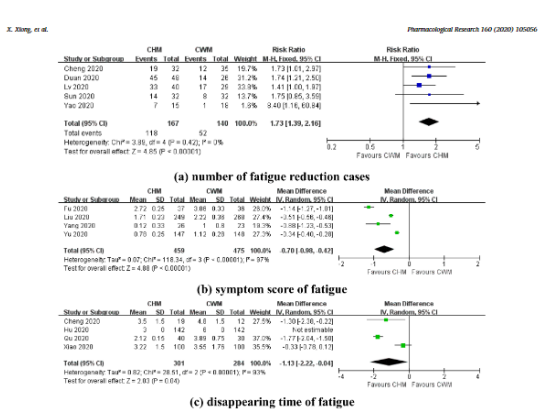


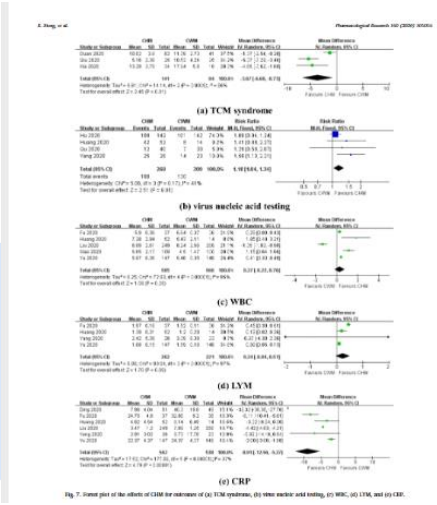
Fig. 6. Forest plot of the effects of CHM for outcomes of (a) number of fatigue reduction cases, (b) symptom score of fatigue, and (c) disappearing time of fatigue.

Fifthly, the most frequently used Chinese herbs were also analysedin this study. In the top 5 herbs, Liquoric Root (Gancao, RadixGlycyrrhizae) is traditionally used as “Shi” to harmonize the propertiesof different drugs. It is also widely used to resolve phlegm and relievecough. Glycyrrhetinic acid (Fig. 10a), the effective component of Li-quoric Root (Gancao, Radix Glycyrrhizae), has a strong antitussive andexpectorant effect with a dose-dependent relationship. Glycyrrhetinicacid and glycyrrhizin also possess obvious antipyretic effect. BaicalSkullcap Root (Huangqin, Radix Scutellariae Baicalensis) can clearaway heat and reduce fire, thus treating febrile diseases, acute upperrespiratory tract infection, and cough yellow phlegm. Baicalin(Fig. 10b) and baicalein (Fig. 10c), the active components of BaicalSkullcap Root (Huangqin, Radix Scutellariae Baicalensis), can inhibitmultiple viruses and bacteria, thus inhibiting inflammation. PinelliaRhizome (Banxia, Rhizoma Pinelliae Tematae) is traditionally used toeliminate phlegm-dampness. In pharmacology, it possessed significantantitussive and expectant effects, with Forsythaside (Fig. 10d) as theactive component. Forsythia Fruit (Lianqiao, Fructus Forsythiae Sus-pensae) could clear away heat and toxic material in TCM theory. It caninhibit a variety of viruses including influenza A virus, human cyto-megalovirus, encephalitis B virus, respiratory syncytial virus, andherpes simplex virus, and inhibit a variety of bacteria including E.coli,staphylococcus aureus, salmonella typhi, escherichia coli, multidrug-resistant acinetobacter baumannii, and staphylococcus epidermidis[39]. Bitter Apricot Seed (Kuxingren, Semen Armeniacae Amarum)could be used to reduce qi, relieve cough and asthma, and relax bowel,thus treating cough, asthma, chest fullness, phlegm, and constipation.The main active ingredient is amygdalin (Fig. 10e), which possesses theeffects of expectorant and antitussive. It is noteworthy that, oxidativestress is a negative effect produced by free radicals in the body, and isconsidered to be an important factor leading to apoptosis, aging anddisease [40–43]. Virus infection is closely related to oxidative injury,and it evokes oxidative stress and intensifies pathological process. Plantpolyphenols include flavonoids, tannins, phenolic acids, etc., whichhave the functions of scavenging free radicals in the body, anti lipidoxidation, delaying the aging of the body, and possess the effects ofbacteriostasis, anti-virus, and anti-tumor [44]. A large number of Chi-nese herb medicine and natural

medicine, including Baical SkullcapRoot (Huangqin, Radix Scutellariae Baicalensis), Forsythia Fruit (Lian-qiao, Fructus Forsythiae Suspensae), Liquoric Root (Gancao, RadixGlycyrrhizae), and so on, possess certain anti-oxidation, anti-bacterialand anti-virus effects, and can be used in treating acute respiratoryinfection. Our study suggested that, CHM can not only improve symp-toms, reduce the number of severe patients, shorten of fever reductiontime and length of hospital stay, and improve pulmonary imaging, butalso possess effects of antiviral and inhibiting inflammatory reaction inthe treatment of COVID-19. That is to say, CHM could be considered totreat patients in both mild and critical stages of the disease, whichembodies the advantages of multi-target and overall regulation byCHM.

4.2. Limitations

Limitations in this review should also be taken into account as below. Firstly, poor methodological design is a very common problemin most of included trials. Significant drawbacks regarding sequencegeneration of randomization, concealment of allocation, reporting onblinding, dropouts, and pre-estimation of sample size should beconsidered in further studies. Secondly, as viral nucleic acid testingturning positive again is very common in the recovery stage of COVID-19, no trial adopted long-term follow-up.



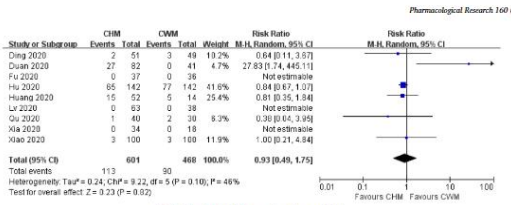


Fig. 8. Forest plot of the reported adverse effects.

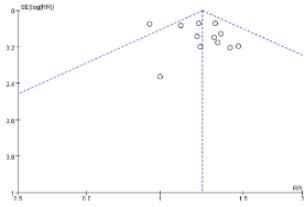


Fig. 9. Funnel plot of outcome of lung CT.

5. Conclusions

In general, this systematic review and meta-analysis suggested thatCHM maybe beneficial for the treatment of COVID-19 in improvingclinical symptoms, imaging, and laboratory indicators, shortening thecourse of disease, and reducing the number of severe cases. However, considering the shortcomings of original trials, further rigorously de-signed trials following CONSORT Statement [45] and CONSORTextension for herbal medicine [46] are warranted to confirm the con-clusions. CHM, especially classical Chinese herbal formulas, could beused as potential candidates for COVID-19 in this battle.

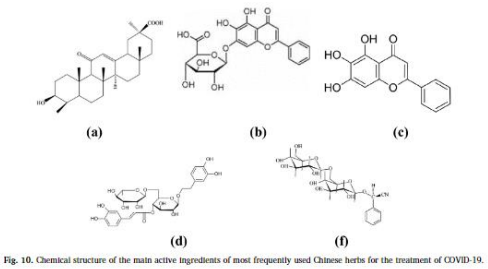


Fig. 10. Chemical structure of the main active ingredients of most frequently used Chinese herbs for the treatment of COVID-19.

Author contributions

X.J.X. designed the paper, extracted data, carried out the statisticalanalysis, produced the tables and figures, and wrote the first edition ofthe paper. P.Q.W. selected the literature. K.L.S. evaluated the metho-dological quality of each trial. Y.W.X. and C.C.W. were consulted andhelped to revise the manuscript. X.J.X. and K.L.S. contributed equally in this paper.

Declaration of Competing Interest

The authors declare no competing financial interests.

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Abstract

A new kind of Pneumonia caused by new corona virus has been widespread in China since winter of 2019. No effective treatment for this disease was verified, so the morbidity and mortality rate were supposed higher than flu. The Traditional Chinese Medicine is widely used in clinical practice in China, but many other countries of the world to deal with diseases that remain clinically challenging.

This new viral pneumonia was named COVID-19, and declared as Public Health Emergency of International Concern by the World Health Organization (WHO) on January 30, 2020. When the COVID-19 broke out, tens of thousands of Chinese people were infected, especially in Wuhan, the capital city of Hubei Province [1], where the infection rate was extremely high. At present, the cumulative number of diagnoses worldwide is 645,957. Chinese government made relevant decisions quickly to control the spread of the epidemic disease. For example, on January 20, 2020, Chinese government classified COVID-19 as a Class B infectious disease and treated it as Class A [2].

Supportive therapies are mainly used in clinical practice, such as oxygen therapy, antiviral therapy and corticosteroid therapy, because there is no specific effective treatment [3]. According to past experiences in the treatment of infectious diseases in China, Traditional Chinese Medicine, including herbal formulas, can be used to prevent and treat such infectious disease [4,5]. Therefore, Traditional Chinese Medicine (TCM) is widely used in the treatment of COVID-19 in China promptly. Here we introduce the herbal formulas that are commonly used in Chinese hospitals.

1. Prevention

According to clinical manifestations of patients who infected with COVID-19, it can be classified as “wet, heat, congestion”, in their lungs. In Traditional Chinese Medicine, we believe that lungs are delicate, so the disease first affects lungs’ function.

“Wet” refers to the factor with sticky and heavy turbidity that can cause a long course of disease and damage the function of the body. “Hot” refers to the factor with hot, dry, and rising turbidity that can cause disease. “Congestion” is a causative factor that can congest blood circulation and cause symptoms such as pain.

Traditional Chinese medicine believes that Qi is the basic substance that constitutes the human body and maintains basic functions. We divide Qi into the healthy Qi and the pathogenic Qi. The healthy Qi refers to substances that maintain the normal operation of our body. The pathogenic Qi refers to substances that can harm the health of our body.

Therefore, the aim of preventive treatment of TCM is to protect lungs. Yupingfeng San, a kind of preventative patent medicine, is chosen because of the nature of the lungs' diseases, listed in Table 1. Yupingfeng San is an ancient herbal medicine in TCM and used to protect lung Qi and avoid pathogenic Qi. In this medicine, there are three herbs: Astragalus, Fangfeng and Atractylodes. Astragalus can improve lung Qi and can reduce phlegm. Fangfeng can relieve the pathogenic Qi, remove dampness and relieve pain. Atractylodes enhances the spleen Qi, which can affect our digestion and absorption. Previous studies has shown that Yupingfeng San could regulate the body's immune function.

Table 1. Herb prescriptions of Yupingfeng San.

Yupingfeng San	Astragalus 20g	Fangfeng 15g	Atractylodes 15g

These medicinal herbs should be mixed and boiled together with 1000ml pure water for about 15 minutes after boiling to get about 600ml tincture. Each tincture may be subdivided into three doses and taken orally 200ml once, three times a day.

2. Treatment of mild infection

Patients with mild infection often has fever, sweat, headache, thirst, cough, sore throat, red tongue tip, thin white or pale yellow coating and floating pulse. We base on above symptoms to diagnose if a patient is a mild one. According to theories of Traditional Chinese Medical, pathogenic Qi hurts lung Qi. If the lung Qi does not work properly, it will cause lung heat and dampness and some typical symptoms, including fever, cough, sore throat and fatigue. So we used the method of “clearing lung heat and dampness” to treat it. Two types of prescriptions, called Sangju yin and Yinqiao san, are commonly used in clinical treatment, and are shown in Table 2. The main function of these two prescriptions is to clear lung heat, expel phlegm, relieve cough, regulate the patient's lungs and restore normal lung function. Clinically, we chose Yinqiao san for patients who had high fever and Sangju yin for patients who had severe cough.

Table 2. Two types of prescriptions for mild patients.

Sangju yin	Mulberry leaf 15g	Chrysanthemum 10g	Forsythia 10g	Almond 9g
	Mint 6g	Chinese bellflower 6g	Reed root 15g	Licorice 3g
Yinqiao san	Forsythia 15g	Chinese bellflower 6g	Honeysuckle 15g	Mint 6g
	Bamboo leaves 6g	Licorice 3g	Nepeta 6g	Light tempeh 5g
	Burdock 6g			

These medicinal herbs should be mixed and boiled together with 1000ml pure water for about 15 minutes after boiling to get about 600ml tincture. Each tincture may be subdivided into three doses and taken 200ml orally once, three times a day.

From some studies, we found that Yinqiao san may have antibacterial and antiviral functions, and can be used to enhance the immune function of upper respiratory tract [6]. These two prescriptions can be used to treat patients with mild infection of COVID-19.

3. Treatment of severe infection

In the early course of treatment for COVID-19, if the infection cannot be controlled, worse respiratory failure, multiple organ failure, and death will occur, then serious infection should be considered. These infectious patients have following main manifestations: high fever, cough, phlegm, difficult breathing, sweating, chest tightness, fatigue, nausea, bloating, red or dark red tongue, yellow coating and slippery or weak pulse. We base on the above symptoms to diagnose if patients are severe ones.

According to TCM, if early treatments are not effective, patients are improperly treated, or the pathogenic Qi is too strong, the healthy Qi (especially the lung Qi) will be damaged severely and lungs will not work. Therefore, more and more sputum will be produced and patients cannot breathe anymore. Maxingshigan tang(decoction) and Baihegujin tang are shown in Table 3 and could be used to benefit the healthy Qi, expel the pathogenic Qi and help the lung to expel sputum and get air.

Table 3. Two types of herb prescriptions for severe patients.

Maxinshigan Tang	Ephedra 15g	Almond 10g	Plaster 20g	Licorice 9g
Baihegujin Tang	Shudihuang 15g	Dihuang 15g	Angelica 15g	White peony 6g

Maxinshigan Tang	Ephedra 15g	Almond 10g	Plaster 20g	Licorice 9g
	Xuanshen 10g	Chinese bellflower 6g	Ophiopogon 6g	Lily 6g
	Beimu 6g	Licorice 3g		

These medicinal herbs should be boiled together with 1000ml pure water for about 30 minutes after boiling to get about 600ml tincture. Each tincture may be subdivided into three doses and taken orally 200ml once, three times a day.

Maxinshigan tang is mainly used to clear lung fever and reduce phlegm. Baihegujin tang can invigorate the lung Qi. We should mix the two prescriptions together when severe infection happens to benefit the healthy Qi and expel the pathogenic Qi.

4. Discussion

In 2003, a lot of descriptions of Traditional Chinese Medicine were used to prevent and treat SARS([7,8]). In 2009, National Administration of Traditional Chinese Medicine of China issued a Traditional Chinese Medicine program for treatment of H1N1 infection [9]. Based on these experiences, we believe that Traditional Chinese Medicine is effective for COVID-19, so we use it during the outbreak of COVID-19, and it works very well clinically.

There is no effective treatment for COVID-19 until now. However, during the treatment of Covid-19 in China, we found that the intervention of traditional Chinese medicine can reduce the severe symptoms of patients. The empirical therapy of Traditional Chinese Medicine is been widely used in Chinese hospitals now, and this therapy might be useful for people all around the world.

Appendix A. Supplementary data

The following is the Supplementary data to this article:Download : Download XML file (266B)

Multimedia component 1.

Research data for this article

Data not available / No data was used for the research described in the article

About research data

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Abstract

Coronavirus disease 2019(COVID-19) has attracted great attentions from the whole world. Traditional Chinese medicine(TCM) has been widely used and shown satisfying efficacies in treating all stages of COVID-19. In this study, the molecular interaction networks of different stages of COVID-19(the early, severe, critical and recovery stage) were constructed using the links among symptoms-related genes collected from TCMIP V2.0(http://www.tcmip.cn/), an integrated pharmacology network-computing platform for TCM. Following the network topological feature calculation and functional enrichment analysis, we found that the molecular targets and pathways related with the "immune-inflammation system" were involved throughout all the stages of COVID-19. The severe stage and the critical period of COVID-19 were occupied by a large proportion of inflammatory factors and pathways, suggesting that there might be a cytokine storm in these periods, along with respiratory disorders, cardiopulmonary dysfunction, nervous system disorders, etc. Accordingly, the therapeutic targets and pathways hit by the recommended prescriptions against COVID-19 were also aimed to regulate the balance of immune-inflammation system, nutrient absorption and metabolism, abnormal energy metabolism, the cardio-pulmonary function, nerve system function, etc., which may be related to the therapeutic effects of these prescriptions in terms of several clinical symptoms, such as expiratory dyspnea, chest tightness and shortness of breath, abdominal distension and constipation, sweating and limb cold, dizziness, and irritability, etc. The above findings reflect the integrative actions of TCM characterizing by multiple-components, multiple-targets, multiple-pathways, and multiple-effects. This study systematically constructed the molecular networks of different TCM syndromes during the development and progression of COVID-19 and uncovered the biological basis for symptomatic treatment of TCM. Furthermore, our data revealed the pharmacological mechanisms and the scientific connotation of recommended prescriptions, which may provide supports for the prevention and treatment of COVID-19 using TCM.

Keywords: TCMIP V2.0; coronavirus disease 2019; network pharmacology; recommended prescription; traditional Chinese medicine syndromes.

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Abstract

Currently, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, formerly known as 2019-nCoV, the causative pathogen of Coronavirus Disease 2019 (COVID-19)) has rapidly spread across China and around the world, causing an outbreak of acute infectious pneumonia. No specific anti-virus drugs or vaccines are available for the treatment of this sudden and lethal disease. The supportive care and non-specific treatment to ameliorate the symptoms of the patient are the only options currently. At the top of these conventional therapies, greater than 85% of SARS-CoV-2 infected patients in China are receiving Traditional Chinese Medicine (TCM) treatment. In this article, relevant published literatures are thoroughly reviewed and current applications of TCM in the treatment of COVID-19 patients are analyzed. Due to the homology in epidemiology, genomics, and pathogenesis of the SARS-CoV-2 and SARS-CoV, and the widely use of TCM in the treatment of SARS-CoV, the clinical evidence showing the beneficial effect of TCM in the treatment of patients with SARS coronaviral infections are discussed. Current experiment studies that provide an insight into the mechanism underlying the therapeutic effect of TCM, and those studies identified novel naturally occurring compounds with anti-coronaviral activity are also introduced.

Key words: SARS-CoV-2, Traditional Chinese Medicine (TCM), coronavirus pneumonia

Introduction

In December 2019, there was an outbreak of unexplainable pneumonia in Wuhan city, Hubei province, China [1]. By Jan 7, 2020, it was confirmed that a new type of coronavirus named SARS-CoV-2 (formerly named as 2019-nCoV) had emerged [2]. The World Health Organization (WHO) named the Wuhan pneumonia as Coronavirus Disease-2019 (COVID-19) on Feb 11, 2020 [3]. The COVID-19 patients showed typical respiratory symptom (such as cough, fever, and lung damage) and some other symptoms such as fatigue, myalgia, and diarrhea [4, 5]. As of February 17, 2020, a total of 73,332 cases of the SARS-CoV-2 infected pneumonia has been reported in China and 25 other countries, of which 72,528 cases was found in China [6]. Due to the rapid spread of SARS-CoV-2 through human-to-human transmission, the cases currently continue to rise.

SARS-CoV-2 extracted from patients with pneumonia in Wuhan is an enveloped single stranded RNA-type beta-coronavirus [7]. The genome sequences of SARS-CoV-2 shared 79.5% sequence identity to severe acute respiratory syndrome-related coronaviruses (SARS-CoV) [8, 9]. In addition, the spike (S) protein of SARS-CoV-2 and SARS-CoV enters human alveolar epitelial cells through binding angiotensin-converting enzyme 2 (ACE2) receptor [8]. COVID-19 can be diagnosed by either chest CTradiography or a laboratory testing. Unfortunately, specific antiviral drugs or vaccines currently have not been available for the treatment [10, 11]. According to coronaviral will be summarized and analyzed, including the laboratory studies that provide an insight into molecular basis of therapeutic benefits.

Conventional treatment of SARS-CoV-2: is there a room for Chinese medicine? Due to the absence of a specific antiviraltherapeutics and vaccine, main treatment strategy for COVID-19 is supportive care, which is supplemented by the combination of broad-spectrum antibiotics, antivirals, corticosteroids and convalescent plasma [16] (Table 1). HIV protease inhibitors ritonavir and lopinavir have been used, typically in combination with appropriate antibiotics or with IFNα-2b, in the treatment of SARS-CoV-2 infected patients [7, 17]. Nucleoside analogs such as ribavirin [12] may be potentially beneficial for the treatment of COVID-19, since ribavirin was approved for treating respiratory syncytial virus (RSV) infection [18] and used extensively during

the SARS and MERS outbreak [10]. However, ribavirin had severe side effects such as anemia [18] and whether it had sufficient antiviral activity against SARS-CoV-2 is unclear. Nucleoside analogs favipiravir (T-705) can effectively inhibit the activity of RNA polymerase of RNA viruses such as influenza [19]. A recent in vitro study found that it had the anti-SARS-CoV-2 activity [20], but the in vivo effect remains elusive. Remdesivir may be the most promising antiviral drug for treating COVID-19. It has in vitro and in vivo antiviral activity against a wide array of RNA viruses including SARS and MERS [21], and could decrease viral loads and pathology of lungs in animal models [22]. A study showed remdesivir markedly inhibited the infection of SARS-CoV-2 in Vero E6 cells [20], and most symptoms of the first US patient infected with SARS-CoV-2 had resolved swiftly after intravenous administration with remdesivir [23]. Currently, it is under clinical trial to evaluate the safety and efficacy of intravenous remdesivir for patients with SARS-CoV-2 infection [24]. Oral oseltamivir has been used for the treatment of the cases with SARS-CoV-2 [7], while its efficacy currently remains uncertain.

Host-targeted small molecules approved for other human diseases may modulate the virus-host interactions of SARS-CoV-2. Chloroquine, a potential broad-spectrum antiviral drug [25, 26], was shown by a recent study had anti-SARS-CoV-2 activity [20]. Its clinical efficacy is under study in an open-label trial (ChiCTR2000029609) [12]. IFNα (5 million U) atomization inhalation was recommended as antiviral therapy to treat SARS-CoV-2 [16]. A trial testing IFNα-2b combination of the approved anti-HCV inhibitors has been initiated [17], however, whether it could act synergistically against SARS-CoV-2 is unclear. Corticosteroids were frequently used to suppress the elevated cytokine levels in patients with SARS-CoV [27, 28] and MERS-CoV [29, 30]. However, there are no evidence showing that the mortality of SARS and MERS patients was reduced by the treatment with corticosteroids, while the clearance of viral was delayed by such treatment [31-33]. Consequently, corticosteroids are not suggested to systemically use in SARS-CoV-2 infected patients [34, 35].

Previously, it was shown that, either in severe influenza or SARS-CoV infection, convalescent plasma treatment could significantly decrease viral load and reduce the mortality [31, 36]. Convalescent plasma has been used for severe SARS-CoV-2 infection in China [22], although promising, the efficacy and safety need to becarefully further evaluated. Consistent with previous analysis, WHO alsoconcluded "to date, there is no specific medicine recommended to prevent or treat SARS-CoV-2" [37]. TCM has been used in control of infectious diseases for thousands of years. There is a clear room for the intervention of TCM as a complementary therapy for COVID-19 patients. It is reported that the patientswith SARS-CoV infection have benefited from TCM treatment [38], including amelioration of side effect of conventionaltherapeutics [39, 40]. Based on these factors, there is a general expectation that TCM would be a valuable weapon in the armory against SARS-CoV-2.

Traditional Chinese Medicine in the treatment of patients infected with SARS-CoV: clinical evidence

Application of TCM in the treatment of SARS-CoV-2 is largely inspired by the treatment of SARS caused by outbreak of SARS coronavirus (SARS-CoV) in the late of 2002 in the Guangdong Province of China which spread rapidly during the 2003, with the cumulative number worldwide of over 8,000 [41-43]. Ranging from case reports, case series, controlled observational studies and randomized clinical trials, clinical studies aiming to examine the effect of TCM on SARS have been carried out and reported. There are quite compelling evidences support the notion that TCM has beneficial effect in the treatment or prevention of SARS. For example, the rate of fatality in Hong Kong and Singapore was approximately 18%, while the rate for Beijing was initially more than 52% until the 5th of May and decreased gradually to 4%-1% after the 20th of May in 2003. The dramatic reduced fatality from late May in Beijing was believed to be associated with the use of TCM as a supplement to the conventional therapy [44]. Lau and colleagues reported that, during SARS outbreak, 1063 volunteers including 926 hospital workers and 37 laboratory technicians working in high-risk virus laboratories used a TCM herbal extract, namely Sang Ju Yin plus Yu Ping Feng San. Compared with the 0.4% of infection in the control group, none of TCM users infected. Furthermore, there was some evidence that Sang Ju Yin plus Yu Ping Feng San could modulate T cells in a manner to enhance host defense capacity [45, 46]. In a controlled clinical study, the supplementary treatment with TCM resulted in marked improvement of symptoms and

shortened the disease course [47]. The clinical beneficial effect of TCM appears to be supported by laboratory studies. For example, a high-profile research published in the Lancet reported that glycyrrhizin, a major active constituent liquorice root which is the most frequently used Chinese herb, potently inhibited the replication of clinical isolates of SARS virus [48]. Another independent study confirmed the antivirus activity of glycyrrhizin by plaque reduction assays and this study found that another Chinese herbal compound baicalin also had the anti-SARS activity [49]. Furthermore, Wang et al. found MOL376, a compound derived from TCM, may become a lead compound for SARS therapy by inhibition of cathepsin L, a target for the treatment of SARS [50]. There is a myriad of literature on TCM treatments for SARS published after the SARS epidemicin China. A critical analysis of thesepublications would be useful to confirm the beneficial effect of TCM. Liu et al. systematically reviewed eight randomized controlled trials, and concluded that, by combination with conventional medicine, TCM showed the beneficial effects such as decrease of mortality and relief of symptom, as well as control of fungal infections in patients with SARS. However, the evidence is not sufficient enough due to the poor quality of methodology used in the trials [13]. Leung analyzed 90 peer-reviewed papers with reasonable quality from 130 publications and concluded that TCM used together with conventional treatment had some positive effects, including better control of fever, quickerclearance of chest infection and other symptoms. However, such beneficial effect of TCM is not conclusive and more high-quality clinical studies are required [15].

In another thorough literatura analysis, Liu and colleagues concluded that there was no benefit of adjuvant treatment with TCM in terms of mortality [39]. Due to the lack of high quality TCM trials and biases that influenced the validity of results, Wu and colleagues suggested to re-run clinical trials of TCM for the treatment of acute respiratory tract infections (ARTIs) [51].

Identification of anti-novel coronaviral compound from Traditional Chinese Medicine Natural products used in TCM remains to be a wealthy source for the identification of noveltherapeuticagents for the treatment of human diseases [52]. In the past decade, scientists have made a considerable effort to identify multiple component herbal formulae in TCM with anti-SARS-CoV activity (Table 2). Further identification of chemical entities contained in TCM herbs responsible for the anti-SARS- CoV effect was also pursued (Table 3). Due to the homology of SARS-CoV and SARS-CoV-2, these previous studies may shed light on the naturally occurring compounds with the capacity to inhibit SARS-CoV-2. 3- chymotrypsin-like protease (3CLpro) is vital for replication of virus, and thusrepresents apromising drug target for the development of therapeutics agents for SARS-CoV as well as other human coronaviruses including SARS-CoV-2. It was reported that following TCM herbal extracts had the capacity to inhibit the enzymatic activity of SARS 3CLpro: Chinese Rhubarb extracts (IC50: 13.76 ± 0.03 μg/mL) [53], water extract of Houttuynia cordata [54, 55], flavonoid extracted from litchi seeds [56] and beta-sitosterol (IC50: 1210μM) extracted from the root extractof Isatis indigotica [57]. Further, following herb-derived naturally occurring compounds including sinigrin (IC50: 217μM), indigo (IC50: 752μM), aloe-emodin (IC50: 366 μM), hesperetin (IC50:8.3 μM)[57], quercetin (IC50:73μM), epigallocatechin gallate (IC50: 73μM), gallocatechin gallate (IC50: 47 μM) [58], herbacetin, rhoifolin and pectolinarin [59] were able to inhibit the SARS 3CLpro activity. Moreover, the flavonoids namely herbacetin, isobavaschalcone, quercetin 3-β-D-glucoside, and helichrysetin had the potential to block the enzymatic activity of MERS-CoV 3CL protease [60]

Table 2. TCM herb formulae used for the Treatment of SARS-CoV infection

TCM Formula	Composition	Therapeutics effect	Reference
Yin Qiao San	*Fructus Forsythiae, Flos Lonicerae, Radix Platycodonis, Herba Menthae, Herba Lophatheri, Radix Glycyrrhizae, Herba Schizonepetae*, Fermented soybean, *Fructus arctii*, and *Rhizoma Phragmitis*	"Disperses wind-heat, clears heat, and relieves toxicity", according to TCM theory Treatment of upper respiratory tract infection. Improvement of the function of upper respiratory mucosal immune system	[111, 112]
Yu Ping Feng San	*Astragali radix, Astragalus membranaceus, Atractylodis macrocephala*, and *Saposhnikoviae Radix*	"Tonifying qi" to protect from external pathogens", according to TCM theory Reportedly have antiviral, anti-inflammatory and immunoregulatory effects	[113-115]
Sang Ju Yin and *Yu Ping Feng San*	*Sang Ju Yin* [made with *chrysanthemum*, mulberry leaf, and 6 other herbs] and *Yu Ping Feng San*	Reportedly have anti-viral and immunoregulatory effects	[46]
Lian Hua Qing Wen Capsule	*Forsythia suspensa, Ephedra sinica, Lonicera japonica, Isatis indigotica, Mentha haplocalyx, Dryopteris crassirhizoma, Rhodiola rosea, Gypsum Fibrosum, Pogostemon cablin, Rheum palmatum , Houttuynia cordata, Glycyrrhizae, uralensis*, and *Armeniaca sibirica*	"Clear heat and detoxify, remove lung hotness", according to TCM theory Reportedly have antiviral, anti-inflammatory and immunoregulatory effects.	[82, 83]
Shuang Huang Lian	*Lonicera japonica*, Scutellaria baicalensis, and *Forsythia suspensa*	"Clear heat and detoxify, remove lung hotness", according to TCM theory Reportedly has anti-SARS-CoV-2 activity Reportedly has immunosuppressive effects	[78, 80, 116]
Ma Xin Gan Shi Tang	*Ephedrae herba, Armeniacae semenamarum*), *Glycyrrhizae radix et rhizome, Gypsum fibrosum*, and *De Yuan Yin* (*Arecae semen, Magnoliae officinalis cortex, Tsuuka fructus,Anemarrhenae rhizoma, Dioscoreae rhizoma, Scutellariae radix*, and *Glycyrrhizae radix et rhizoma*)	"Facilitate the flow of the lung "qi" and clear away heat", according to TCM theory Reportedly have anti-SARS-CoV activity	[117, 118]

Table 3. TCM herbal extracts or TCM-derived Compounds with anti-HCoV Activity

TCM Compound (s)	Mode of action	Reference
Plant-derived phenolic compounds and Root extract of *Isatis indigotica*	Inhibit the cleavage activity of SARS-3CLpro enzyme	[57]
Water extract of *Houttuynia cordata*	Inhibit the viral SARS-3CLpro activity Block viral RNA-dependent RNA polymerase activity (RdRp) Immunomodulation	[54, 55]
Scutellarein and myricetin	Inhibit nsP13 by affecting the ATPase activity	[61]
Glycyrrhizin from *Glycyrrhizae radix*	Inhibit viral adsorption and penetration	[48, 75]
Herbacetin, quercetin, isobavachalcone, 3-β-D-glucoside and baliicbysastin	Inhibit cleavage activity of MERS-3CLpro enzyme	[60]
Tetrandrine, fangchinoline, and cepharanthine	Inhibit the expression of HCoV- OC43 spike and nucleocapsid protein. Immunomodulation	[106, 119]
Chinese Rhubarb extracts	Inhibit SARS-3CLpro activity	[53]
Flavonoids (For example: extracted from litchi seeds, herbacetin, rhoifolin, pectolinarin, quercetin, epigallocatechin gallate, and gallocatechin gallate)	Inhibit SARS-3CLpro activity	[56, 58, 59]
Quercetin and TSL-1 from *Toona sinensis* Roem	Inhibit the cellular entry of SARS-CoV	[76]
Emodin derived from genus Rheum and *Polygonum*	Inhibit interaction of SARS-CoV Spike protein and ACE2 Inhibit the 3a ion channel of coronavirus SARS-CoV and HCoV-OC43	[67, 72]
Kaempferol derivatives	Inhibit 3a ion channel of coronavirus	[73]
Baicalin from *Scutellaria baicalensis*	Inhibit Angiotensin-converting enzyme (ACE)	[44, 68]
Saikosaponins	Prevent the early stage of HCoV-229Ev infection, including viral attachment and penetration	[74]
Tetra-O-galloyl-β-D-glucose and luteolin, from *Galla chinensis* and *Veronicalina riifolia* respectively	Avidly binds with surface spike protein of SARS-CoV	[71]

Du Bu Fei Tang (IC50:471.3 μg/mL), Sinomenium acutum (IC50:198.6 μg/mL), Coriolus versicolor (IC50:108.4 μg/mL) and Ganoderma lucidum (IC50:41.9 μg/mL) inhibited SARS-CoV RdRp in a dose- dependent manner [54]. Wu et al. performed large- scale screening of existing drugs, natural products, and synthetic compounds (>10000 compounds) to identify effective anti-SARS-CoV agents through a cell-based assay with SARS virus and Vero E6 cells [62]. They found that ginsenoside-Rb1 isolated from Panax ginseng, aescin isolated from the horse chestnut tree, reserpine contained in the genus Rauwolfia and extracts of eucalyptus and Lonicera japonica inhibited SARS-CoV replication at non-toxic concentrations [62].

Same as SARS-CoV and HCoV-NL63, SARS-CoV-2 uses host receptor ACE2 for the cellular entrance [63-66]. Therefore, TCM with the capacity to target ACE2 holds the promise to prevent theinfection of SARS-CoV-2. Emodin from genus Rheum and Polygonum [67], baicalin from in Scutellariabaicalensis [44,68], nicotianamine from foodstuff (especially "soybean ACE2 inhibitor (ACE2iSB)") [69], scutellarin [70], tetra-O-galloyl-β-D-glucose (TGG) from Galla chinensis and luteolin from Veronicalina riifolia [71] markedly inhibited the interaction of SARS-CoV S-protein

and ACE2. However, the anti-SARS-CoV activity of these compounds remain to be evaluated. In addition, inhibition of the 3a ion channel by emodin [72] or kaempferol derivatives- juglanin [73] could potentially prevent the viral release from the infected cells. Saikosaponins [74], glycyrrhizin [48, 75], quercetin and TSL-1 extracted from Toona sinensis Roem [76] purportedly had potent anti-SARS-CoV effects by inhibition of viral cellular entry, adsorption, and penetration. Overwhelming inflammatory responses are attributable to the deaths of patients with infection of SARS-CoV, or MERS-CoV, or COVID-19. Thus, anti-inflammatory agents presumably could reduce the severity and mortality rate [77]. Shuang Huang Lian, a TCM herbal product prepared from Lonicerae japonicae Flos, Scutellariae radix and Fructus Forsythiae, purportedly had the activity to inhibit SARS-CoV-2 [78]. Interestingly, We have shown that this herbal preparation potently inhibited staphylococcal toxic shock syndrome toxin 1 (TSST-1)-induced production of cytokines (IL-1β, IL-6, TNF-α, IFN-γ) andchemokines (MIP-1α, MIP-1β and MCP-1) by peripheral blood mononuclear cell (PBMC) [79]. In line with our results, this herbal product was shown to markedly reduced the transcriptional and translational levels of inflammatory cytokines TNF-α, IL-1β, and IL-6 in lipopolysaccharide-stimulated murine alveolar macrophages [80]. Indirubin is an active ingredient of a TCM preparation Dang Gui Long Hui Pill, had strong antiviral and immunomodulatory effects, as shown by a study based on the observation of influenza H5N1 virus-infected human macrophages and type-I alveolar epithelial cells [81]. Lian Hua Qing Wen Capsule was reported to have in vitro activity in inhibition of propagation of various influenza viruses. This TCM herbal product not only blocked the early stages of influenza virus infection but also inhibited virus-induced gene expression of IL-6, IL-8, TNF-a, IP-10, and MCP-1 [82]. Additionally, a study by Dong et al. reported that the levels of IL-8, TNF-α, IL-17, and IL-23 in the sputum and of IL-8 and IL-17 in the blood were markedly decreased after Lian Hua Qing Wen Capsule treatment in patients with acute exacerbation of chronic obstructive pulmonary disease [83]. A self-control study by Poon et al. showed that the administration of the TCM herbal formulas (Sang Ju Yin and

Yu Ping Feng San) may have beneficial immunomodulatory effects for the prevention of viral infections including SARS-CoV [46].

Moreover, a number of anti-coronaviral agents have been identified from TCM herbs, although the mechanisms of action have not yet been elucidated. For example, extracts from Lycoris radiata, Artemisia annua, Pyrrosia lingua, and Lindera aggregate possessed the anti–SARS-CoV activity [84], 3β-Friedelanol isolated from Euphorbia neriifolia [85], Blancoxanthone isolated from the roots of Calophyllum blancoi [86] exhibited anti-HCoV-229E activity.

Traditional Chinese Medicine used in the treatment of SARS-CoV-2-infected patients: the current situations TCM is highly valued by the government of China in their campaign to contain and eradiate SARS-CoV-2. For example, Health Commission in 26 provinces have officially declared that TCM should be used in combination with conventional medicine in the treatment of COVID-19 patients. On 17, February, National Health Commission (NHC) of the People's Republic of China reported that 60,107 confirmed COVID-19 patients (85.20% of total confirmed cases) had been treated with TCM [87]. As for March 1, 2020, a total of 303 ongoing clinical trials aiming to evaluate the efficacy and safety of treatments for CoV-19 patients have been launched in China. Among them, 50 trials (16.5%) are about the use of TCM, including 14 cases (4.6%) to examine the effect of combined treatment with TCM and Western medicine. In 22 TCM trials (7.3%), the effect of self-made herbal preparations such as Xin Guan-1 Formula, Xin Guan-2 Formula and Qing Yi-4 are examined. In another 14 TCM trials (4.6%), commercially available TCMproducts such as Tan Re Qing Injection and Lian Hua Qing Wen Capsule are studied (Table 4). To date, NHC has published 6 editions Guidelines of Diagnosis and Treatment for COVID-19 [88]. Since the fourth versions, different herbal medicinesused in TCM system has been recommended for the treatment of COVID-19, based on the stage of disease and symptom differentiation [89]. According to the latest edition of Guideline [88], following multiple componentChinese herbal products are recommended for the patients in the medical observationperiod, presumably as apreventive measure: Huo Xiang Zheng Qi Shui, Lian Hua Qing Wen Capsule, Shu Feng Jie Du Capsule and Jin Hua Qing Gan Granule. In the clinical treatment period, Qing Fei Pai Du Tang, Xi Yan Ping Injection, Xue Bi Jing injection, Re Du Ning Injection, Tan Re Qing Injection Xing Nao.

Jing Injection and some other Chinese medicineformulae should beselected [90]. Inaddition, for the patients in critical condition, Shen Fu Injection, Sheng Mai Injection, Shen Mai Injection, Su He Xiang Pill and An Gong Niu Huang Pill should be administered (Table 5). Through analysis of the frequency of TCM used in 23 provinces, Luo, et al. [37] concluded that Astragalus membranaceus, Glycyrrhizae uralensis, Saposhnikoviae divaricata, Rhizoma Atractylodis Macrocephalae, Lonicerae Japonicae Flos, Fructus forsythia, Atractylodis Rhizoma, Radix platycodonis, Agastache rugosa, and Cyrtomium fortune J. Sm were 10 most commonly used Chinese herbs in the treatment of COVID-19. Xu, et al. [91] reported that Astragalus membranaceus and Yu Ping Feng were used in the 13 prevention programs (in Beijing, Tianjin, et al.) for "reinforcing vital qi", a terminology used in TCM that is similar to boosting host defense capacity.

Ophiopogon japonicas and Scrophularia ningpoensisand are TCM herbs which were most frequently used for"nourishing yin" in northern China, while Atractylodis Rhizoma, Agastache rugosa and other Chinese medicinal herbs with the property of "aromatic dehumidification" were commonly used in southern China (Table 6).

Table 4. Ongoing TCM Clinical Trials for the treatment of SARS-CoV-2 infection

Registration number	Design type	Title	TCM herbal medicine	Sample size	Phase
ChiCTR2000029432	CCT	A real world study for the efficacy and safety of large dose Tanreqing Injection in the treatment of patients with novel coronavirus pneumonia (COVID-19)	*Tan Re Qing Injection*	72	4
ChiCTR2000029434	RCT	A randomized, open-label, blank-controlled trial for Lian-Hua Qing-Wen Capsule/Granule in the treatment of novel coronavirus pneumonia (COVID-19)	*Lian Hua Qing Wen Capsule/Granule*	400	4
ChiCTR2000029487	CCT	Clinical study for Gu-Biao-Jie-Du-Ling in preventing of novel coronavirus pneumonia (COVID-19) in children	*Gu Biao Jie Du Ling*	200	0
ChiCTR2000029589	CCT	An open, prospective, multicenter clinical study for the efficacy and safety of Reduning injection in the treatment of ovel coronavirus pneumonia (COVID-19)	*Re Du Ning Injection*	60	0
ChiCTR2000029605	RCT	A randomized, open-label, blank-controlled, multicenter trial for Shuang-Huang-Lian oral solution in the treatment of novel coronavirus pneumonia (COVID-19)	*Shuang Huang Lian Oral Liquid*	400	4
ChiCTR2000029780	RCT	A multicenter, randomized, open, controlled trial for the efficacy and safety of Shen-Qi Fu-Zheng injection in the treatment of novel coronavirus pneumonia (COVID-19)	*Shen Qi Fu Zheng Injection*	160	4
ChiCTR2000029781	RCT	A multicenter, randomized, open and controlled trial for the efficacy and safety of Kang-Bing-Du granules in the treatment of novel coronavirus pneumonia (COVID-19)	*Kang Bing Du Granules*	160	4
ChiCTR2000029822	RCT	A randomized controlled trial for honeysuckle decoction in the treatment of patients with novel coronavirus (COVID-19) infection	*Jin Yin Hua Tang*	110	0
ChiCTR2000029991	RCT	A randomized, open-label, controlled trial for the safety and efficiency of Kouating syrup and Keqing capsule in the treatment of mild and moderate novel coronavirus pneumonia (COVID-19)	*Ke Su Ting Syrup /Ke Qing Capsule*	72	4
ChiCTR2000030043	RCT	Shen-Fu injection in the treatment of severe novel coronavirus pneumonia (COVID-19): a multicenter, randomized, open-label, controlled trial	*Shen Fu Injection*	300	4
ChiCTR2000030117	RCT	A multicenter, randomized, open, parallel controlled trial for the evaluation of the effectiveness and safety of Xiyanping injection in the treatment of common type novel coronavirus pneumonia (COVID-19)	*Xi Yan Ping Injection*	348	4
ChiCTR2000030255	RCT	Efficacy and safety of Jing-Yin Granule in the treatment of novel coronavirus pneumonia (COVID-19) wind-heat syndrome	*Jing Yin Granule*	300	4
ChiCTR2000030388	RCT	Efficacy and safety of Xue-Bi-Jing injection in the treatment of severe cases of novel coronavirus pneumonia (COVID-19)	*Xue Bi Jing Injection*	60	0
ChiCTR2000029813	RCT	Clinical Trial for Tanreqing Capsules in the Treatment of Novel Coronavirus Pneumonia (COVID-19)	*Tan Re Qing Capsules*	72	0

Notes: RCT: randomized controlled trial; CCT: controlled clinical trial.

Table 5. TCM recommended by 6th editions Guidelines of Diagnosis and Treatment for COVID-19 [88].

Stage of disease	Symptom	Recommended Chinese patent medicine
Medical observation period	Fatigue with gastrointestinal discomfort	*Huo Xiang Zheng Qi Shui*
	Fatigue with fever	*Lian Hua Qing Wen Capsule, Shu Feng Jie Du Capsule, Jin Hua Qing Gan Capsule*
Clinical treatment period (Confirmed patients)	Mild cases	*Qing Fei Pai Du Tang*
	General cases	*Qing Fei Pai Du Tang*
	Several cases	*Xi Yan Ping Injection, Xue Bi Jing Injection, Re Du Ning Injection, Tan Re Qing Injection, Xing Nao Jing Injection, Qing Fei Pai Du Tang*
	Critical cases	*Xue Bi Jing Injection, Re Du Ning Injection, Tan Re Qing Injection, Shen Fu Injection, Sheng Mai Injection, Shen Mai Injection, Su He Xiang Pill, An Gong Niu Huang Pill*

Table 6. Frequently used TCM herbs for the Prevention of COVID-19 infection

Reported by	Herbs (Latin name)	Herbs (Chinese Pin Yin)	Applicable regions
Luo, *et al.* [37]	*Astragalus membranaceus*	Huangqi	23 provinces covered Northeast, North, Central (including Wuhan), South, East, Northwest, and Southwest China.
	Glycyrrhizae uralensis	Gancao	
	Saposhnikoviae divaricata	Fangfeng	
	Rhizoma Atractylodis Macrocephalae	Baizhu	
	Lonicerae Japonicae Flos	Jinyinhua	
	Fructus Forsythiae	Lianqiao	
	Atractylodis Rhizoma	Cangzhu	
	Radix platycodonis	Jiegeng	
	Agastache rugosa	Huoxiang	
	Cyrtomium fortune J. Sm	Guanzhong	
Xu, *et al.* [91]	*Astragalus membranaceus*	Huangqi	Beijing, Tianjin, Shandong, Shaanxi, Gansu, Hebei, Shanxi, Henan, Hubei, Jiangxi, Hunan, and Yunnan
	Atractylodis Rhizoma	Cangzhu	Five regions in southern China (Hubei, Jiangxi, Hunan, Yunnan, and Wuhan)
	Eupatorii Herba	Peilan	
	Agastache rugosa	Huoxiang	
	Ophiopogon japonicas	Maidong	Eight regions in northern China (Beijing, Tianjin, Hebei, Henan, Shaanxi, Shanxi, Gansu, and Shandong)
	Scrophularia ningpoensis	Xuanshen	
	Rhizoma phragmitis	Lugen	
	Adenophora stricta Miq	Shashen	
	Dendrobium nobile Lindl.	Shihu	

According to the report of National

Administration of Traditional Chinese Medicine, up to February 5th, 2020, 214 COVID-19 patients were treated with Qing Fei Pai Du Tang in Shanxi, Hebei, Heilongjiang and Shaanxi Provinces with overall effective rate ≥ 90%. Among them, the symptoms of majority of patients (≥60%) were markedly improved, while illness of others (30%) was stabilized [92]. After that, 701 COVID-19 patients were treated with Qing Fei Pai Du Tang in 10 provinces in China. The result showed that 130 patients (18.5%) were completely cured after treatment. The treatment also resulted in the disappearance of characteristic symptoms ofCOVID-19 such as fever and cough in 51 patients (7.27%). In addition, symptom improvement or stabilization were observed in 268 patients (38.2%), and in 212 patients (30.2%), respectively [87]. Yao, et al. and Lu, et al. [93, 94] retrospectively analyzed the clinical efficacy of Lian Hua Qing Wen Capsule in treatment of confirmed and suspected COVID-19 patients. The results indicated that this herbal product could markedly relieve major symptoms such as fever and cough and had the capacity to promote the recovery. Some patients with mild illness in the early stage could suddenly progress to severe disease, and eventually died due to septic shock with multiple organ dysfunction syndrome (MODS), which was associated with cytokine storm [95]. There is compelling evidence that some TCM herbal products or its components have potent immunosuppressive effects, as shown by our own and other's studies [79, 96-103]. For example, Wang, et al. [104] reported that Shen Fu Injection could inhibit the lung inflammation and decrease the levels of IL-1β, IL-6 and other cytokines. Chang, et al. [105] reported that Re Du Ning Injection could markedly reduce the levels of IL-1β, TNF-α, IL-8, IL-10, and some other cytokines ofLPS-induced model of acute lung injury in rats. We recently reported that tetrandrine,a compoundisolated from an anti-rheumatic Chinese herb, could potently inhibit proinflammatory Th1, Th2 and Th17 responses in LPS-challenged mice [106]. Therefore, TCM with the capacity to inhibit cytokine storm and its devastating consequences may be harnessed in the treatment of severe COVID-19 patients. Currently, the laboratory study on the effect ofT CM isapparently lagging behind the clinical application of TCM in the treatment of COVID-19 patients. Nevertheless, some scientists have started to examine the effect of TCM products or its components on SARS-CoV-2 in their laboratories. For example, an in vitro study showed that Shuang Huang Lian Oral Liquid had the inhibitory effect on SARS-CoV-2 [78]. However, its clinical efficacy and safety for thetreatment of COVID-19 patients has not been evaluated. We noticed that this TCM product was not recommended by HNC's Guideline [89]. Same as SARS-CoV, SARS-CoV-2 uses receptor ACE2 for the cellular entrance [8]. Theoretically, blockade of ACE2 can prevent the infection of SARS-CoV-2. Chen and Du thus performed the molecular docking study and they found that TCM-derived compounds, including as baicalin, scutellarin, hesperetin, glycyrrhizin and nicotianamine could interact with ACE2 [107]. Therefore, these compoundsas well as herbs containing these ingredients may have the capacity to inhibit the infection of SARS-CoV-2. We anticipate more experiment studies showing anti-SARS-CoV-2 activity of TCM or its components will be published in the near future. Closing remarks TCM TCM hasaccumulatedthousand-of-year's experiences in the treatment of pandemicandendemic diseases. Providing complementary and alternative treatments are still urgently needed for the management of patients with SARS-CoV-2 infection, experiencesin TCM is certainly worth learning.

Fighting against current epidemics also provide an opportunity to test the true value of TCM in treating emerging contagious diseases. Randomized, double-blind and placebo-controlled studies is the best way to provide the most reliable evidence for a therapy,including TCM. It is encouraging that thecontrolled clinical studies to evaluate the efficacy of TCM in the treatment of SARS-CoV were conducted and reported. However, the most of these studies were found to be poorly designed and the results could lead to potential biasesin evaluating theeffectiveness of TCM treatment [13]. Hopefully, current clinical study to evaluate the effect of TCM on COVID-19 will use more strict protocols, concealment of allocation, and double-blinding, in order to ensure the compliance of international acceptable standards. Furthermore, standardized products of TCM, rather than self-prepared formulations, should be used in clinical study. Experiment study may be able to elucidate the mechanism underlying the therapeutic effect of TCM in the treatment of COVID-19. The further study of TCM may lead to the identification of novel anti human coronavirus compounds that may eventually prove to be useful in the treatment of SARS-CoV-2 or other emerging fatal viral diseases as conventional therapeutic agents. The safety of TCM in the treatment of emerging coronavirus diseases was not included in the observation on SARS patients [13]. It was reported that some herbs used in TCM contain nephrotoxins and mutagens [108], while the toxicological features of the most of Chinese herbal medicines remain to be fully understood [109]. Furthermore, herbs used in TCM can mimic, or magnify, or oppose the effect of conventional medicines [110]. Thus, the safety of TCM used in treatment of emerging coronavirus infections should be carefully evaluated. It is particularly important to avoid toxicity or interfere with the efficacy of conventional treatment caused by herb-drug interaction.

Supplementary Material Supplementary figures and tables.

http://www.ijbs.com/v16p1708s1.pdf

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Competing Interests

The authors have declared that no competing interest exists.

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Abstract

The Coronavirus Disease 2019 (COVID-19) pandemic has become a huge threaten to global health, which raise urgent demand of developing efficient therapeutic strategy. The aim of the present study is to dissect the chemical composition and the pharmacological mechanism of Qingfei Paidu Decoction (QFPD), a clinically used Chinese medicine for treating COVID-19 patients in China. Through comprehensive analysis by liquid chromatography coupled with high resolution mass spectrometry (MS), a total of 129 compounds of QFPD were putatively identified. We also constructed molecular networking of mass spectrometry data to classify these compounds into 14 main clusters, in which exhibited specific patterns of flavonoids (45 %), glycosides (15 %), carboxylic acids (10 %), and saponins (5 %). The target network model of QFPD, established by predicting and collecting the targets of identified compounds, indicated a pivotal role of Ma Xing Shi Gan Decoction (MXSG) in the therapeutic efficacy of QFPD. Supportively, through transcriptomic analysis of gene expression after MXSG administration in rat model of LPS-induced pneumonia, the thrombin and Toll-like receptor (TLR) signaling pathway were suggested to be essential pathways for MXSG mediated anti-inflammatory effects. Besides, changes in content of major compounds in MXSG during decoction were found by the chemical analysis. We also validate that one major compound in MXSG, i.e. glycyrrhizic acid,

inhibited TLR agonists induced IL-6 production in macrophage. In conclusion, the integration of in silico and experimental results indicated that the therapeutic effects of QFPD against COVID-19 may be attributed to the anti-inflammatory effects of MXSG, which supports the rationality of the compatibility of TCM.

1. Introduction

Coronavirus Disease 2019 (COVID-19), which is caused by the novel coronavirus acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), has become a serious threaten for public health worldwide [1]. This novel coronavirus has relatively fast transmission speed, which make COVID-19 a highly contagious disease [2,3]. As of March 26, 2020, the epidemic has been sweeping through over 100 countries or regions, and the pandemic has spread to all continents except Antarctica [4], and a total of 531,630 people worldwide were infected with the virus, of whom 24,065 died [5].

Cytokine storm is considered to be one of the leading causes of the clinical deterioration of COVID-19. Through laboratory blood cell counts, lymphopenia was detected in 83.2 % of the patients upon admission, the proportion of which was higher in those with severe illness than non-severe cases [6]. Meanwhile, 36.2 % of patients had thrombocytopenia, and 33.7 % of patients had leukopenia [6]. The reduction of white blood cells and platelets in the bloodstream may be a result of the over-mobilization and aggregation of immune cells in the lesion area for inflammation control. The occurrence of immune cells detachment from blood vessels increases the risk of excessive immune response and directly leads to cytokine storms [7]. When a large number of immune cells are concentrated in the lesion area, a great amount of cytokines are secreted through positive feedback stimulation between each other. Consequently, the immune cells will be activated excessively, which cause tissue damage in the lesion area, as well as severe immune overreaction in the body. This pathological process may directly leads to hypoxemia, shock, and multiple organs failure [8]. Therefore, reducing the excessive aggregation and response of immune cells is important for the prevention of cytokine storms and is beneficial for patient outcomes.

According to a World Health Organization (WHO) commentary, there are currently no effective drugs for the treatment of COVID-19 [9], and the four most promising coronavirus treatments are still undergoing clinical trials [10]. Moreover, for controlling cytokine storms, only hormones and artificial liver blood purification systems are available at present [11]. However, the use of glucocorticoids cannot alleviate the progression of lung injury during COVID-19 infection, yet the side effects of glucocorticoids may significantly reduce life quality of patients [12,13]. Meanwhile, the application of artificial liver blood purification systems also faces challenges as this technology has not been widely used in clinical. Therefore, the successful treatment of COVID-19 patients, especially those severe cases with severe cytokine storms, were impeded.

Since the outbreak of COVID-19, traditional Chinese medicine (TCM) has been used as first-line drugs to treat patients in China and has obtained positive curative effects. As of February 22, 2020, the rate of TCM treatment of COVID-19 in China was 87 %, and the total effective rate of TCM treatment was 92 %, of which only 5% of patients have worsened clinical manifestations [14], [15], [16]. The Chinese government decided TCM as one of the recommended therapeutic options for the treatment of COVID-19 in the third version *COVID-19 treatment guidelines*, which was published on January 23, 2020 [17]. Besides, based on the theoretical system of TCM, physicians can combine and adjust prescriptions based on the symptoms of patients diagnosed. After nearly one month of clinical experimental observation, Qingfei Paidu Decoction, a formula consisting of 21 components including both herbs and mineral drugs, has been included in the 6th edition of the guidelines as the primarily recommended formulae [18]. According to the 6th and 7th edition of *COVID-19 treatment guidelines* [19,20], Qingfei Paidu Decoction (QFPD) is effective for patients at all stages, and the total effective rate is 92.09 % [21]. However, whether QFPD treat COVID-19 through regulating immune status and preventing cytokine storms has not been fully elucidated.

Here, we integrated multidisciplinary technologies to explore the pharmacological mechanism and to identify the major constituents of QFPD. The chemical constituents in QFPD was dissected by liquid chromatograph-mass spectrometry (LC–MS), and a substance-based intervention network was established through network pharmacology. At the same time, a disease network of patients based on their description of TCM symptoms was built via the SymMap database. Then, through network analysis, we analyzed the regulating pathways of QFPD and conducted biological verification via an *in vitro* macrophage activation model. Moreover, the chemical composition and pharmacological effects of a main component of Qingfei Paidu Decoction, Ma Xing Shi Gan Decoction, was further verified by transcriptomics. In summary, our study

demonstrated Qingfei Paidu Decoction regulates the level of cytokine storms during the infection, which may be key mechanism underlying its therapeutic effects in COVID-19. These results may also guide future researches on TCM in infectious diseases and hyperimmune-related diseases.

2. Experimental

2.1. Materials and reagents

The reference compounds wogonin, cryptochlorogenic acid, neochlorogenic acid, chlorogenic acid, rosmarinic acid, mangiferin, irisflorentin, amygdalin, baicalin, iristectorin, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, rutin, naringin, narirutin, neohesperidin, hesperidin, isoacteoside, saikosaponin B2, saikosaponin A, wogonoside were purchased from Shanghai Winherb Medical Technology Co., Ltd (Shanghai, China). Quercetin, isoliquiritin, isoliquiritin apioside, acteoside, 25-O-methylalisol A, alisol C monoacetate, glycyrrhizic acid were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Quercitrin was were obtained from Aladdin Industrial Corporation (Shanghai, China). Ephedrine and pseudoephedrine were from National Institutes for Food and Drug Control (Beijing, China). HPLC-grade acetonitrile, methanol and formic acid were from Merk (Darmstadt, Germany). Deionized water was prepared with an Elga PURELAB flex system (ELGA LabWater, UK). All other chemicals and reagents used were of analytical grade. The crude drug materials were obtained from China Beijing Tongrentang (Group) Co., Ltd. High-glucose Dulbecco's modified Eagle's medium, fetal bovine serum, trypsin-EDTA and antibiotics (100 U/mL penicillin G and 100 g/mL streptomycin) were obtained from Gibco BRL (Grand Island, NY, USA). Poly(I:C) and Pam3CSK4 were purchased from InvivoGen (Carlsbad, CA, USA). IL-6 ELISA kit was obtained from Boster Biological Technology Co., Ltd (Wuhan, China).

2.2. Sample preparation

Qingfei Paidu Decoction was prepared from 21 herbs, *Herba Ephedrae* (Ma Huang), *Radix Glycyrrhizae* (Gan Cao, baked), *Semen armeniacae amarum* (Ku Xing Ren), *Gypsum fibrosum* (Sheng Shi Gao), *Ramulus Cinmomi* (Gui Zhi), *Rhizoma Alismatis* (Ze Xie), *Polyporus umbellatus* (Zhu Ling), *Atractylodis Macrocephalae Rhizoma* (Bai Zhu), *Poria* (Fu Ling), *Stellariae Radix* (Chai Hu), *Radix Scutellariae* (Huang Qin), *Pinelliae Rhizoma Praeparatum cum Zingibere* (Jiang Ban Xia), *Rhizoma Zingiberis Recens* (Sheng Jiang), *Radix asteris* (Zi Wan), *Flos farfarae* (Kuan Dong Hua), *Rhizoma Belamcandae* (She Gan), *Herba Asari* (Xi Xin), *Rhizoma Dioscoreae* (Shan Yao), *Fructus Aurantii Immaturus* (Zhi Shi), *Pericarpium Citri Reticulatae* (Chen Pi), and *Pogostemon Cablin (Blanco) Benth* (Guang Huo Xiang). Briefly, Sheng Shi Gao was decocted firstly, and then all raw materials were co-decocted in water twice (1 h for each time, the amount of the solvent was ten and eight times of the total weight of herbs, respectively). A total of approximate 1.78 L decoction were collected. The extracts were centrifuged at 10, 000 rpm for 20 min, and the supernatants were subject to LC–MS analysis.

2.3. LC-Q-TOF-MS analysis

An Acquity UPLC system (Waters, Milford, MA, USA) coupled with a Triple TOF 5600plus MS (AB SCIEX, Framingham, MA, USA), which equipped with an ESI source, was employed for chemical identification. Samples were separated on a Waters ACQUITY UPLC HSS T3 (150 mm × 2.1 mm i.d. 1.8 µm) at a flow rate of 0.3 mL/min and a column temperature of 50℃. Mobile phases A and B were 0.1 % formic acid-water and 0.1 % formic acid-acetonitrile, respectively. The linear gradient elution was optimized as follows: 0−2 min, 0−0%B; 2−25 min, 0–30 %B; 25−35 min, 30–95 %B; 35−37 min, 95 %B. The injection volume was 10 µL. The detection wave was 254 nm. Mass spectrometry analysis was performed in both positive and negative mode under following parameters: scan range, *m/z* 100–1500; ion source GS1 55 psi; ion source GS2, 55 psi; curation gas (CUR), 35 psi; temperature, 600℃ for ESI^+ and 550℃ for ESI^-; ionspray voltage (IS), −4.5 kV for ESI^- and 5.0 kV for ESI^+; declustering potential (DP), 100 V; collision energy (CE), 10 V.

2.4. Raw data-preprocessing parameters

The MS/MS data files were converted from the. wiff (Waters) standard data format to. mzXML format using the MSConvert software, within the ProteoWizard package. All. mzXML data were then processed using MZmine 2 v40.1 [22]. The mass detection was performed setting the noise level at 5.0 E4. The ADAP chromatogram builder was used with a set of a minimum group size of scans of 5, a group intensity threshold of 100 000, a minimum highest intensity of 50 000 and a *m/z* tolerance of 0.001 Da (or 10 ppm) [23]. The ADAP wavelets deconvolution algorithm was used using S/N threshold = 10, minimum feature

height = 5.0 E4, coefficient/area threshold = 100, peak duration range 0–1 min, RT wavelet range 0–0.2. MS/MS scans were paired using a *m/z* tolerance range of 0.02 Da and RT tolerance range of 0.1 min. Isotopic peaks grouper algorithm was used with an *m/z* tolerance of 0.001 Da (or 10 ppm) and an RT tolerance of 0.01 min. Peak alignment was performed using the join aligner module with *m/z* tolerance =0.001 Da (or 10 ppm), weight for *m/z* = 1, weight for RT = 1.0, absolute RT tolerance 0.1 min. The peak list was gap-filled with Peak finder module using *m/z* tolerance of 0.001 Da (or 10 ppm) and then filtered using Peak list row filter with retention time 0−42 min. Eventually, the mgf data file and its corresponding. csv metadata file including Peaks height and areas integration were exported.

2.5. Molecular networking

Corresponding molecular networking was created according to the online workflow at GNPS (http://gnps.ucsd.edu) with a parent mass tolerance of 0.02 Da and an MS/MS fragment ion tolerance of 0.02 Da, the minimum cluster size of 1, run MScluster and filter precursor window tools were turned off [24]. The network created with cosine score above 0.7 and more than four matched peaks. The spectra in the network were then searched against GNPS spectral libraries with a cosine score above 0.7 and at least 4 matched peaks. The molecular networking data were visualized using Cytoscape (ver.3.7.2).

2.6. Qingfei Paidu Decoction networking

After the compound network was created, potential therapeutic targets network for Qingfei Paidu Decoction were further established as previous studies [[24], [25], [26], [27], [28], [29]]. First, we query and standardize the identified compound information by the Pubchem database [30]. As a result, all compounds were listed with CAS number or PubChem ID, which are for subsequent query work. Next, we collected the target points of each compound in the list through the ETCM [31] and SymMap databases [32]. At the same time, we searched the SMILE structure corresponding to each compound through the Pubchem database, entered the structure into the SWISS Target Prediction database [33] to predict possible targets, and collected targets with scores above 0.75 in the results. Finally，we used the DAVID database [34] to enrich the included target genes and visualized the enrichment results with Cytoscape (ver.3.7.2) and Power BI (ver. 2.79.5773.0).

2.7. COVID-19 disease network

At present, there are limited diagnostic indicators for COVID-19, and the variability in the detection process of the existing indicators directly leads to significant false negatives in the test. In comparison, the diagnostic system established according to the diagnosis of TCM mostly avoid false-negative results. Therefore, we established a COVID-19 disease network through the SymMap database according to the classification and symptoms from the TCM system in the 7th edition of *COVID-19 treatment guidelines*. First, we used the symptoms and their synonyms in the7th edition of *COVID-19 treatment guidelines* as search terms and entered them into SymMap. According to the symptom-drug-compound-target cycle, the database returns the target information corresponding to the symptoms. According to the classification of syndrome types in the 7th edition of *COVID-19 treatment guidelines*, we merged all collected targets and then performed enrichment analysis on targets through the DAVID database. After the enrichment analysis, we visualized the enriched results through the Enhancement Map and Annotation in Cytoscape (ver.3.7.2). Enrichment Map results only include those that contain more than 20 pathways or progresses.

2.8. The establishment of Qingfei Paidu Decoction therapeutic network for COVID-19

After getting the potential therapeutic targets of Qingfei Paidu Decoction and the target-genes of COVID-19, we further established the intervention network between Qingfei Paidu Decoction and COVID-19. In order to further focus the curative effect of Qingfei Paidu Decoction on the regulation of the immune system, especially in the regulation process of innate immunity, we mapped the drug-disease network of Qingfei Paidu Decoction and COVID-19 on the toll-like signaling pathway. Results were visualized with wikipathway plugin of Cytoscape software.

2.9. *In vivo* transcriptome experiment of Ma Xing Shi Gan Decoction

Male SD rats (110 ± 10 g, 8–9 weeks) were purchased from SPF (Beijing) Biotechnology Company. During housing, the animals were kept in standard polypropylene cages, and the room temperature and the humidity were maintained at 12/12-h light-dark cycle. Animals eat and drink freely.

After three days of adaptive breeding, the weight of all rats was measured. 50 SD rats were randomly divided into five groups, ten rats/group. The groups were named as normal group (Normal), model group (Model), Ma Xing Shi Gan Decoction (MXSG), antibiotic group (moxifloxacin), and hormone group (prednisone acetate). Next, the Model, MGXG, Antibiotic, and Hormone were received given 0.5 mg/mL LPS nebulization intervention, 30 min per day for three consecutive days. At the same time, on the day after the first atomization, the MGXG group, hormone group, and antibiotic group were given the oral treatment of Ma Xing Shi Gan Decoction, moxifloxacin, and prednisone acetate, which were administered orally once a day for three consecutive days. All doses were converted according to the equivalent dose of 0.018 for humans and rats with reference to clinical guidelines. After the last treatment, all the rats were on the condition of free drinking but without food for 12 h, then anesthetized with 10 % chloral hydrate and further sacrificed for liver, lung thymus, and kidney samples. The tissues were quickly collected, weighed, and the tissue/weight index was calculated and then placed in liquid nitrogen for quick freezing, and then placed in a −80 °C refrigerator for later tests.

The total RNA from each of the different specimens was isolated from the lungs of rats of three groups (normal group, model group, and MXSG group) simultaneously using the RNeasy Mini-Kit (QIAGEN, Valencia, CA), as per the manufacturer's instructions. After extracting total RNA from the sample and digesting the DNA with DNase, enrich it with magnetic beads with Oligo (dT); then add a disruption reagent to break the mRNA into short fragments, use the disrupted mRNA as a template, and randomly use six bases. The primers are used to synthesize one-strand cDNA, and then a two-stranded reaction system is prepared to synthesize the two-stranded cDNA, and the double-stranded cDNA is purified; the purified double-stranded cDNA is then subjected to end repair, added with A tail, and connected to a sequencing adapter, and finally, PCR amplification is performed. After the library was qualified with the Agilent 2100 Bioanalyzer, it was sequenced using the Illumina HiSeqTM 2 sequencer to generate 125bp or 150bp double-ended data. After passing the quality inspection, sequencing was performed using an Illumina sequencer, and bioinformatics analysis were performed based on the results.

Gut microbiota of rats in different group were analyzed as previous study [35]. This study used a larger set of gut microbiota profiles that were generated alongside those described in a recent study by Goodrich et al. [36], which reported a smaller sample as it considered only complete twin pairs. The processing of fecal samples has been described previously [37]. Briefly, samples were collected by the individual at home and either bought to a clinical visit or posted on ice to the clinical research department on ice where it was stored at −80 °C. Frozen samples were extracted to obtain DNA, the V4 region of the 16S rRNA genes amplified, and amplicons sequenced using a multiplexed approach on the Illumina MiSeq platform. Sample reads were demultiplexed and paired-ends merged using a 200 nt minimum overlap.
De novo chimera removal was carried out on the 16S rRNA gene sequencing per sample using UCHIME [38]. Remaining reads were collapsed to de novo operational taxonomic units (OTUs) at 97 % identity using SUMACLUST within QIIME version 1.9.0 [39,40]. OTU taxonomy was assigned by aligning representative sequences to the Greengenes v13_8 database using UCLUST in QIIME. Analyses were adjusted for sequencing depth throughout by using sample read count as a covariate. Taxonomic abundances were generated by collapsing OTU counts at appropriate levels, followed by conversion to log-transformed relative abundances. Three alpha diversity metrics, namely the Shannon index, phylogenetic diversity, and raw OTU counts, were calculated using QIIME. Beta diversity was calculated as both weighted and unweighted UniFrac metrics, and principal coordinate analysis of the beta distances was carried out using the vegan package.
For histological evaluation, the formaldehyde-fixed lower lobe of the left lung was embedded in paraffin wax, serially sectioned, and stained with hematoxylin and eosin. Histologic changes, including alveolar wall edema, congestion, hemorrhage, and inflammatory cell infiltration, were evaluated under a light microscope to assess pulmonary inflammation according to the previous report [41].

2.10. Anti-inflammatory effect of glycyrrhizic acid in macrophage activation model

The RAW264.7 cell lines were cultured in high glucose DMEM with 10 % FBS and antibiotics (100 units/mL penicillin and 100 µg/mL streptomycin). The cells were seeded in 12-well plates at a density of 100,000 cells. After 1 day, the cells were co-incubated with LPS (100 ng/mL) or Poly(I:C) (10 µg/mL) + Pam3CSK4 (100

ng/mL) and test drug for 24 h. Dexamethasone acts as positive drug. After that, the cell culture medium was collected for IL-6 concentration measurement by enzyme-linked immunosorbent assay (ELISA) kit.

3. Results

3.1. Characterization of chemical constituents in Qingfei Paidu Decoction

Representative chromatograms obtained by UPLC-Q-TOF/MS are shown in Fig. 1. After analyzing the raw data, 151 peaks were caught as individual compounds. Among these, a total of 129 constituents were identified or tentatively characterized from QFPD based on literature and database matching. The compounds identified from QFPD were listed in Supplementary Table S1, including 58 flavonoids, 20 glycosides, 13 carboxylic acids, 7 saponins, 6 alkaloids, 4 terpenes, and other types of compounds. The main peaks in LC–MS chromatogram were labeled with chemical structures from different herbs (Supplementary Fig. S1). Among them, 29 compounds were unambiguously identified by comparisons with reference standards in terms of retention time and mass spectra (Supplementary Fig. S2). The number of chemical constituents of each herb and representative constituents chosen from identified list of QFPD was shown in Fig. 2, which suggested that GC, CH, HQ are the main sources of identified compounds.

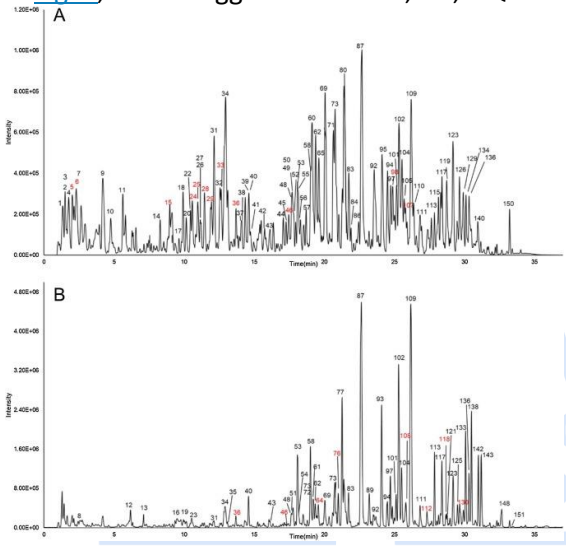

Fig. 1. Mass spectrum chromatograms of Qingfei Paidu Decoction (A) Negative mode (B) Positive mode. Red numbers represent unidentified compounds.

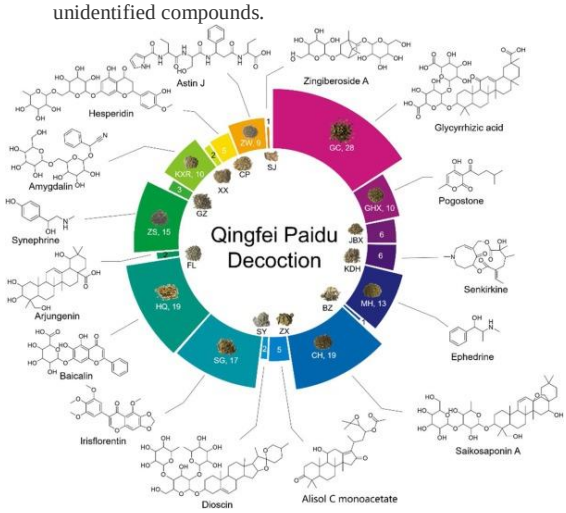

Fig. 2. The number of chemical constituents of each herbs and representative constituents structure identified from Qingfei Paidu Decoction.

MS/MS data were used to construct molecular networking of QFPD according to the classification of compound structures and its similarity in MS^2 spectra. As shown in Fig. 3, the colors of the nodes in the molecular networking represent the sources herb of the compounds, which clearly indicated the contribution of MH, GC, CH, SG, ZX to the chemical composition of QFPD. The sectorial area of color corresponding to the peak area of each compound. The size of nodes corresponds to the peak height of each

compound in the whole decoction. Based on the networking, we labeled the represent compounds on the nodes, as shown in Fig. 4. Flavonoids aggregated to form the largest cluster, while glycosides and saponins also formed typical clusters.

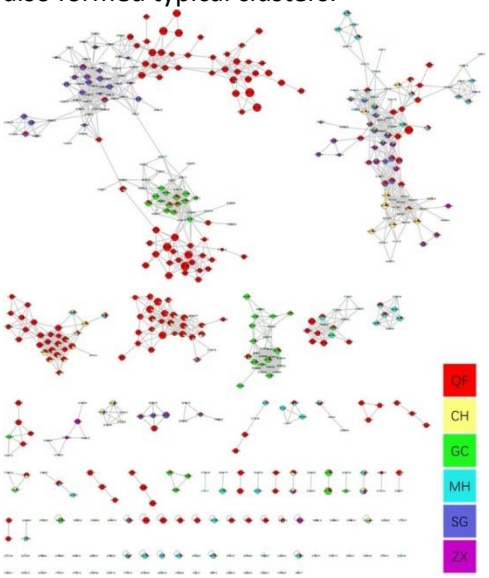

Fig. 3. Molecular networking of Qingfei Paidu Decoction.

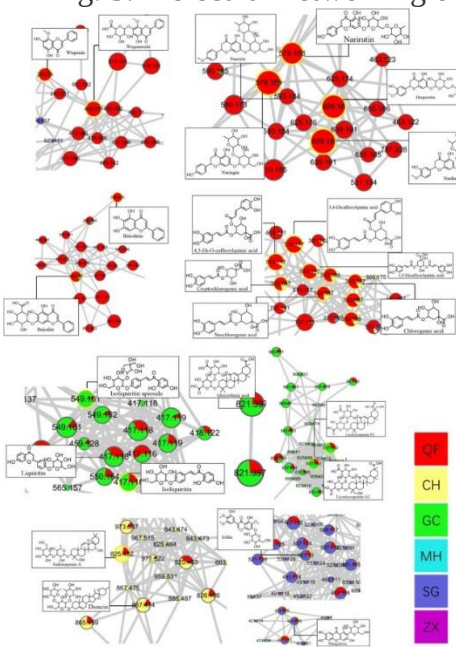

Fig. 4. Representative clusters of molecular networking of Qingfei Paidu Decoction.

3.2. Characterization of potential therapeutic targets of Qingfei Paidu Decoction

The potential therapeutic target-network of Qingfei Paidu Decoction was presented in Fig. 5. We firstly predicted and collected the targets of compounds identified by LC–MS. Qualified target information was obtained for 40 flavonoids, 7 glycosides, 5 terpenes, 3 carboxylic acids, 3 cholestanes, 2 saponins, 1 xanthenes, 1 heterocyclic, 1 benzopyrans, 1 benzofurans and 1 alkaloid. The links among the herbs, compound categories, and compounds were shown in Fig. 5A. Then, we counted the number of targets corresponding to each drug based on the compound information contained in the herbal medicine (Fig. 5B). The results showed that MH, GC, CH, HQ, ZW were the herbs with the highest number of corresponding targets. After merging all the target information and removing the duplicate target content, we next performed GO and KEGG enrichment analysis on the target information (Fig. 5C and D). As a result, in the GO enrichment analysis, QFPD played an interventional role by interfering with cellular processes and metabolic processes. The interventional effect was mainly on organelles and membrane structures, and the main targeted molecules were involved in protein binding process and the function of catalysis. KEGG analysis

suggested that the effect of QFPD was mainly to interfere with viral infection-related pathways and cancer-related pathways. All the herb-compound-target information is in Supplementary Material 2.

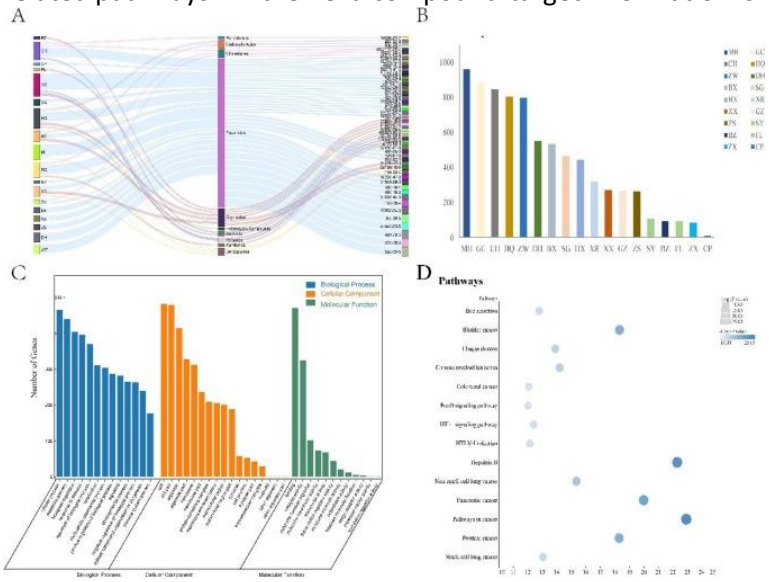

Fig. 5. The therapeutic network of Qingfei Paidu Decoction. (A) the distribution of compounds from different Chinese herbs. (B) the summary of potential targets genes from different herbs. (C) the GO enrichment of herbal-related genes. (D) the top 15 pathways enriched by the KEGG method. MH, *Herba Ephedrae* (Ma Huang); GC, *Radix Glycyrrhizae* (Gan Cao, baked); CH, *Stellariae Radix* (Chai Hu); HQ, *Radix Scutellariae* (Huang Qin); ZW, *Radix asteris* (Zi Wan); DH, *Flos farfarae* (Kuan Dong Hua); BX, *Pinelliae Rhizoma Praeparatum cum Zingibere* (Jiang Ban Xia); SG, *Rhizoma Belamcandae* (She Gan); *Pogostemon Cablin (Blanco) Benth* (Guang Huo Xiang); XR, *Semen armeniacae amarum* (Ku Xing Ren); XX, *Herba Asari* (Xi Xin); GZ, *Ramulus Cinmomi* (Gui Zhi); ZS, *Fructus Aurantii Immaturus* (Zhi Shi); SY, *Rhizoma Dioscoreae* (Shan Yao); BZ, *Atractylodis Macrocephalae Rhizoma* (Bai Zhu); FL, *Poria* (Fu Ling); ZX, *Rhizoma Alismatis* (Ze Xie); CP, *Pericarpium Citri Reticulatae* (Chen Pi).

3.3. The COVID-19 disease network

The construction of the COVID-19 symptom target network is based on TCM diagnostic system. According to the diagnostic criteria in the 7th edition of *COVID-19 treatment guidelines*, TCM classified COVID-19 patients into four categories based on patients' pathological process, including mild, moderate, severe, and critical. Among them, the mild type included the syndrome of cold fluid-retention suspend in lung and the syndrome of stagnant and jamming dampness-heat in the lung; the moderate type was divided into the syndrome of dampness toxin and depression of lung and the syndrome of accumulation of cold fluid in the lung; the severe type contained the syndrome of dirty-toxicity blocking lung and the syndrome of flaring heat in qifen (also known as defense qi, a term describes qi is "fierce, bold and uninhibited" and unable to contained by the vessels and therefore flowing out side them) and yingfen (also known as construction qi, which refer to the qi that forms the blood and flows with it in the vessels, helping to nourish the entire body) [42]; the critical type had only the syndrome of internal blockade and external collapse. We next collected the targets corresponding to the symptoms in the SymMap database according to the symptoms of different syndromes and the synonyms of the symptoms. The results showed that there were 1029 no-duplicate targets information in mild cases, of which the syndrome of cold fluid-retention suspend in lung contains 576 targets, whereas the syndrome of stagnant and jamming dampness-heat in lung contains 905 targets. For the moderate type, there were 638 targets for the syndrome of dampness toxin and depression of lung, and 611 targets for the syndrome of accumulation of cold fluid in the lung. For the severe type, 937 targets were collected for the syndrome of dirty-toxicity blocking lung, and 667 targets were collected for the syndrome of flaring heat in qifen and yingfen. Finally, for the critical type, 443 targets were included for the syndrome of the internal blockade and external collapse (Table 1).

Table 1
The TCM symptoms of COVID-19 and potential therapeutic targets.

Class	Category	Symptoms	Gene number
Mild	Syndrome of cold fluid-retention suspend in lung	Fever, fatigue, sore body, cough, expectoration, chest tightness, shortness of breath, appetite, nausea, vomiting, and sticky stools. The tongue is pale, with creases or redness, the fur is white and thick, rotten or greasy, and the pulses are smooth or slippery	576
	Syndrome of stagnant and jamming dampness-heat in lung	Low or no fever, slight chills, fatigue, heavy head and body, muscle aches, dry sputum, sore throat, dry mouth, do not want to drink more or accompanied by chest tightness, no sweat or sweating, Nausea, diarrhea, or sticky stools. Reddish tongue, thick white or yellowish fur, slippery pulses	904
Moderate	Syndrome of dampness toxin and depression of lung	Fever, less cough, and sputum, or yellow sputum, tightness, shortness of breath, bloating, and constipation Dark red tongue, fat tongue, yellow greasy or yellow dry fur, slippery pulses or slippery strings	638
	Syndrome of accumulation of cold fluid in the lung	Low fever, no heat, or no heat, dry cough, less phlegm, fatigue, fatigue, chest tightness, nausea, or nausea. The tongue is pale or red, the fur is white or greasy, and the veins are floating and soft.	611
Severe	Syndrome of dirty-toxicity blocking lung	Fever redness, cough, yellowish phlegm stickiness, or blood in sputum, shortness of breath, shortness of breath, tiredness, dry mouth, and sticky mouth, nausea, poor stool, short urination. Red tongue, greasy yellow fur, slippery pulses	937
	Syndrome of flaring heat in qifen and yingfen	High fever and thirst, shortness of breath, shortness of breath, slang fainting, blurred vision, or spotted rash, or vomiting blood, bleeding, or convulsions in the limbs. Tongue ridges with little or no moss, fine pulse sinking or floating large	667
Critical	Syndrome of the internal blockade and external collapse	Dyspnea, dyspnea, or need mechanical ventilation, fainting, irritability, cold sweaty extremities, dark purple tongue, thick or dry fur, and floating pulses	443

We further analyzed these targets from COVID-19. The distribution of targets from different categories was shown in Fig. 6A. The results suggested that at different pathological stages (both class of mild, moderate, severe, and critical, or their divided categories), the targets that change during the pathological process are different. We then deduplicated, merged the target genes of the same class, and exhibited the results in the form of Venn diagrams (Fig. 6B). There are 233 targets in all four stages, and 376 targets appear in three stages, suggesting that in the pathological process of COVID-19, many biological processes may be shared (Supplementary Material 3). At the same time, in the transition phase, that is, mild phase to moderate phase, moderate phase to severe phase, severe phase to critical phase, the number of common targets appeared is relatively large, 77 between the mild and the moderate, 124 between the moderate and the severe, and between 82 the severe and the critical. To further find the regular of these targets, we merged, with no repetition, all the targets and then enriched these genes by GO and pathway (using the Reactome database) method. As a result, we found that targets were enriched in the regulation of a variety of biological processes and participated in the immune response process; the corresponding molecular functions of these genes were the docking, catalysis and molecular activity regulation of proteins; meanwhile, the positions of these targets correspond to subcellular organelles and cell membrane (Fig. 6C). This result was similar to the target network results of Qingfei Paidu Decoction, suggesting that Qingfei Paidu Decoction could be used as a promising formula to intervene in the pathological process of COVID-19. Moreover, we enriched the results of the biological process from GO and the results of pathways from Reactome database, and discover the immune regulation cells was the core physiological and pathological process of the pathological process of COVID-19 (Fig. 6D), which indicated that controlling the level of the immune response during the disease process was one of the crucial intervention to treating COVID-19.

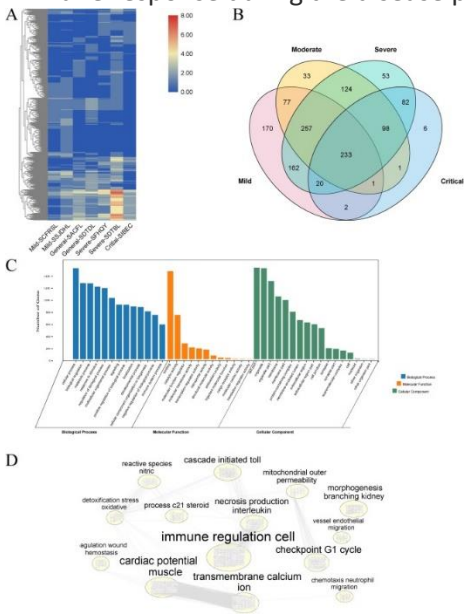

Fig. 6. The network of COVID-19. (A) the heatmap of different categories in diverse pathological classes based on TCM Diagnostic System. Each row refers to a target of COVID-19, while each column stand for a category of COVID-19 The color variety showed the frequency of the targets in each category. (B) Potential genes distribution of diverse pathological classes. (C) the enrichment of genes

with GO method. (D) the enrichment of signaling pathways. SCFRSL, syndrome of cold fluid-retention suspend in the lung; SSJDHL, syndrome of stagnant and jamming dampness-heat in the lung; SACFL syndrome of dampness toxin and depression of lung; SDTDL, syndrome of accumulation of cold fluid in the lung; SFHQY, syndrome of dirty-toxicity bocking lung; SDTBL, syndrome of flaring heat in qifen and yingfen; SIBEC, syndrome of the internal blockade and external collapse.

3.4. The intervention of Qingfei Paidu Decoction for COVID-19 based on toll-like signaling pathway

As the Toll-like signaling pathway was one of the most critical pathways in the immune response to viral infections, we further investigate the effect of Qingfei Paidu Decoction for COVID-19 based on this pathway. We combined the potential therapeutic network of QFPD and the target network of COVID-19, and merged the result with the genes of the Toll-like signaling pathway. As a result, we found that a total of 24 genes are promising target genes of QFPD for COVID-19 (Fig. 7). Among the genes interfered by QFPD, 6 genes are reported as disease-related genes; 3 genes are target genes related to drug therapy, 12 genes are both disease-related genes and target genes, and 3 genes are background genes. When dissecting the compound-target relationships in the context of Toll-like receptor pathway, it is interesting to find out MH and GC, which belongs to Ma Xing Shi Gan Decoction, chiefly regulated the similar targets of QFPD.

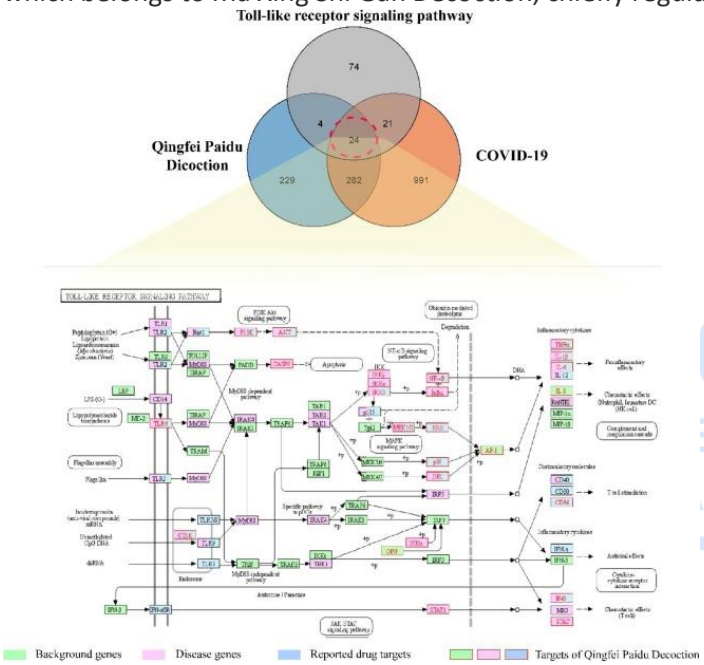

Fig. 7. The regulation of Qingfei Paidu Decoction on the Toll-like receptor signaling pathway.

3.5. Components variation of co-decoction of Ma Xing Shi Gan Decoction

Ma Xing Shi Gan Decoction, which consists of *Herba Ephedrae* (MH), *Semen armeniacae amarum* (Ku Xing Ren), *Gypsum fibrosum* (SSG) and *Radix Glycyrrhizae* (GC), was considered as the main efficacy part of the whole decoction of QFPD. We also analyzed its chemical composition in parallel to the same LC–MS condition and investigated the synergistic effects on the content of main components, when different herbs were co-decocted and decocted individually (Fig. 8A). Based on the result of element analysis, it was found that the concentration of Manganese (Mn) and Nickel (Ni) arrived ppb level in the decoction after *Gypsum fibrosum* (SSG) was extracted for the first 30 min. It is interesting that the co-decoction of *Herba Ephedrae* (MH), *Gypsum fibrosum* (SSG), and *Radix Glycyrrhizae* (GC) lead to the increased yield rate of ephedrine (Fig. 8B). Conversely, the dissolution of glycyrrhizic acid was reduced during the co-decoction period, as shown in Fig. 8C, suggesting that the potential interaction between alkaloids (such as ephedrine) and glycyrrhizic acid may affects the chemical composition of Ma Xing Shi Gan Decoction.

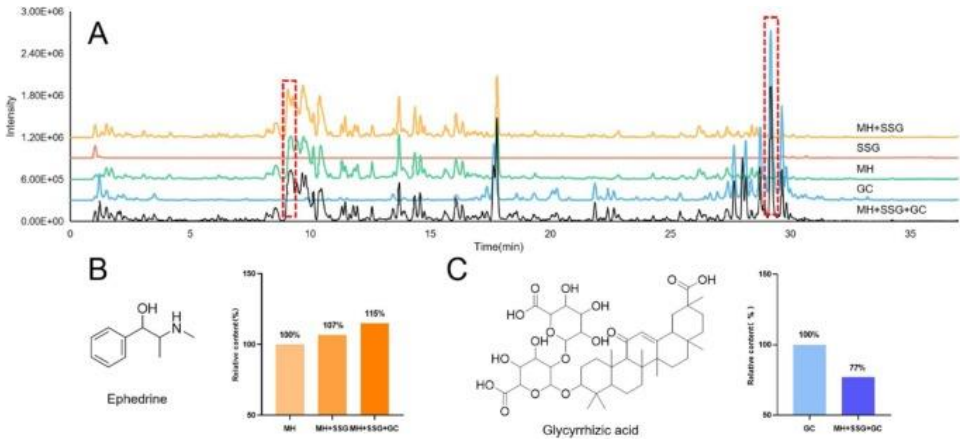

Fig. 8. The effects of components variation in Ma Xing Shi Gan Decoction on different decoction methods. (A) Comparison of mass spectrum chromatograms from four decoctions. (B) Effects of decoction methods on the relative content of ephedrine. (C) Effects of decoction methods on the relative content of glycyrrhizic acid.

3.6. The therapeutic effect of Ma Xing Shi Gan Decoction

We further validated the therapeutic effects of Ma Xing Shi Gan Decoction in a rat model of LPS induced pneumonia. Ma Xing Shi Gan Decoction significantly reduced the pulmonary inflammation response *in vivo* observed through pathological results (Supplementary Fig. S3A–E). The HE results showed that Ma Xing Shi Gan Decoction attenuated the LPS-induced inflammation in lung tissue.

The transcriptome of lung tissue results also showed that it was primarily related to complement and coagulation cascades, NOD-like receptor signaling pathway, antigen processing and presentation, cytosolic DNA-sensing pathway, RIG-I-like receptor signaling pathway, natural killer cell-mediated cytotoxicity, and IL-17 signaling pathway. These signaling pathways are closely related to the regulatory mechanism of the immune system (Supplementary Fig. S5). Vital proteins in these pathways, such as F12, F13b, F9, AT3, etc., were mainly concentrated in the blood coagulation system. These proteins were involved in the conversion of zymogen to serine protease, and eventually form thrombin, which is responsible for converting soluble fibrinogen into insoluble fibrin clot, affecting the regulation of innate immunity. The results suggested that the thrombin system might be one of the essential ways for Ma Xing Shi Gan Decoction to interfere with infection.

Besides, compared with the hormone and antibiotic groups, Ma Xing Shi Gan Decoction has higher safety. Safety evaluation based on HE staining, organ index, and intestinal flora were presented in Supplementary Fig. S4.

3.7. Glycyrrhizic acid attenuated the inflammatory response of macrophage

We further validated the anti-inflammatory effects of glycyrrhizic acid in cellular models of macrophage activation induced by different TLRs stimuluses. LPS stimulation is Toll-like receptor 4 (TLR4)-mediated response, while the combination of Poly(I:C) and Pam3CKS4 represents activation of TLR2 and TLR3. As shown in Fig. 9, both LPS or combination of Poly(I:C) and Pam3CKS4 stimulation significantly elevated the cytokine IL-6 levels in RAW264.7 cells. Glycyrrhizic acid (5 μM) significantly reduced the IL-6 release of macrophage after activation with both LPS (Fig. 9B) and Poly(I:C)/ Pam3CKS4 (Fig. 9C), suggesting that glycyrrhizic acid may exerts moderate anti-inflammatory effects through Toll-like receptor pathways

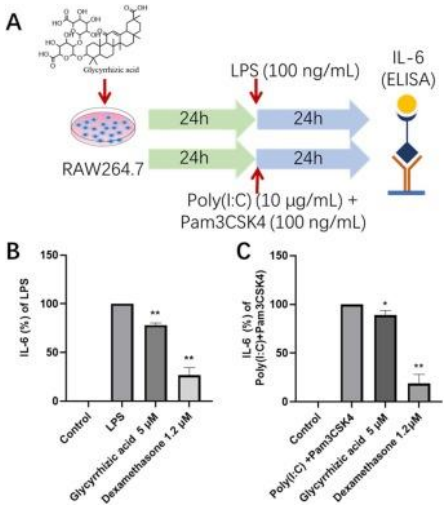

Fig. 9. Glycyrrhizic acid inhibits TLR agonists induced IL-6 production in RAW264.7. (A) Scheme of TLR agonists induced macrophage activation. Glycyrrhizic acid reduced the concentrations of IL-6 released by macrophage activated by LPS (B) or Poly(I:C)/ Pam3CKS4 (C), compared with LPS or Poly(I:C)/Pam3CKS4, $^{*}P < 0.05$, $^{**}P < 0.01$.

4. Discussion

COVID-19 has brought an enormous threat to human security. As of March 26, 2020, the number of deaths has been up to 24,065, which is about 3.4 % of the total confirmed cases [43]. In this present study, we construct a TCM symptom-target disease network of COVID-19 based on the diagnostic system of TCM, which is in 7th edition of *COVID-19 treatment guidelines* and has been used in the clinical treatment of COVID-19 in China. We found that in addition to the immune response and activation of multiple immune cells, the pathological processes of COVID-19 also involve in the coagulation system.

Using *in silico* approaches including network pharmacology and molecular networking of MS data, we elucidated the chemical composition and potential mechanism of QFPD against COVID-19. A total of 129 compounds were identified from QFPD, while the targets of these compounds in the context of COVID-19 disease network l were generated. As a result, we found that Toll-like signaling pathway plays important roles in pharmacological mechanism of QFPD for treating COVID-19. Compound from QFPD may directly interfere with Toll-like receptor 4 and regulate the downstream signaling pathways, including the NF-κB signaling pathway and MAPK signaling pathway, leading to the inhibition of release of proinflammation factors, such as TNF-α, IL-1β, IL-8. These cytokines may further induce the inflammation storm in COVID-19 [44], which is one of the most critical signs of the severe and critical condition of COVID-19, and the leading cause of patients death. Interestingly, based on the results of network pharmacology, QFPD may also regulate the downstream positions except for the Toll-like receptor 4, like PI3K, AKT, and CASP8, without interfering Toll-like receptor 2/3. Similarly, Qingfei Paidu Decoction also affects the INF-α response induced by viral DNA or RNA invasion. These results indicate that QFPD may interfere with COVID-19 through multiple signaling pathways by multiple constituents from different TCMs.

As one of the components of QFPD, Ma Xing Shi Gan Decoction has been vastly used in the treatment of multiple epidemics caused by viruses [[45], [46], [47]]. Previous studies showed that Ma Xing Shi Gan Decoction can alleviate viral infection by regulating the expression of multiple cytokines, activating multiple innate immune-related cells, and preventing pathogens from entering targeted host cells [[48], [49], [50]]. In this present study, LC–MS results suggested that the compatibility of Ma Xing Shi Gan Decoction increase the dissolution of ephedrine while reducing the dissolution of glycyrrhizic acid. As one of the primary material foundations of Ma Xing Shi Gan Decoction, ephedrine has an anti-platelet aggregation effect [51]. Combined with the results of lung transcriptomics in this study, Ma Xing Shi Gan Decoction may enhance the effect of the whole prescription through intervention on the coagulation system.

Meanwhile, glycyrrhizic acid, an indispensable compound in the whole recipe of Ma Xing Shi Gan Decoction, is a compound with a glucocorticoid-like effect [52]. Clinical data shown that glycyrrhizic acid has excellent anti-inflammatory, anti-oxidant and other suppressive immune response [53]. However, on the other hand, it may exerts side effects with potent cytotoxicity and bone damage characteristics [54]. This suggests that the therapeutic window of glycyrrhizic acid may be narrow. However, the co-decoction of Ma Xing Shi Gan

Decoction lead to the controlled content of glycyrrhizic acid within a suitable dose range to exert anti-inflammatory effects without significant side effects. Due to limitation of time, whether glycyrrhizic acid and other major constituents of QFPD regulated other nodes and pathways in COVID-19 disease model are not fully investigated. Further research on the synergistic and additive effects of those compounds in QFPD will enhance our understanding on its pharmacological mechanism against COVID-19.

5. Conclusion

In this study, we illustrated the major chemical constituents as well as their potential targets of QFPD for treating COVID-19. By establishing a COVID-19 disease network and enriching the key nodes and pathways regulated by the active ingredients of QFPD, we found that Toll-like receptor signaling pathway play important role in pharmacological effects of QFPD. Interestingly, we found that Ma Xing Shi Gan Decoction, the core component of whole recipe may have a major contribution to the overall efficacy of QFPD. Further results of chemical analysis suggested that the contents of ephedrine and glycyrrhizic acid changed after decoction. Transcriptomic analysis of effects of Ma Xing Shi Gan Decoction in a rat model of pneumonia suggested that it regulated the coagulation system in the inflammatory state, which benefits QFPD to intervene the inflammatory storm caused by COVID-19. Our research provides an experimental and computational basis and research ideas for further discovery of TCM formula and botanical drug for the treatment of COVID-19.

Declaration of Competing Interest

The authors declare there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Abstract

Background: In January, national guidelines were developed and recommended for use throughout China to fight coronavirus disease 2019 (COVID-19). Chinese herbal medicine (CHM) was also included as part of the treatment plans at various stages of COVID-19. **Methods:** We conducted a pilot randomized, controlled trial in patients with severe COVID-19 in Wuhan, China. Eligible adult patients were randomly assigned in a 2:1 ratio to receive either CHM plus standard care or standard care alone for 7 days. The primary outcome was the change in the disease severity category of COVID-19 after treatment. **Results:** Between Jan 31, 2020, and Feb 19, 2020, 42 out of 100 screened patients were included in the trial: 28 in the CHM plus standard care group and 14 in the standard care alone group. Among 42 participants who were randomized (mean [SD] age 60.43 years [12.69 years]), 21 (21/42, 50%) were aged ≥65 years, 35 (35/42, 83%) were women, and 42 (42/42, 100%) had data available for the primary outcome. For the primary outcome, one patient from each group died during treatment; the odds of a shift toward death was lower in the CHM plus group than in the standard care alone group (common OR 0.59, 95% CI 0.148-2.352, *P* = 0.454). Three (two from the CHM plus group and one from the standard care alone group) patients progressed from severe to critical illness. After treatment, mild, moderate, and severe COVID-19 disease accounted for 17.86% (5/28) vs. 14.29% (2/28), 71.43% (20/28) vs. 64.29% (9/28), and 0% (0) vs. 7.14% (1/28) of the patients treated with CHM plus standard care vs. standard care alone. **Conclusions:** For the first time, the G-CHAMPS trial provided valuable information for the national guideline-based CHM treatment of hospitalized patients with severe COVID-19. The effects of CHM in COVID-19 may be clinically important and warrant further consideration and studies. **Clinical Trial** Registration: http://www.chictr.org.cn/index.aspx. Uniqueidentifier: ChiCTR2000029418.

Introduction

Approximately 14–16% patients with Coronavirus disease 2019 (COVID-19) suffer from severe diseases like pneumonia, and 5% become critically ill (1, 2). The mortality rate of COVID-19 among those suffering critical illness was reported to be over 50% (2). At present, effective antiviral treatment for COVID-19 is still lacking. Because of continuous widespread and increasing casualties, researchers are racing to find treatments that may speed recovery and lower mortality in COVID-19. The use of Chinese herbal medicine (CHM), such as the classic formula maxingshigantang, yinqiaosan, dayuanyin, xiaochaihutang, et al., in epidemics has a history of thousands of years in China. For example, the use of herbal medicine in malaria ultimately led to the discovery of Artemisinin, an herbal extract from Artemisia annua used as part of the standard treatment worldwide for P. falciparum malaria (3). The herbal formula maxingshigan–yinqiaosan was found to speed fever resolution similarly to oseltamavir for mild H1N1 infection (4). Although showing no mortality benefits, CHM in combination with conventional care might have facilitated pulmonary infiltrate resolution and improved symptoms and quality of life in patients with severe acute respiratory syndrome in the 2002 SARS epidemic (5).

The National Health Commission and the National Administration of Traditional Chinese Medicine of the People's Republic of China developed clinical guidelines for the management of COVID-19 (NHC-NATCM-China guidelines) (6, 7). In these guidelines, CHM was included as part of the treatment plans for severe COVID-19. These recommendations were developed by the consensus of experts. We thus conducted this pilot randomized clinical trial (RCT) to test the potential effectiveness of the guideline-based CHM treatment for severe COVID-19 in Wuhan, China.

Methods

Study Design

This was an open-label, pilot, randomized trial for severe COVID-19. The trial was approved by the ethics committee at Dongzhimen Hospital (No. DZMEC-KY-2020-09). The trial was registered at the Chinese Clinical Trial Registry (ChiCTR2000029418). The trial protocol and protocol amendments are provided in Appendices 1-3.

Patient Enrollment

Patients were screened for eligibility for the G-CHAMPS trial upon admission. During the ongoing epidemic of COVID-19 in Wuhan, China, patients with a confirmatory diagnosis of COVID-19 were directly admitted or transferred to designated COVID-19 hospitals. By Jan 27, 2020, the Chinese government had designated over 40 hospitals for the treatment of COVID-19 in Wuhan. Hubei Provincial Hospital of Integrated Chinese and Western Medicine is one of the hospitals designated by the government for the treatment of COVID-19. Inclusion criteria comprised: adult patients (≥18 years), positive test result for SARS-CoV-2 on a polymerase-chain-reaction (PCR) assay, respiratory rate (RR) ≥30/min or SaO2 ≤ 93% or a PaO2/FiO2 ratio ≤ 300 mmHg (7), and able to provide informed consent. Patients were excluded if known life expectancy was 48 h or less, on home oxygen at baseline, pregnant or lactating, diagnosed with end-stage diseases, or having used immunosuppressants for 6 months or longer. Eligible patients were provided with information about the trial orally and given the opportunity to ask questions. Patients who were willing to take part in the trial were invited for an interview to gather necessary information, including verbal consent; the audio of the interview was electronically recorded.

Randomization and Masking

Eligible participants were randomized in a 2:1 ratio to the CHM plus standard care (CHM plus) group or the standard care alone group using a simple random allocation method. Allocation was concealed from laboratory personnel and outcome assessors.

Procedures

Per NHC-NATCM-China guidelines, all patients received standard care, which included hemodynamic monitoring, laboratory testing, supplementary oxygen, intravenous fluids, and routine pharmaceutical medications and other medical care when deemed appropriate by on-duty physicians. Oral ribavirin/arbidole (not remdesivir) was part of the standard care in China (Appendix 1). Per the NHC-NATCM-China guidelines, patients in the CHM plus group also received CHM within 12 h after randomization (Appendix 1); all interventions were in line with updated NHC-NATCM-China guidelines. The herbal formulas were supplied by Jiangyin Tianjiang Pharmaceutical Co., Ltd. The quality of the herbs was in accordance with the 2015 Chinese Pharmacopeia (8). All herbs were tested for heavy metals, microbial contamination, and residual pesticides to ensure that they met the safety standards in China prior to use. Trained and experienced technicians prepared the decoction from the formulas according to a standardized procedure; each unit of formula yielded 400 mL of decoction, divided into two equal portions. Nurses administered 200 mL of the decoction to patients orally (via feeding tube if needed) twice daily for a total of 7 days in the CHM plus group. Data were retrieved from electronic medical records using the standardized case record forms created by members of the ISARIC (9) (International Severe Acute Respiratory and Emerging Infection Consortium) in collaboration with the World Health Organization.

Outcomes

The primary outcome was the change in the disease severity category of COVID-19 after treatment. The severity of COVID-19 was assessed based on the Six-Point Clinical Status Scale for COVID-19 (COVID-19 severity scale) (Box 1). The Six-Point Clinical Status Scale for COVID-19 was defined according to NHC-NATCM-China guideline and WHO R&D Blueprint. An independent clinical event adjudication committee (CEAC) performed the final outcome assessment based on the pre-specified criteria. Secondary outcomes included the overall survival through last day of treatment, the proportion of patients without improvement (scored 3–5 on the COVID-19 severity scale), the change in serum procalcitonin level after treatment, and the prevalence of antibiotic use during treatment.

0	Hospital discharge or meets discharge criteria	Discharge criteria are defined as: 1 Normal body temperature for more than 3 days; 2 Significantly improved respiratory symptoms: no oxygen supplementation requirement, stable and normal vital signs for longer than 1 day; 3 Lung imaging shows obvious absorption and resolution of acute infiltrates; 4 Negative results of the nucleic acid test for SARS-CoV-2 two times consecutively, with at least a 1-day interval between tests.
1	Mild	Improving and/or mild clinical symptoms and no pneumonia changes in radiological imaging studies.
2	Moderate	Active symptoms like fever and respiratory tract symptoms and pulmonary infiltrates seen in imaging.
3	Severe	Meeting any of the following: 1 Respiratory distress, RR $\geq$30 breaths/min; 2 Pulse oximetry (SpO_2) $\leq$93% on room air at rest state; 3 Arterial partial pressure of oxygen (PaO_2)/oxygen concentration (FiO_2) $\leq$300 mmHg
4	Critical illness	Meeting any of the following: 1 Mechanical ventilation; 2 Shock; 3 Other organ failure complications that require intensive care unit care
5	Death	

Statistical Analysis

Since this is a pilot randomized trial, sample size calculation was not performed. For pharmaceutical interventions, a minimum sample size of 12 per group was usually recommended as a rule of thumb for a

pilot study (10). Considering a dropout rate of 10%, we aimed to recruit a total sample size of 42 patients (standard care group, *n* = 14; CHM plus group, *n* = 28).

We compared the severity of COVID-19 with ordinal logistic regression (shift analysis). The proportion of patients without clinical improvement after treatment was assessed using the generalized linear model. Laboratory findings were evaluated using the Wilcoxon rank-sum test. Hodges–Lehmann estimates of location shift and 95% CIs are presented.

All outcomes were assessed in the intention-to-treat population with no imputation for missing data. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc), with a 2-sided $p < 0.05$ considered significant.

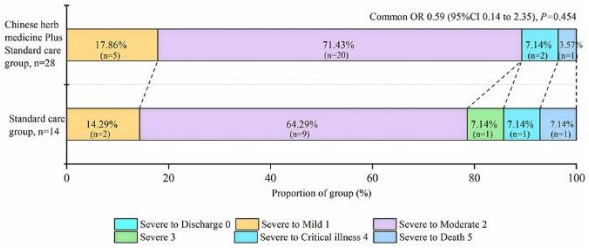
Results

Forty-two out of 100 screened patients were included in the trial (Appendix Figure 1). The two groups were generally well-balanced at baseline, although older patients and more women were enrolled in the CHM plus group than in the standard care alone group (Table 1). Based on symptom-based syndrome differentiation using CHM principles, the included patients in the CHM plus group were divided into the following two syndromes: Lung Blocked by Epidemic Toxin and Inner Blocking Causing Collapse. Correspondingly, the modified formula of maxinshigan–dayuanyin was used in the former, and the shengfutang formula was used in the later syndrome. Lung Blocked by Epidemic Toxin syndrome was found in 20 patients (20/28, 71.43%) and Inner Blocking Causing Collapse in eight patients (8/28, 28.57%) in the CHM plus group. During the G-CHAMPS trial, supportive measures of standard care were similar in the two groups (Appendix 1).

	CHM plus standard care ($n = 28$)	Standard care ($n = 14$)
Characteristics		
Age,-yr	65 (53.5–69)	59 (47–67)
Age ≥65 yr, - no. (%)	16 (57)	5 (36)
Age <65 yr, -no. (%)	12 (43)	9 (64)
Sex, no. (%)		
Men	2 (7)	4 (29)
Women	25 (93)	10 (71)
Current smoker, no. (%)	0	0
Heart rate, per min	89 (70–92.5)	97 (90–105)
Blood pressure, mm Hg		
Systolic pressure, mm Hg	129 (110–140)	115.5 (110–119)
Diastolic pressure, mm Hg	85 (74.5–90)	80.5 (75–90)
Body temperature, °C	37 (36.6–37.1)	36.4 (36.2–37)
Respiratory rate >24 breaths, per min	28 (100)	14 (100)
SaO_2	89 (86–90.5)	89 (87–90)
Transfer from other hospitals-no. (%)	2 (7.41)	4 (28.57)
Onset of symptoms to hospital admission, days	9 (6.5–11.5)	9.5 (6–14)
Hospital admission to randomization, days	1 (0.5–2)	0.5 (0–1)
Any Comorbidity-no. (%)		
Chronic heart disease, including congenital heart disease (except hypertension)	8 (28.57)	3 (21)
Chronic lung disease (except asthma)	2 (7.14)	2 (14)
Asthma	1 (3.57)	0
Mild liver disease	3 (10.71)	2 (14)
Chronic nervous system diseases	2 (7.14)	0
Malignant tumor	0	1 (7.14)
Diabetes without complications	1 (3.57)	3 (21.43)
Hypertension	12 (42.86)	7 (50.00)
Hyperthyroidism	0	1 (7.14)
Presenting Symptoms and Signs-no. (%)		
History of fever*	27 (96)	9 (75)
Cough	23 (82)	12 (86)
Sputum	10 (36)	4 (29)
Sore throat	1 (4)	0
Rhinorrhea	0	1 (7)
Loss of appetite	25 (89)	12 (86)
Insomnia	20 (71)	10 (71)
Wheezing	5 (18)	1 (7)
Chest pain	2 (7)	1 (7)
Muscle pain	8 (29)	6 (43)
Arthralgia	0	1 (7)
Fatigue	26 (93)	14 (100)
Shortness of breath (dyspnea)	5 (18)	5 (36)
Headache	2 (7)	1 (7)
Vomiting/nausea	6 (21)	1 (7)
Diarrhea	3 (11)	3 (21)
Chest x-ray and CT Findings**		
Ground-glass opacity	15 (79)	7 (78)
Local patchy shadowing	0	1 (11)
Bilateral patchy shadowing	4 (21)	1 (11)

*CHM= Chinese herbal medicine. Data are presented as median (IQR) unless otherwise indicated. *Two participants in the standard care group had no baseline record of fever. **Chest x-ray and CT findings (standard of care plus CHM, n = 19; standard care group, n = 9). Transfer here was considered as new admission in this trial.*

For the primary outcome, one patient from each group died during the first 3 days of treatment; the odds of a shift toward death was lower in the CHM plus group than in the standard care group (common OR 0.589, 95% CI 0.148–2.352 P = 0.454; Figure 1). The results for the changes shown by imaging studies are listed in Table 2. For secondary outcomes, 11% (3/28) of patients in the CHM plus group and 21% (3/14) of patients in the standard care alone group had no clinical improvement (difference −10.71 (−35.07 to 13.64), P = 0.350) after treatment. More secondary outcomes and safety outcomes are provided in Appendix



Tables 1–5.

Chest X-ray and CT findings, *n* (%)	CHM plus standard care (*n* = 28)	Standard care (*n* = 14)
No pneumonia change	2(8.7)	0
Pneumonia change	21(91.3)	12(100)
Missing data	5	2

Discussion

To our best knowledge, this is the first prospective randomized trial to investigate the effect of NHC-NATCM-China guideline-based CHM in patients with severe COVID-19. In this trial, the odds of a shift toward death or critical illness at 7 days after treatment was lower in the CHM plus group at a non-significant level. The result was collaborated with the universal normalization or near normalization of leukocytes and different inflammatory markers. In a retrospective study with data of 1,099 patients with COVID-19, 5% (55/1,099) of the patients were admitted to the ICU, 2% (25/1,099) underwent invasive mechanical ventilation, and 1% (15/1,099) died, whereas the composite of these endpoints occurred in 25% of the patients with severe disease (11). In our trial, 12% (5/42) of the patients with severe COVID-19 required ICU care, and 5% (2/42) died within 7 days. That retrospective study collected data from 30 provinces around China, while our trial data are from Wuhan. Disease severity is an important factor when considering treatment for COVID-19 and likely contributed to the differences between these two studies. An ongoing trial of Gilead Sciences' Remdesivir utilized a category ordinal scale to define its primary outcome (NCT04257656).

Although COVID-19 is caused by a virus and will heal without treatment in the majority of patients, most patients in the G-CHAMPS trial received antibiotics. The percentages of antibiotic use are comparable to the previous study (80%) (11).

Animal studies found that the Chinese herbal medicine maxingshigan could decreased lung cell apoptosis and reduced the serum content of TNF-α in acute lung injury from H1N1 infection (12). During the 2002 SARS outbreak, Poon et al. (13) found that herbal medicine had immunomodulating effects in regulating the subgroups of T lymphocytes. Changes in the inflammatory markers seem to aid the hypothesis of a lung-protective effect of CHM in COVID-19. These results of the present trial of CHM in COVID-19 were consistent with previous findings that CHM like maxingshigan can speed up patient recovery in respiratory epidemics (4).

Our study has several limitations, including an open-label design and a small sample size. As with other small studies, a natural manifestation of disease development may influence clinical outcome despite close monitoring. Additionally, this study lacks long-term outcomes, and the COVID-19 disease severity scale deserves further investigation. There is nothing wrong with conducting a well-designed small trial, it just needs to be interpreted carefully. Despite these substantial limitations, the G-CHAMPS trial provided an important opportunity to better understand the use of CHM for severe COVID-19.

For the first time, the G-CHAMPS trial provided valuable information for national guideline-based CHM treatment for hospitalized patients with severe COVID-19. As effective antiviral treatment is still lacking for COVID-19, and SARS-CoV-2 continues to spread outside of China (14), all potentially effective treatments, including CHMs, are worth vigorous further investigation. Adequately powered clinical trials of CHMs are needed to further assess their efficacy and safety for the treatment of severely ill hospitalized COVID-19 patients.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author on reasonable request. Participant data without names and identifiers will be made available after approval from the corresponding author and the National Health Commission. After publication of the study findings, the data will be available to others on request. The research team will provide an email address for communication once the data are approved to be shared with others. A proposal with detailed description of study objectives and statistical analysis plan will be needed for evaluation of the reasonableness of the request for our data. The corresponding author and the National Health Commission will make a decision based on these materials. Additional materials may also be required during the process.

Ethics Statement

The studies involving human participants were reviewed and approved by ethics committee of Dongzhimen Hospital. Patients who were willing to take part in the trial were invited for an interview to gather necessary information, including verbal consent; the audio of the interview was electronically recorded.

Author Contributions

YY, CA, and HS: concept and design. TL, XH, YZ, TW, JD, XG, WH, CJ, DJ, HW, WX, and ZZ: acquisition, analysis, or interpretation of data. CZ and YL: drafting protocol. CZ, YL, KZ, and HS: drafting of the manuscript. CZ, KZ, YL, and HS: critical revision of the manuscript for important intellectual content. YL: statistical analysis. All authors: final approval of the version to be published and dedicated large amounts of time to the study, in the hope of improving care for patients during COVID-19 outbreak. GT, XZ, XW, YC, HD, JZ, and XZ: administrative, technical, or material support. YY: agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately

investigated and resolved. In addition to the core writing group, the group members also contributed substantively to the conduct of the G-CHAMPS trial. These authors contributed equally to this work (Appendix 4 the G-CHAMPS collaborative group). All members: read and approved the final report. All authors agree, as the G-CHAMPS group members, to submit this article.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00256/full#supplementary-material

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Abstract

Background: The novel coronavirus named COVID-19, which originated in Wuhan, China, has spread to many countries around the world. Currently, no effective medical treatment exists to combat this disease. Traditional Chinese herbal medicines (CHM) have unique roles in the treatment of viral infections. In this article we analyzed the effectiveness and possible molecular mechanisms of CHM formulas for the prevention of COVID-19.

Methods: The active ingredients and action targets of CHM formulas were obtained from the TCMSP database. Genes related to severe acute respiratory syndromes (SARS) and Middle East respiratory syndrome (MERS) were queried on the GeneCards database. The action mechanisms of these genes were predicted using a Gene Ontology (GO)-based functional enrichment and annotation tool and the Kyoto Encyclopedia of Genes and Genomes (KEGG).

Results: CHM formulas played a positive role in preventing COVID-19 and warrant further application.

Conclusions: Our research provides new evidence to support the possible value of CHM formulas for the prevention of COVID-19. However, further clinical studies with large sample sizes are required to verify their effectiveness.

Keywords: COVID-19; Novel coronavirus; network pharmacology; traditional Chinese herbal medicines (traditional CHM).

Introduction

As 2019 drew to a close, several cases of pneumonia of unknown etiology were reported in Wuhan, the capital of Hubei province in China (1). Up to now, the outbreak of COVID-19, caused by a new coronavirus, Genes related to severe acute respiratory syndromes (SARS) and Middle East respiratory syndrome (MERS) were queried in the GeneCards database (2). As of February 20, 2020, based on data released by China CDC (http://2019ncov.chinacdc.cn/nCoV/), the total number of confirmed cases was 74,680, with 2,122 of these cases resulting in death. At present, no drug exists to effectively treat or prevent COVID-19 (3). In addition, immune response to the new coronavirus differs according to the individual, leading to a variety of clinical symptoms, disease course, and treatment response to drugs and vaccines (4). Based on the *National Health Commission of China Diagnosis and Treatment Protocol for COVID-19 Infection* (Sixth Edition) as well as the experience of managing the virus in Hubei province (especially in Wuhan), the *Hubei Province Diagnosis and Treatment Protocol for COVID-19* has been developed and released. In our current study, we analyzed data relevant to exploring the effectiveness and possible action mechanism of traditional Chinese herbal medicine (CHM) formulas for the prevention and treatment of the novel coronavirus.

MethodsOther Section

Collection of active ingredients

Two CHM formulas were obtained from the *Hubei Province Diagnosis and Treatment Protocol for COVID-19*: Formula A: Rhizoma Atractylodis, Flos Lonicerae, Pericarpium Citri Reticulatae, Rhizoma Phragmitis, Folium Mori, and Radix Astragali seu Hedysari; and Formula B: Radix Astragali seu Hedysari, Rhizoma Atractylodis Macrocephalae, Radix Saposhnikoviae, Cyrtomium fortunei J. Sm., Flos Lonicerae, Eupatorium fortunei Turcz., and Pericarpium Citri Reticulatae.

Screening for active ingredients and target genes

Oral bioavailability (OB) reflects the rate of absorption of an orally administered drug that enters the circulation via the liver after absorption into the gastrointestinal tract. Drug-likeness (DL) refers to the structural similarity of herbal ingredients to a known drug. There is a positive correlation between these two descriptors. With the help of the TCMSP data platform (5) (http://tcmspw.com/tcmsp.php), the chemical constituents in the compounds in Formulas A and B that had OB ≥30% and DL ≥0.18 were retrieved as the active ingredients in the study. A high OB value often reflects better DL (6).

Screening of disease targets

No data on COVID-19-related genes were available in the GeneCards (https://www.genecards.org/) database at the time of study. Since the new coronavirus is highly similar to SARS-CoV and MERS-CoV (7,8), especially the bat SARS-like coronavirus (Genbank accession number MG772933), “Severe Acute Respiratory Syndromes” (SARS) and “Middle East Respiratory Syndrome” (MERS) were used in our study to search for genes that may be associated with the new coronavirus.

Screening of COVID-19-related genes acted by the active CHM ingredients

A Venn diagram was created to visualize the amount of overlap between the genes related to the active CHM ingredients and COVID-19-related genes.

Construction of protein-protein interaction (PPI) networks and screening of its core network

The protein-protein interaction core network (PPICN) refers to the correlation between compounds and disease-related protein molecules, taking into account biochemistry, signal transduction, and genetic networks. The obtained intersection genes were uploaded onto STRING11.0 (http://string-db.org/cgi/input.pl) to obtain the relationships of PPIs.

Comparison and analysis of target pathways

We analyzed the core genes screened out from the two formulas, as well as their roles in signaling pathways, to explore their functions. To achieve this, a Gene Ontology (GO)-based functional enrichment and annotation tool and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were used. Data were obtained from the website (http://bioconductor.org/biocLite.R) and the results were visualized with Rstudio.

Results

Active compounds

Some of the compounds (i.e., those with the 10 highest OB values) in these two formulas were retrieved through the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). OB ≥30% and DL ≥0.18 served as the criteria (*Tables 1,2*).

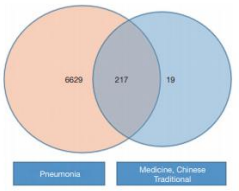
Table 1 Basic information of some active compounds in Formula A

No.	Chemical composition	Relative molecular weight	OB%
MOL000379	9,10-dimethoxypterocarpan-3-O-b-D-glucoside	462.49	0.92
MOL000729	Oxysanguinarine	347.34	0.87
MOL000211	Mairin	456.78	0.78
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	428.82	0.78
MOL000184	NSC63551	412.77	0.76
MOL000186	Stigmasterol 3-O-beta-D-glucopyranoside_qt	412.77	0.76
MOL000092	Daucosterin_qt	414.79	0.76
MOL000094	Daucosterol_qt	414.79	0.76
MOL003036	ZINC03978781	412.77	0.76
MOL000449	Stigmasterol	412.77	0.76

Table 2 Basic information of some active compounds in Formula B

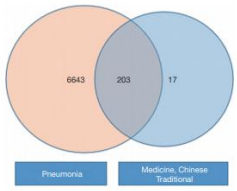
No.	Chemical composition	Relative molecular weight	OB%
MOL000379	9,10-dimethoxypterocarpan-3-O-b-D-glucoside	462.49	0.92
MOL000592	Dammaradienyl acetate	468.84	0.82
MOL000211	Mairin	456.78	0.78
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	428.82	0.78
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	428.82	0.78
MOL000028	β-Amyrins	426.8	0.76
MOL003036	ZINC03978781	412.77	0.76
MOL000449	Stigmasterol	412.77	0.76
MOL000449	Stigmasterol	412.77	0.76
MOL000296	Hederagenin	414.79	0.75

OB, oral bioavailability.



Prescription 1

Figure 1 The intersection generated between genes related to the effects of the active ingredients of Formula A and COVID-19-associated genes.



Prescription 2

Figure 2 The intersection generated between genes related to the effects of the active ingredients of Formula B and COVID-19-associated genes.

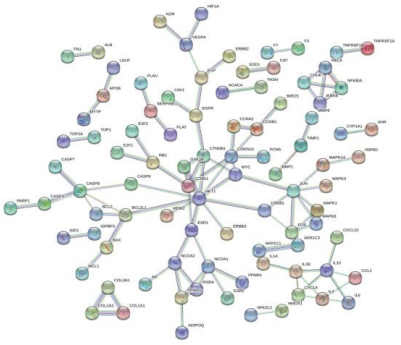


Figure 3 The PPICN between Formula A and COVID-19. Each node represents a protein and the connections represent the interaction between two proteins. A thicker connection represents higher correlation. PPICN, protein-protein interaction core network.

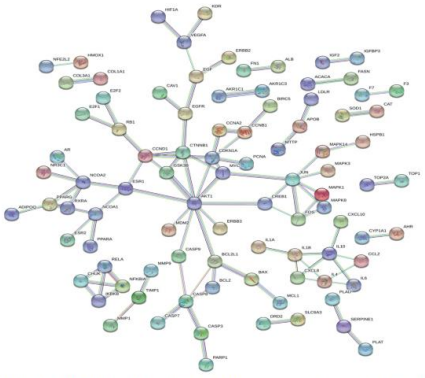


Figure 4 The PPICN between Formula B and COVID-19. Each node represents a protein and the connections represent the interaction between two proteins. A thicker connection represents higher correlation. PPICN, protein-protein interaction core network.

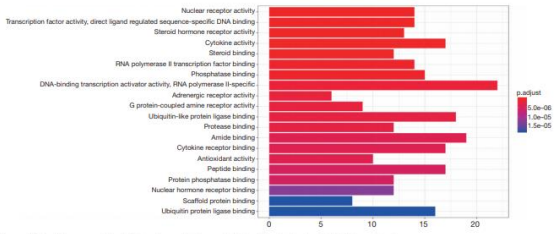


Figure 5 The 20 most enriched GO pathways in Formula A (ranking by P value). GO, Gene Ontology.

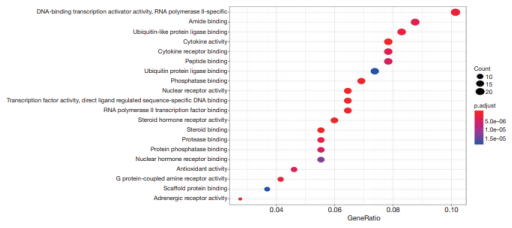


Figure 6 The 20 most enriched GO pathways in Formula A (ranking by number of enriched genes). GO, Gene Ontology.

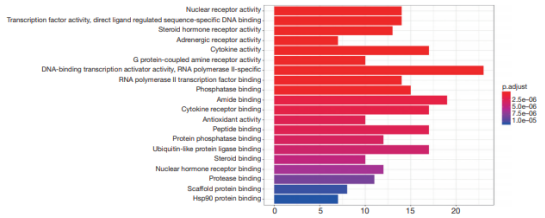


Figure 7 The 20 most enriched GO pathways in Formula B (ranking by P value). GO, Gene Ontology.

Core action genes in the CHM formulas

Aided by the TCMSP database, the genes linked to the effects of the active ingredients in these two CHM formulas were obtained. Genes related to COVID-19 were retrieved from the GeneCard database using the keywords “SARS” and “MERS”. Gene intersections were generated based on the intersection results from these two groups (*Figures 1,2*).

Construction and screening of PPICN between CHM formulas and COVID-19

The 217 and 203 intersected genes were analyzed using the STRING software, with a coefficient of 0.990 indicating correlation. The PPICN (PPI correlation network) of Formula A contained 217 nodes and 99 connections, with the average degree of nodes being 0.912. Formula B contained 203 nodes and 98 connections, with the average degree of nodes being 0.314 (*Figures 3,4*).

Target pathways

GO-based functional enrichment and annotation of Formula A yielded 192 GO entries (P<0.05). The 20 pathways with the highest GO enrichment are shown in *Figures 5* and *6*.

GO-based functional enrichment and annotation of Formula A yielded 192 GO entries (P<0.05). Twenty pathways with highest GO enrichment are shown in *Figures 7* and *8*. The KEGG pathways of Formulas A and B were enriched and screened to obtain the 20 most enriched signal pathways (P<0.05), including the hepatitis B pathway, Kaposi sarcoma-associated herpesvirus (KSHV) infection-related pathways, and human cytomegalovirus infection-related pathways (*Figures 9-12*).

Discussion

In the absence of drugs to effectively treat COVID-19 infection, CHM offers unique antiviral benefits. For instance, astragalus polysaccharide at a non-cytotoxic concentration (30 μg/mL) significantly suppresses the expressions of two early viral proteins (Zta and Rta) in the Epstein-Barr virus lytic cycle to exert an antiviral effect (9). CHM can directly inhibit respiratory pathogens or coordinate immune system activity to prevent or alleviate respiratory infections (10). A combination of traditional Chinese and Western medicine was capable of promoting the absorption of pulmonary infiltrates in SARS patients (11). In addition to their antiviral benefits, CHMs can also be applied at the rehabilitative stage (12). Therefore, CHM may be effective for the treatment of COVID-19 (13).

By using the technology offered by network pharmacology, we analyzed the molecular mechanisms of two CHM formulas for the prevention of COVID-19. Although different herbal drugs were used in these two formulas, the results of the PPI networks, GO-based enrichment analysis, and KEGG enrichment analysis were fairly similar, which is not coincidental.

The internal regulation of the body involves a complex regulatory network rather than a single signaling pathway. Signal transduction exists among different signaling pathways and targets; therefore, the therapeutic effects of drugs are not just a result of direct targeting. Instead, they more commonly directly regulate the target while indirectly regulating other targets. Data on PPIs facilitate our understanding of the regulatory roles of targets. Akt, as the core target of CTHM formulas, plays an important role in containing coronavirus infections. In SARS patients, the phosphorylation level of the cell survival protein Akt is downregulated in cells expressing M protein. Meanwhile, the overexpression of 3-phosphoinositide-dependent protein kinase-1 (PDK1), an upstream kinase for Akt, inhibits M protein-induced apoptosis, indicating that M-protein perturbs the PDK1 and PKB/Akt cell survival signaling pathway (14). Through Akt activation, which inhibits apoptosis, CHM formulas can suppress viral replication (15), reduce apoptosis, and thereby repair damage to the body. Based on PPI networks, we can further investigate the targets via which

CHM formulas affect the occurrence and development of COVID-19, map the PPI target networks, and thus pave the way for further network analysis and investigation of their underlying mechanisms.

In the GO-based enrichment analyses, the most enriched biological processes of these two CHM formulas included nuclear receptor activity, transcription factor activity, and direct ligand regulated sequence-specific DNA binding pathways. Nuclear receptors (NRs) are a superfamily of ligand-dependent transcription factors that regulate a variety of biological processes including growth and development, metabolism, and inflammation (16,17). After coronavirus infection, some of the NR-encoded proteins (e.g., nsp1) can inhibit the translation of the host without seriously affecting the expressions of viral genes (18). Fortunately, the targets of CHM formulas are enriched in NR activity, which, to a certain extent, can alleviate the damage caused by coronavirus infection. However, whether viral replication can be suppressed via the activation of coronavirus membrane fusion, which takes place through a receptor-driven ratcheting mechanism, remains unclear and further verification is required (19).

The KEGG analysis showed that a vast majority of the most enriched pathways in these two formulas were associated with viral infections. These pathways included the cytomegalovirus infection, hepatitis B virus infection, and P13K/Akt signaling pathways. Notably, the P13K/Akt pathway serves as a central regulator of many important processes that control translation, metabolism, and apoptosis. Active PI3K/Akt signals can meet the needs of replication for many viruses. When the “proviral” kinase is activated, it is also involved in the host’s response to viral infection and ultimately inhibits viral replication (20).

Limitations and prospects

CHMs have been widely recognized not only for their multiple biologically active ingredients and multiple pharmacological activities, but also their production of other biologically active or inactive metabolites when delivered *in vivo*. Therefore, it is difficult to determine whether the antiviral activity of a CHM is a consequence of a single and precise action mechanism or a result of a synergistic therapeutic effect. Furthermore, the medicinal properties or toxicities of CHM may be influenced by many other factors, including the methods of processing, combining, and frying involved with some medicines (21,22).

In summary, a network pharmacology method was used in our current study to investigate the effectiveness of CHM for COVID-19. Although the results were promising, more clinical trials are warranted for our findings to be confirmed.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Abstract

The global outbreak of coronavirus disease 2019(COVID-19) has further spread, and there is an increasing number of confirmed cases in many countries. On February 28, 2020 of Geneva time, the World Health Organization has raised global risk level to the very high level in view of outbreak of COVID-19. Since some patients' condition appeared to deteriorate rapidly after infection of this 2019 novel coronavirus(2019-nCoV), a variety of treatments should be considered. Holistic view and syndrome differentiation are the two characteristics of traditional Chinese medicine(TCM). Therefore, under the guidance of the holistic view, syndrome diffe-rentiation of TCM has achieved good effects in the treatment of COVID-19. This treatment mainly aimed at eliminating pathogens and strengthening overall health, regulating the balance of body and coordinating various of functions of Zangfu organs. In addition, modern medical proposes host-directed therapy(HDT), a strategy aims to interfere with host cell mechanism, enhance immune responses, and reduce exacerbated inflammation. To some extent, the combined application of HDT and antiviral therapy is highly consistent with the therapeutic concept of the holistic view of TCM. Therefore, under the guidance of the holistic view, syndrome differentiation of TCM uses treatments, such as clearing heat, detoxification, relieving asthma, clearing damp and phlegm, together with Lianhua Qingwen Capsules, Maxing Shigan Decoction, and Haoqin Qingdan Decoction under the guidance of these therapeutic methods. These therapeutic methods and prescriptions intervened with both virus and host at the same time in the treatment of COVID-19, which has important implications for the effective clinical treatment of COVID-19.

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JIM-02-2020-OA-ER-0077 In silico screening of Chinese herbal medicines with the potential to directly inhibit 2019 novel coronavirus

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ABSTRACT

Objective: In this study we execute a rational screen to identify Chinese medical herbs that are commonly used in treating viral respiratory infections and also contain compounds that might directly inhibit 2019 novel coronavirus (2019-nCoV), an ongoing novel coronavirus that causes pneumonia. Methods: There were two main steps in the screening process. In the first step we conducted a literature search for natural compounds that had been biologically confirmed as against sever acute respiratory syndrome coronavirus or Middle East respiratory syndrome coronavirus. Resulting compounds were cross-checked for listing in the Traditional Chinese Medicine Systems Pharmacology Database. Compounds meeting both requirements were subjected to absorption, distribution, metabolism and excretion (ADME) evaluation to verify that oral administration would be effective. Next, a docking analysis was used to test whether the compound had the potential for direct 2019-nCoV interaction. In the second step we searched Chinese herbal databases to identify treatments containing the selected compounds. Plants containing 2 or more of the compounds identified in our screen were then checked against the catalogue for classic herbal usage. Finally, network pharmacology analysis was used to predict the general in vivo effects of each selected herb. Results: Of the natural compounds screened, 13 that exist in traditional Chinese medicines were also found to have potential anti-2019-nCoV activity. Further, 125 Chinese herbs were found to contain 2 or more of these 13 compounds. Of these 125 herbs, 26

are classically catalogued as treating viral respiratory infections. Network pharmacology analysis predicted that the general in vivo roles of these 26 treatments were related to regulating viral infection, immune/inflammation reactions and hypoxia response. Conclusion: Chinese herbal treatments classically used for treating viral respiratory infection might contain direct anti-2019-nCoV compounds.

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Correspondence: Prof. Deng-hai Zhang; E-mail address: shanghai_zhang@hotmail.com 1. Introduction

Toward the end of December 2019, a novel coronavirus (2019-nCoV) with human-to-human transmission and severe human infection, originating in Wuhan, China, was identified [1]. This virus has affected many persons in China and spread to other countries in a very short time. On January 30, 2020, the Director-General of the World Health Organization declared that the outbreak of 2019-nCoV constitutes a public health emergency of international concern and issued temporary recommendations under the International Health Regulations [2]. According to the Daily Report of China National Health Commission, as of this writing on February 2, 2020, 14,488 cases, including 304 deaths, have been confirmed in China; 146 cases, including 1 death, have also been reported among 23 other countries. This pandemic is still ongoing, so it is urgent to find new preventive and therapeutic agents as soon as possible. In addition, commensurate with the risk, strong measures for early detection, isolation and treatment of cases, as well as minimization of transmission through social interaction must be implemented.

While specific vaccines and antiviral agents are the most effective methods to prevent and treat viral infection, there are not yet effective treatments that target the 2019-nCoV. Development of this treatment may require months or years, meaning that a more immediate treatment or control mechanism should be found if possible. Herbs used in traditional Chinese medicine present a potentially valuable resource to this end. The effectiveness of herbal treatment to control contagious disease was demonstrated during the 2003 severe acute respiratory syndrome (SARS) outbreak [3]. As such, the Chinese government is encouraging the use of herbal plants in fighting this new viral pneumonia. However, the application of herbal treatment is mainly guided by the type of herb (based on the catalogue of classic literature on herbs) and the patient's symptoms or signs. There is often not enough information to predetermine whether the herbs in question can directly target the viral cause, in other words, herbal usage is generally not guided by viral pathology. We think more detailed knowledge about the direct antiviral effects of different plants would be greatly helpful to the doctors selecting them. In fact, after the outbreak of SARS, many groups dedicated themselves to finding anti-coronavirus agents, including some natural compounds that exist in traditional Chinese herbal medicines [4–12]. The coronavirus encodes more than one dozen proteins, some of which are essential to viral entry and replication. Among these proteins, the most well-studied are papain-like protease (PLpro), 3C-like protease (3CLpro) and spike protein. Coronavirus PLpro not only processes the viral polypeptide onto functional proteins but is also a deubiquitinating enzyme that can dampen host anti-viral response by hijacking the ubiquitin (Ub) system. For example, SARS PLpro cleaves ISG15, a two-domain Ub-like protein, and Lys48-linked polyUb chains, releasing diUbLys48 products [13,14]. SARS-3CLpro is a cysteine protease indispensable to the viral life cycle [15]. Coronavirus spike protein uses angiotensin-converting enzyme 2 as a receptor to help the virus enter cells [16]. These three proteins make attractive targets for drug development. Through in silico and biological processing, a series of small molecules, including those from natural compounds, have been screened and confirmed to directly inhibit these important proteins in SARS or Middle East respiratory syndrome (MERS) coronavirus [17–23]. The gene sequence of 2019-nCoV has been released, which suggests high similarities between the main proteins in this virus and those previously identified in SARS-Cov or MERS-Cov [24,25]. In this sense, previously reported anti-SARS-Cov or anti-MERS-Cov natural compounds may

become a valuable guide to finding anti-coronavirus (2019-nCoV) herbal plants among the traditional Chinese herbs used to treat viral pneumonia. It is a challenge to screen out the herbs containing anti-coronavirus (2019-nCoV) compounds from the large number of those possibly being used for patients infected with this pathogen, especially in very short time. Here, we propose two principles to guide such work: oral effectiveness and compatibility. The first principle refers to the fact that most Chinese herbal plants are orally ingested after boiling with water, meaning that the anti-coronavirus (2019-nCoV) ingredients in selected plants should be absorbable via oral preparation. The second principle recognizes that candidate plants should be consistent with the type classifications for traditional herbal usage, since type-guided applications are integral to herbal use, as mentioned above. Following these two principles, we used a 6-step selection process (3 for each principle), including drug-likeness, evaluation of oral bioavailability, molecular docking, network pharmacology analysis and other methods to identify herbs that have both a high possibility of containing effective anti-coronavirus (2019-nCoV) compounds and are classified as treating virus-caused respiratory infection .

2. Materials and methods

2.1. Literature search and compound selection PubMed literature concerning natural compounds against SARS or MERS coronavirus activity was selected using the query “coronavirus AND inhibitor AND (SARS OR MERS OR SARS-CoV OR MERS-CoV).” After careful reading of the studies returned by this search, the natural compounds that had biologically confirmed antiviral activities were compared with the Traditional Chinese Medicine Systems Pharmacology database (TCMSP, http://www.tcmspw.com/browse.php?qc=herbs), the Encyclopedia of Traditional Chinese Medicine (ETCM, http://www.nrc.ac.cn:9090/ETCM/) and SymMap (https://www.symmap.org/). Natural compounds both associated with antiviral activity and contained in herbs were examined in the next step of our study. 2.2. ADME screening of natural compounds Since Chinese herbal treatments are always taken orally after boiling with water, an in silico integrative model of absorption, distribution, metabolism and excretion (ADME) was used to screen for natural compounds that may be bioactive via oral administration. The indices used for the screening include evaluation of oral bioavailability, Caco-2 permeability, drug-like value, and drug half-life. The threshold values indicating effectiveness for these four indices were > 30%, > –0.4, > 0.18 and > 3 h, respectively, as recommended by Hu et al [26]. The values of these four indices can be obtained from the TCMSP database. 2.3. Protein-molecular docking We used molecular docking software AutoDock 4 to perform protein compound docking analysis, according to the following procedure: (1) We built three-dimensional (3D) structure files of the proteins. We used the online server SWISS-MODEL (https://swissmodel.expasy.org/) to build the 3D structures of the proteins of interest by template-based modeling, these template structures being the reported 3D structures of the corresponding proteins from SARS-CoV. The models built were of Protein Data Bank (PDB) format. (2) To retrieve the required 3D structure files of compounds, the structure data file (SDF) format of compounds were retrieved from the PubChem website and then converted to PDB format by Discovery Studio. (3) AutoDock 4.2 was used to prepare PDBQT format files for target and ligand screening (Target.pdbqt and Ligand.pdbqt) and grid and docking parameter files (a.gpf and a.dpf). (4) Molecular docking was performed using AutoDock in Cygwin and finally the results were analyzed. The process and parameters used were detailed by Rizvi et al [27]. 2.4. Plant selection Herbs were selected through three steps. (1) Primary selection: molecules chosen from the above steps were used as input for the TCMSP, ETCM and SymMap to search for plants containing that input and the plants were filtered by the numbers of antiviral compounds they contain. Those containing 2 or more antiviral compounds were selected for the next step. (2) Classic usage catalogue cross-reference: only herbs traditionally used to treat viral respiratory infection were retained for further study. (3) Predication of general effects in vivo with network pharmacology analysis, which is detailed as follows. 2.5. Network pharmacology analysis The TCMSP provided the main components of each herb and the protein targets for each. We identified the reported chemical constituents for each plant in the final analysis and used the ADME indices listed above to find the orally absorbable and drug-like compounds for the plant. The protein targets of these compounds were downloaded from the TCMSP database. All protein targets for each individual plant were

used as input for the String online server (https://string-db.org/) to perform protein-protein interaction analysis and pathway enrichment. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched (with $P < 0.01$) by the input were downloaded.

All data were processed using the statistical language R (3.6.2), unless otherwise specified.

3. Results

3.1. Overlapping of natural compounds biologically confirmed to be anti-SARS or anti-MERS coronavirus in literature and in Chinese herbal database

We received 261 hits from conducting our search in the PubMed database. After careful evaluation of the abstracts from these citations we downloaded and carefully analyzed the full text of 23 highly relevant papers. The natural compounds reported to have biologically confirmed anti-coronavirus activity were identified and then compared to the ingredients listed in TCMSP. The result was 115 overlapping ingredients, which we used for further testing (Fig. 1).

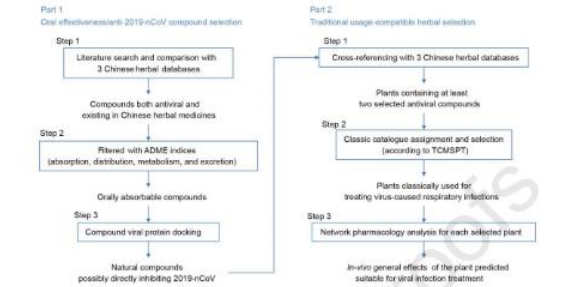


Fig. 1. Workflow scheme. The work is divided into two main parts, natural compound selection and herbal plant selection. Each part consists of three steps. As detailed in the text, oral effectiveness is important in compound selection, while in the plant selection portion, the selected herbs should be compatible with the classic usages of herbal treatment in traditional Chinese medicine. TCMSP: Traditional Chinese Medicine Systems Pharmacology.

3.2. Filtration of compounds selected by ADME

The antiviral activities of the 115 natural compounds were reportedly confirmed with enzyme-based (cell-free) or cell-based experimental systems. To be utilized as a Chinese herbal medicine, they must be absorbable via oral prescription. Therefore, we performed ADME screening for the 115 natural compounds, reducing the number of candidates to 13. 3.3. Docking between selected compounds and their reported targets To perform the docking analysis, the 3D structure files of 2019-nCoV PLpro, 3CLpro and spike proteins were built based on the corresponding SARS-CoV templates, i.e., PDB 5e6j, 1uj1 and 6cad, respectively. Then, molecule-protein docking was carried out between the molecules and their reported targets. If the molecules were reported to inhibit viral entry, they were docked with spike proteins (Table 1). Each separate analysis returned positive results (Table 1, Fig. 2 and online supplementary Fig. S1), indicating the natural compounds we selected might directly inhibit 2019-nCoV. The molecules selected to target PLpro (M2, M3, M7, M9, M10, M11 and M13) mainly bound in the region between the thumb and palm domains, which might interfere with substrate entering this enzyme's active sites, located at the bottom of the two domains [29]. The molecules reported to inhibit 3CLpro (M1, M2, M3, M4, M5, M7, M8, M10, M11, M12 and M13) mainly entered the region between domains 2 and 3, and this region is important for 3CLpro to form a dimmer [30]. M6 was reported to inhibit viral entry, accordingly it bound the fusion cone of spike protein; this cone structure is important for viral membrane fusion [31] (Fig. 2 and online supplementary Fig. S1).

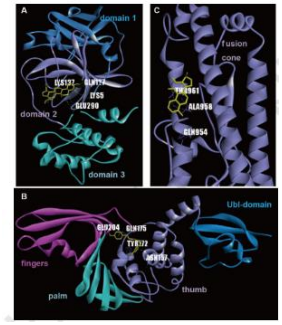
Table

Table 1. The molecules and their docking proteins, binding energy (kcal/mol).

No.	Molecular name	Targets or inhibition	Reference	Docking (binding energy)		
				PLpro	3CLpro	Spike
M1	Betulinic acid	Replication, 3CLpro	[16]	Undo	−4.23	Undo

M2	Coumaroyltyramine	PLpro and 3CLpro	[11,20]	−3.22	−4.18	Undo
M3	Cryptotanshinone	PLpro and 3CLpro	[18]	−5.25	−6.23	Undo
M4	Desmethoxyresenpine	Replication, 3CLpro, and entry	[6]	Undo	−3.52	Undo
M5	Dihomo-γ-linolenic acid	3CLpro	[7]	Undo	−3.88	Undo
M6	Dihydrotanshinone I	Entry, and spike protein	[28]	Undo	Undo	−5.16
M7	Kaempferol	PLpro and 3CLpro	[11]	−2.15	−6.01	Undo
M8	Lignan	Replication, 3CLpro	[16]	Undo	−4.27	Undo
M9	Mougisnamide	PLpro	[20]	−3.05	Undo	Undo
M10	N-cis-feruloyltyramine	PLpro and 3CLpro	[11,20]	−3.11	−4.31	Undo
M11	Quercetin	PLpro and 3CLpro	[20]	−4.62	−6.25	Undo
M12	Sugiol	Replication, 3CLpro	[16]	Undo	−6.04	Undo
M13	Tanshinone IIa	PLpro and 3CLpro	[18]	−5.02	−5.17	Undo

3CLpro: 3C-like protease; PLpro: papain-like protease.



3.4. Selection of antiviral herbal plants The 13 molecules passing the three-round selection process were then compared to the three Chinese herbal databases, and we found 230 herbs containing these molecules (online supplementary file Table S1). We then evaluated these herbs for those containing 2 or more of the 13 natural compounds, leaving 125 results. We cross-referenced the 125 results with the classic categorizations for herbal usage in the TCMSP database, finally choosing 11 types that are traditionally used to treat viral respiratory infections. There are 26 herbal plants within the 11 types. The timeframe during the course of a viral infection that each of these 26 herbal plants (Table 2) should be used was also documented by seeking advice from senior practitioners of traditional Chinese medicine. For example, plants catalogued as antipyretic detoxifying drugs, qi-reinforcing drugs, antitussive antiasthmatics, pungent cool diaphoretics and phlegm-resolving medicines may all be used throughout the course of infection, whereas drugs belonging to the interior warming group may be best utilized in prevention.

Table 2. The 26 Chinese herbals screened and the possible time for usage.

No.	Herbal name		Numb	Classic catalogue		Time to use
	Latin	Chinese	er*	Latin/English	Chinese	
1	*Forsythiae fructus*	连翘	3	Antipyretic detoxifying	清热解毒药	Full course
2	Licorice	甘草	3	Qi-reinforcing	补气药	Full course
3	*Mori cortex*	桑白皮	3	Antitussive antiasthmatics	止咳平喘药	Full course
4	*Chrysanthemi flos*	菊花	2	Pungent cool diaphoretics	辛凉解表药	Full course
5	*Farfarae flos*	款冬花	2	Antitussive antiasthmatics	止咳平喘药	Full course
6	*Lonicerae japonicae flos*	金银花	2	Antipyretic-detoxifying drugs	清热解毒药	Full course
7	*Mori folium*	桑叶	2	Pungent cool diaphoretics	辛凉解表药	Full course
8	*Pogostani radix*	前胡	2	Phlegm-resolving medicine	化痰药	Full course
9	*Rhizoma fagopyri cymosi*	金荞麦	2	Antipyretic detoxifying	清热解毒药	Full course
10	*Tamaricis cacumen*	西河柳	3	Pungent-warm exterior-releasing medicine	辛温解表药	Early
11	*Erigeron breviscapus*	灯盏细辛	2	Pungent-warm exterior-releasing medicine	辛温解表药	Early
12	*Radix bupleuri*	柴胡	2	Pungent cool diaphoretics	辛凉解表药	Early
13	*Coptidis rhizoma*	黄连	2	Heat-clearing and dampness drying medicine	清热燥湿药	Middle
14	*Houttuyniae herba*	鱼腥草	2	Antipyretic-detoxifying	清热解毒药	Middle
15	*Hoveniae dulcis semen*	枳椇子	2	Antipyretic-detoxifying	清热解毒药	Middle
16	*Inulae flos*	旋覆花	2	Phlegm resolving medicine	化痰药	Middle
17	*Eriobotryae folium*	枇杷叶	3	Antitussive antiasthmatics	止咳平喘药	Middle and later
18	*Hedysarum multijugum maxim.*	黄芪	3	Qi-reinforcing	补气药	Middle and later
19	*Lepidii semen descurainiae semen*	葶苈子	3	Antitussive antiasthmatics	止咳平喘药	Middle and later
20	*Ardisiae japonicae herba*	矮地茶	2	Antitussive antiasthmatics	止咳平喘药	Middle and later
21	*Asteris radix et rhizoma*	紫菀	2	Antitussive antiasthmatics	止咳平喘药	Middle and later
22	*Euphorbiae hellenicopiae herba*	泽漆	2	Diuretic dampness-excreting	利水渗湿药	Middle and later
23	*Ginkgo semen*	白果	2	Antitussive antiasthmatics	止咳平喘药	Middle and later
24	*Anemarrhenae rhizoma*	知母	3	Fire-purging	清热泻火药	Later
25	*Epimedii herba*	淫羊藿	2	Yang-reinforcing	补阳药	Later
26	*Fortunes houyfern rhizome*	贯众	2	Warming interior	温里药	Prevention

*: the number of antiviral natural compounds contained in the plant.

3. 5. Network analysis of possible effects or mechanisms Each of the potentially effective herbal remedies contains many ingredients in addition to the antiviral ones found here. To evaluate the possible general in vivo effects of each of our identified herbs, we used the ADME indices listed above to examine each of the orally absorbable and drug-like ingredients recorded in the TCMSP database for each plant. We then extracted the target proteins for each ingredient which had passed the screening process. All proteins belonging to a single plant were combined as input on the online protein-protein interaction analysis server, String, to find the pathway enrichment. For the 26 herbs, about 1/3 of the top 30 KEGG-enriched pathways (mean = 11) were related to regulating viral infection, immune/inflammatory reactions and hypoxia response, indicating that they are potentially effective treatments for viral respiratory infection (Fig. 3 and online supplementary Fig. S2). Note that some of the herbal plants selected here had been reported to be effective for SARS-CoV infection in 2003 (online supplementary Table S2).

Thus, the general effects of each plant should be examined by combining the effects of all of the orally absorbable and biologically active ingredients in it.

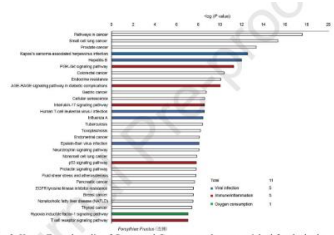


Fig. 3. Kyoto Encyclopedia of Genes and Genomes pathways enriched for the herb *Forsythiae Fructus* in network pharmacology analysis. The top 30 pathways are shown. The blue, red and green bars represent the pathways related to viral infection, immune/inflammation response, and hypoxia response, respectively. EGFR: epidermal growth factor receptor; AGE: advanced glycation end product; RAGE: receptor for AGE.

4. Discussion

In this work, we undertook a multiple step selection process and screened out 26 herbal plants with a high probability of directly inhibiting the novel coronavirus (2019-nCoV), possibly providing instant help in the prevention and treatment of the pneumonia that it can cause. While mainly in China at this point, viral spread is ongoing and has affect persons worldwide. Two principles guided our screening work. The first is that the anti-coronavirus (2019-nCoV) components contained in the source plants should be absorbable via oral prescription. This principle requires that the herbs selected should contain biologically proven anti-coronavirus (2019-nCoV) ingredients, and that these natural compounds should pass the drug-likeness and oral bioavailability evaluations. Therefore, we conducted a three-step screening process. First, we extracted natural compounds verified in PubMed as being effective in treating SARS or MERS coronavirus and then cross-checked these compounds in the Chinese herbal databases. There were 115 overlapping compounds. This method was an expeditious way to identify natural components both pre-existing in Chinese herbal treatment and having a high possibility of anti-coronavirus (2019-nCoV) activity. This is important, as the anti-coronavirus effects of the selected compounds have been biologically confirmed, and the genetic similarities between coronavirus (2019-nCoV) and SARS or MERS coronavirus are high [24,25]. The anti-coronavirus effects of the natural compounds screened by the above method have been mainly confirmed in vitro by direct loading onto cultured cells, thus it does not guarantee their effectiveness in vivo, especially with oral preparation—the principal way in which Chinese herbals are administered. Therefore, to meet the first principle, we ran ADME filters on the natural compounds selected by 4 indices, as used by Hu et al [26]. Among the 115 compounds highlighted by our first step, only 13 passed this screening, showing the necessity of such a test. The novel coronavirus has some mutations when compared to SARS or MERS coronavirus, so the natural compounds effectiveness against the two previous coronaviruses might not be present in the new virus. To reduce this risk, and as the third step of our first principle, we reconstructed the 3D structure of the new coronavirus using the reported structures of SARS and MERS coronavirus proteins as a guide, and then used molecular docking technology to simulate whether the 13 natural compounds selected could combine with the structures we constructed for the new coronavirus proteins. All 13 compounds could bind to the proteins as predicted for the new coronavirus. We believe that the high success rate of our docking screening was due to the high genetic similarity between the new coronavirus and the SARS or MERS virus [24,25]. Our second principle for screening should also be emphasized and elaborated upon. It states that the selected herbal plants must conform to traditional usages. There are many kinds of Chinese herbs that have been used for thousands of years. Based on this rich history and experience, Chinese herbal medicines are divided into different types, each type dedicated to certain kinds of diseases. Ignoring these grouping guidelines can lead to serious side effects. Therefore, as a further condition for the medicine screened here, we verified that they have been routinely used to treat viral pneumonia. To meet this principle, we conducted another three-step screening process for the herbal plants. First, we searched the Chinese medicine database for herbs containing the 13 natural compounds identified. Herbs containing at least 2 of these potentially useful compounds were selected, and a total of 125 herbal plants were identified. The second step in targeted plant selection was based on type classification. Of the 125 results, only 26 herbs were found to be routinely used in treating viral respiratory infection. Finally, network pharmacological analysis was performed to predict the possible therapeutic effects of these 26 plants. Because Chinese herbal medicines contain many ingredients, and

multiple absorbable ingredients might exert their effects on the body, the general effects of herbs may be dictated by all of the absorbable ingredients they contain. With this consideration in mind, we extracted the recorded ingredients of each of the plants selected from the Chinese medicine database and screened these ingredients for drug-likeness and oral availability (via ADME filter) [26,32]. The target proteins of all ingredients passing ADME selection were used for network enrichment to predict the general effects of the herbal plants. For all the plants analyzed, nearly half of the top 30 pathways enriched in KEGG are related to antiviral , immune/inflammatory responses and hypoxia response indicating that these herbs are suitable for anti-viral usage. In fact, some of the herbal plants selected here had been reported effective in against SARS-CoV infection in 2003 (online supplementary Table S2). We thought that the general antiviral and immune/inflammation effects predicted for the 26 plants are correlated with the fact that these plants were selected according to Chinese herbal type classifications. Of course, it should be pointed out that Chinese herbs that have not been identified through this screening process may still have beneficial effects. Further, considering that the biologically validated natural compounds reported in the literature cannot cover all antiviral natural compounds, and the natural compounds included in the Chinese medicine database are not complete, the process that we have followed may have excluded herbs that would be well suited to this treatment. Nevertheless, the purpose of this screening was to provide a rational approach for selecting Chinese herbal medicines with a high potential efficacy in treating 2019-nCoV and related viruses. The specific dosage and usage of each herb should be determined based on patients' manifestations. Finally, the key step in this screening was molecular docking. The 3D structures of the proteins used here are based on reported gene sequences. If the virus mutates during transmission, a new screening is recommended. In conclusion, this work has identified several Chinese medicinal plants classified as antiviral/pneumonia-effective that might directly inhibit the novel coronavirus, 2019-nCoV. Additionally, we propose screening principles and methods which may provide guidance in screening antiviral drugs from other natural drug databases.

Authors' contributions

DZ conceived the study, participated in its design, coordination, and all the work processes. KW participated in herbal selection. XZ participated in data collection and network pharmacology analysis. SD helped to collect data. BP helped to draft the manuscript.

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Abstract

Background:

At present, accumulative attention has been paid to coronavirus disease 2019 (COVID-19) due to its global prevalence. Acupuncture may play a beneficial role in patients with breathlessness in COVID-19. This study is designed to determine the efficacy and safety of acupuncture for breathlessness in COVID-19.

Methods:

Randomized controlled trials (RCT) will be searched from 7 electronic databases, with the last search update being 30 June 2020. Studies by registers of clinical trials will be additionally searched. Two investigators will independently select studies, extract data and evaluate study quality. Finally, a meta-analysis will be used to evaluate the pooled intervention effect if possible.

Results:

Our present findings will indicate the application of acupuncture as an adjunctive treatment for dyspnea in COVID-19, which will be published in a peer-reviewed journal.

Conclusion:

Our study will provide a reference foundation for clinical optimization of treatment.

1 Introduction

Coronaviruses (CoVs) is a type of enveloped positive-sense RNA virus that is diversely found in humans and wildlife.[1] Human coronaviruses (HCoVs) have been proven to be non-essential pathogens for a long period, which can result in “common cold” in other healthy population.[1,2] Nevertheless, two highly pathogenic HCoVs from animal reservoirs have led to worldwide pandemics with striking morbidity and mortality, including severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV).[2] At present, a new strain called the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus has become another outbreak. The coronavirus disease 2019 (COVID-19) was recognized in Wuhan, China in December, 2019, causing increasing morbidity and mortality ever since.[2] The ongoing COVID-19 pandemic is an exceptional challenge for the health systems throughout the world. Through the continuous accumulation of research data, the biological, epidemiological, and clinical characteristics of COVID -19 have been gradually improved. International attention has been paid to COVID-19 due to the quickly increasing number of diagnosed patients along with subsequently rising secondary

outbreaks in various global regions. Hopefully, the rapid in-depth sequencing of viral genomes has enabled the development and research of diagnostic tests, as well as the initiation of vaccine and therapeutics research.[3] However, so far, no effective vaccine or causative therapy is available.[3] Existing studies have readily confirmed the interpersonal transmission of SARS-CoV-2,[3,4] with an incubation period varying from 1 to 14 days (median: 5–6 days), which could be 24 days under extreme conditions.[3,5] Additionally, patients with COVID-19 have a higher proportion of men, the elderly, patients with hypertension and/or diabetes.[6] There are also investigations concerning the risk factors as well as clinical outcomes in admission and intensive care unit. In an early study of patients with COVID-19 in China and Italian, older men, patients with smoking and cardiometabolic diseases were associated with poor prognosis.[7] In general, the clinical presentation of COVID-19 includes myalgias, fatigue dry cough and fever, as well as less common ones (including abdominal pain, breathlessness, headache diarrhea and sore throat).[3,8] A recent descriptive study found that there were 56.7% men among elderly diagnosed COVID-19 patients were male, and common symptoms included fatigue (23.3%), dyspnea (30.0%), cough (56.7%), and fever (78.3%).[8] In general, older population are more vulnerable to COVID-19 infection, and their mortality rate is higher, which deserves more attention.[8] The COVID-19 pandemic has triggered prevalent research interest in therapeutic and preventive interventions. However, due to the lack of specific antiviral therapeutics and vaccine, adjuvant therapy has become the major therapeutic strategy for COVID-19, along with the administration of corticosteroids, antivirals, broad-spectrum antibiotics and convalescent plasma.[9] At present, diverse trials have been launched, such as tocilizumab, losartan, hydroxychloroquine, remdesivir as well as convalescent plasma.[10]

Traditional Chinese medicine (TCM) plays an important role in the prevention and treatment of various infectious diseases. To be specific, the application of TCM has also obtained significant therapeutic efficacy during the SARS epidemic in 2003.[11] The combination of TCM and Western medicines could relieve symptoms, enhance life quality, absorb lung infiltration, while attenuate the corticosteroid dosage among SARS patients.[11] Similarly, recent clinical practice has also revealed the significant therapeutic effect of TCM in COVID-19.[12] China has issued guidelines for the diagnosis and treatment of COVID-19, recommending the use of conventional treatment methods plus TCM.[9,13] In the current Chinese medical system, licensed practitioners of TCM are allowed to prescribe western medicines after a formal course of study.[14] Therefore, the participation of TCM practitioners in the treatment of COVID-19 is legal and supported by the government. During the entire treatment period of COVID-19, Chinese medical staff volunteered to join the designated hospitals in Hubei Province,[12] who comprehensively adopted acupuncture, Chinese patent medicine, decoction and other characteristic therapies of TCM, shedding novel light on the control and prevention of COVID-19.[12] In total, 303 ongoing clinical trials concerning the assessment of the therapeutic safety and efficacy for COVID-19 patients have been launched in China by March 1, 2020, 50 of which focus on TCM, including 14 clinical trials aimed at evaluating the efficacy of TCM combined with Western medicine.[15] At present, there are various types of evidence for TCM treatment of COVID-19,[9,13,15] as shown in Figure 1.

Figure 1:

Types of current evidence on TCM treatment of COVID-19.

Acupuncture, a main component of TCM, has been widely adopted to treat respiratory diseases in clinical practice,[16,17] whose efficacy has been assessed by a number of randomized controlled trials (RTCs).[18] Breathlessness is one of the prevalent symptoms in COVID-19 patients.[8,19] Acupuncture may play a role in the prevention, treatment and rehabilitation of the COVID-19 and relieve the symptoms caused by COVID-19. Acupuncture has been demonstrated to effectively relieving common symptoms in supportive and palliative care, including anxiety disorders, nausea, insomnia, leukopenia, fatigue as well as vomiting,[20–25] which might also effectively treat abdominal pain and abdominal distension.[26,27] Coyle et al have proposed that acupuncture is an effective therapeutic approach for COPD-associated breathlessness.[28] Possible related symptoms of COVID-19 treated with acupuncture is shown as Figure 2. The recent systematic review and meta-analysis show that acupuncture can relieve breathlessness in subjects with advanced diseases.[16] Therefore, in this meta-analysis review, our goal is to systematically review the efficacy of acupuncture in relieving breathlessness, subsequently improving the physiological function and quality of life of patients with COVID-19 combined with dyspnea.

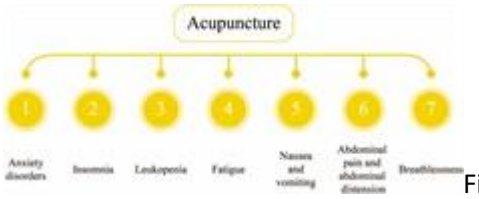

Figure 2:

Possible related symptoms of COVID-19 treated with acupuncture.

2 Methods

The study will be conducted in accordance with the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P).[29]

2.1 Inclusion criteria

2.1.1 Study type

Randomized controlled trials (RCTs) will be included in the review, without restriction on language or publication date.

2.1.2 Participant types

Patients with breathlessness due to lab-confirmed COVID-19 will be included, regardless of age, race, sex. Diagnosis of COVID-19 is based on the international or Chinese diagnostic criteria for COVID-19.[3,30,31]

2.1.3 Types of interventions

Acupuncture will be performed in the treatment group, combined with other treatments, including routine therapy and so on.[9] Patients in the control group will receive other therapeutic approaches other than acupuncture, including routine therapy, placebo, etc.

2.1.4 Types of outcomes

The primary outcomes include the changes on any subjective measurement of breathlessness severity made on a validated rating scale from baseline to endpoint, including visual analogue scale, numerical rating scale and the Borg Scale.[32,33]

Secondary outcomes include the assessment of quality of life activities using any validated questionnaire. In addition, anxiety will be evaluated by any validated scale. The safety of treatment will be assessed by the incidence and degree of adverse events.[34–36]

2.2 Search methods to identify studies

2.2.1 Search strategy

Two reviewers will independently search seven databases: EMBASE, Medline, Cochrane Central Register of Controlled Trials, Web of Science, Wan Fang Data, China National Knowledge Infrastructure as well as Chinese Scientific Journal Database (VIP) from inception until 30 June 2020 (search date). The same terms in China will be conducted in Chinese databases. The searching strategy for Medline is shown in Table 1.

#1 Coronavirus disease 2019 [Abstract/Title]
#2 COVID-19 [Abstract/Title]
#3 2019 novel coronavirus [Abstract/Title]
#4 2019-nCoV [Abstract/Title]
#5 Coronavirus disease-19 [Abstract/Title]
#6 Severe acute respiratory syndrome coronavirus 2 [Abstract/Title]
#7 SARS-CoV-2 [Abstract/Title]
#8 Novel coronavirus [Abstract/Title]
#9 COVID19 [Abstract/Title]
#10 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9
#11 Dyspnea [MeSH]
#12 Dyspneas [Abstract/Title]
#13 Shortness of Breath [Abstract/Title]
#14 Breath Shortness [Abstract/Title]
#15 Breath Shortnesses [Abstract/Title]
#16 Breathlessness [Abstract/Title]
#17 Breathlessnesses [Abstract/Title]
#18 #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17
#19 Acupuncture [MeSH]
#20 Electroacupuncture [Abstract/Title]
#21 Dermal needle [Abstract/Title]
#22 Acupoint [Abstract/Title]
#23 #19 OR #20 OR #21 OR #22
#24 Randomized controlled trial [PT]
#25 Controlled clinical trial [PT]
#26 Randomized [Abstract/Title]
#27 Placebo [Abstract/Title]
#28 Clinical trials as topic [Abstract/Title]
#29 Randomly [Abstract/Title]
#30 Trial* [Abstract/Title]
#31 #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30
#32 Animals [MeSH]
#33 #31 NOT #32
#34 #10 AND #18 AND #23 AND #33

Table 1:

Search strategy for Medline.

2.2.2 Searching other resources

Similar retrieval methods will be applied in the Clinical Trials.gov to obtain unpublished studies. There is no restriction on publication regions or language.

2.3 Data collection and analysis

2.3.1 Selection of studies

After searching studies, 2 investigators will review titles and abstracts, or full text if necessary. All investigators will reach agreement on the identification of study inclusion after evaluating their eligibility, and we will also record reasons why studies are eliminated. The selection process is summarized using PRISMA flow diagram. Details of the selection procedure for studies are shown in a PRISMA flow chart (Fig. 3). Any inconsistency is resolved by discussing with a third investigator.

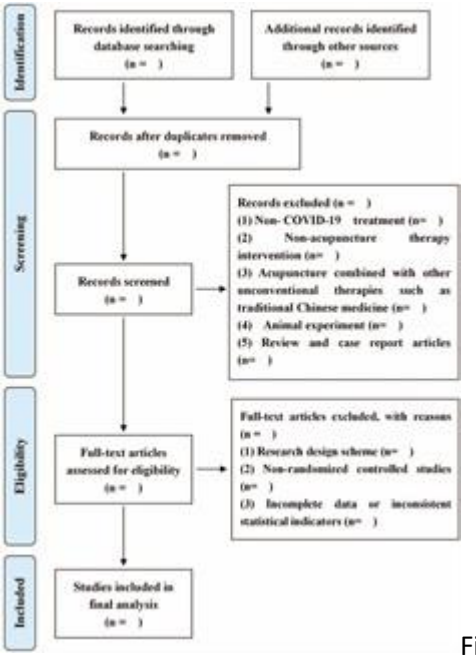

Figure 3:

Study selection flow diagram.

2.3.2 Data extraction and management

Two investigators will extract relevant data independently, including study design, general information, characteristics of patients, comparison interventions, and outcomes. For articles with incomplete or uncertain data, the authors will be contacted for complete data. And the study will be further excluded without adequate information. If there is any dispute in the data extraction process, it will be submitted to a third researcher for processing.

2.3.3 Evaluation of bias risk in included studies

Two investigators will independently evaluate the bias risks among enrolled researches according to the Cochrane Collaboration.[37] Discrepancy will be resolved by discussion and judgment by an arbiter. The following seven aspects in all RCTs will be evaluated: outcome assessment blinding, participants and personnel blinding, selective outcome reporting, allocation concealment, inadequate outcome data, generation of random sequences as well as other potential sources of bias.[37] The bias risk in each aspect will be assessed and divided into 3 levels: low risk, high risk, and unclear risk.

2.3.4 Measurement of therapeutic effect

Review Manager (RevMan 5.3) software will be used for statistical analysis if a meta-analysis is allowed. Risk ratio will be calculated for dichotomous data. The intervention effect will be shown as the mean difference for continuous outcomes. Additionally, 95% confidence intervals will be calculated.

2.3.5 Heterogeneity evaluation

After stratifying the study according to the therapeutic duration and region, the chi-square test will be utilized to evaluate the heterogeneity and depressive symptoms. I2 statistic will be utilized for quantification of heterogeneity degree, where I2 >50% indicates the significant heterogeneity.[37–40]

2.3.6 Publication bias

The publication bias will be evaluated by funnel plots by determining whether there are 10 or more studies with the same outcome. In the case of asymmetric funnel plot, subgroup analysis or sensitivity analysis will be performed to investigate possible causes.[37,41]

2.3.7 Data synthesis

RevMan 5.3 software will be utilized for statistical analysis. The fixed-effects model will be employed to analyze data in the case of insignificant heterogeneity (I2 < 50%). In the case of heterogeneity (I2 ≥ 50%), subgroup analysis will be further conducted to decrease the clinical heterogeneity by taking into consideration of possible factors. If the heterogeneity is still significant, the random-effect model or qualitative description will be used.

2.3.8 Sensitivity analysis

Sensitivity analysis will be conducted by sequential omission of single study at a time, followed by simultaneously excluding 2 studies for identification of factors making the most contribution to heterogeneity.

2.3.9 Assessment of evidence quality

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) evaluation method of evidence quality will be used to evaluate the primary and secondary results of this study. The evidence quality will be categorized into high, moderate, low or very low according to five parameters (publication bias, indirectness, inconsistency, imprecision, and study limitations).

2.4 Ethics and publication

Since this study does not involve the patient privacy, ethical approval is not required. Our research results will be shared and shown through conference reports and peer-reviewed journals.

3 Discussion

The pathogenesis and clinical symptoms related to severe respiratory disease were described many years ago in TCM texts.[42] There are many studies on current application of TCM in COVID-19,[42,43] such as the clinical outcome, pathogenesis and the current application of TCM on COVID-19. The strength of our review includes the following 3 points. First, it is the first systematic review concerning the safety and effectiveness of acupuncture for breathlessness in COVID-19. Second, only RCTs are included in our systematic review, which are more likely to provide unbiased information than other study designs. Third, the comprehensive search strategy renders in-depth searching lists as well as trial registries associated with acupuncture and COVID-19.

However, the intrinsic methodological challenges among these enrolled trials will limit our systematic review. As a manipulated intervention, it is difficult to implement the blindness of therapeutic modes on acupuncturists.[44–48] Acupuncture therapy could be further categorized into manipulation and needling instrument. In addition, there might be great variation on acupuncture therapy in these enrolled studies. Although the above problem might be resolved and the consistency of interventions might be ensured by subgroup analysis, the comparability of enrolled researches will be decreased and the difficulty in meta-analysis will be increased.

Author contributions

Conceptualization: Baozhen Zhang and Kai Zhang.

Data curation: Kai Zhang and Zhenzhen Han.

Formal analysis: Kai Zhang and Zhenzhen Han.

Investigation: Baozhen Zhang and Kai Zhang.

Methodology: Baozhen Zhang.

Software: Qilin Tang and Kaihang Sun.

Supervision: Qilin Tang.

Writing – original draft: Baozhen Zhang, Kai Zhang, and Kaihang Sun.

Writing – review & editing: Qilin Tang.

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Abstract

Since the outbreak of Corona Virus Disease 2019 (COVID-19) in Hubei province, the epidemic scale has increased rapidly, and no effective antiviral drug therapy has been identified yet. This study aimed to evaluate the adjuvant efficacy of Natural Herbal Medicine (NHM) combined with Western medicine in the treatment of COVID-19. We performed a retrospective, 1:1 matched, case-control study of the first cohort of hospitalized COVID-19-confirmed cases (January 17, 2020 to January 28, 2020). A total of 22 of the 36 confirmed patients were included in this study, split into two groups of 11: the NHM group (NHM combined standard Western medicine treatment) and control group (standard Western medicine treatment alone). All patients received appropriate supportive care and regular clinical and laboratory monitoring. Main evaluation indicators included improvement of clinical symptoms such as fever, cough and diarrhea after hospitalization; pathogen nucleic acid test result of respiratory tract and fecal specimens of the patient after hospitalization, and change of chest CT examination after hospitalization. The duration of fever in the NHM group ([Formula: see text] days) was significantly shorter than that in the control group ([Formula: see text] days) ([Formula: see text]). During the whole hospitalization period, the number of cases with diarrhea in the NHM group (two cases) was less than that in the control group (eight cases) ([Formula: see text]). Compared with the control group ([Formula: see text]), the duration for improvement (DI) of chest CT in the NHM group ([Formula: see text]) was significantly shorter ([Formula: see text]). Our results suggest that NHM could improve the clinical symptoms of COVID-19 patients and may be effective in treating COVID-19; thus, a larger, prospective, randomized, controlled clinical trial should be conducted to further evaluate the adjuvant efficacy of NHM in the treatment of COVID-19.

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Abstract

The outbreak of emerging infectious pneumonia caused by 2019 Novel Coronavirus (2019-nCoV) has posed an enormous threat to public health, and traditional Chinese medicine (TCM) have made vast contribution to the prevention, treatment and rehabilitation of coronavirus disease 19 (COVID-19) among Chinese population. As an indispensable part of TCM, Chinese patent medicines (CPMs) are highly valued and critically acclaimed in their campaign to contain and tackle the epidemic, they can achieve considerable effects for both suspected cases under medical observation period, and confirmed individuals with serious underlying diseases or critical conditions. Given this, based on the Guideline on Diagnosis and Treatment of Coronavirus Disease 2019 in China, the present review summarized the basic information, clinical evidence and published literatures of recommended CPMs against COVID-19. The details were thoroughly introduced involving compositions, therapeutic effects, clinical indications, medication history of CPMs and the profiles of corresponding research. With regard to infected patients with different stages and syndrome, the preferable potentials and therapeutic mechanism of CPMs were addressed through the comprehensive collection of relevant literatures and on-going clinical trials. This study could provide an insight into clinical application and underlying mechanism of recommended CPMs against COVID-19, with the aim to share the Chinese experience in clinical practice and facilitate scientific development of TCM, especially CPMs in the fierce battle of COVID-19.

1. Introduction

From its beginning in December 2019, a cluster of unexplained pneumonia cases have witnessed and reported in Wuhan City, Hubei Province, China [1,2]. The cause of the novel coronavirus disease (COVID-19) is identified as a novel betacoronavirus, named the 2019 novel coronavirus (2019-nCoV), the spread of severe acute respiratory syndrome has been almost entirely driven by 2019-nCoV via respiratory droplets, human-to-human transmission, posing pandemic potential with unfortunate characteristics of strong infectiousness, rapid dissemination, long incubation period, and general susceptibility [[3], [4], [5]]. In subsequent months, the ongoing outbreak of COVID-19 is rapidly spreading globally and declared as a public health emergency by the World Health Organization (WHO) [[6], [7], [8]]. As of April 4, 2020, more than 200 countries sufferred from the expansive spread of the virus 2019-nCoV in a global pandemic with massive and urgent crisis on healthcare, economic and social systems [[9], [10], [11], [12]], there were over one million confirmed cases with COVID-19 worldwide, of these, a total of 277,965 subjects were from the United States [13,14]. According to official release on April 4, 2020 by National Health Commission of the People’s Republic of China, more than 80,000 cases had been confirmed as COVID-19 with 3326 death cases [15].

Currently, the global pandemic of COVID-19 continues to accelerate and escalate, the health-care systems of different countries are beavering away to contain the virus, therefore, there is urgent need to seek for effective or adjuvant therapies with excellent safety profiles against COVID-19 [[16], [17], [18]]. In the theory of traditional Chinese medicine (TCM), 2019-nCoV infected pneumonia was deemed to the category of "Pestilence", and the characteristics of its pathogenesis was "dampness, toxin, stasis and closure" [19,20]. Over the past few months, TCM exhibited remarkable benefits against COVID-19 in China, and it was convinced that TCM achieved satisfactory therapeutic superiority for patients infected by 2019-nCoV with regard to preventive treatment of diseases, comprehensive therapies and rehabilitation by the accumulation of clinical experience and scientific evidence [21,22]. As recommended in *the Guideline on Diagnosis and Treatment of Coronavirus Disease 2019* (Revised 7th version) which was officially released by National Health Commission of the People's Republic of China, TCM could exert favorable effects for patients with different syndromes and distinct stages of COVID-19, contributing to infections in the periods of both medical observation and clinical treatment [23]. Recently, the latest official data showed that 91.5 % of confirmed subjects with COVID-19 (74,187 cases) received TCM in China, and increasing results of clinical observation indicated that the total effective rate of TCM reached more than 90 % [24]. These promising advantages were associated with its unique therapeutic principles including syndrome differentiation and treatment, boosting the individual's endogenous healing ability, balancing *Yin* and *Yang*, various therapies and personalized treatment in first-line clinic [25,26].

With the development of technology in TCM domain, Chinese herbal products were transformed into varied dosage forms, Chinese patent medicines (CPMs) were consumer-near and popular "folk medicine" that contributed to widely application in clinical practice among Chinese citizens [27,28]. Compared to herbal decoction, CPMs had the advantages of stable quality, curative efficacy, considerable safety, rapid absorption, high bioavailability, convenience of taking, carrying and storing [29,30]. Similarly, as an indispensable part of TCM, CPMs were substantial utilized in combination with western medicine for the management of COVID-19, and proposed as adjunctive and therapeutic options to fight the public health emergency of 2019-nCoV by national and provincial guidelines in China [[31], [32], [33]]. Given the paucity of published English research concerning CPMs against COVID-19 currently, the present review performed a descriptive analysis of recommended CPMs from both clinical trials and published literatures, based on the following aspects: information retrieved from their instructions (compositions, therapeutic effects, and clinical indications), medical evidence, pharmacodynamic mechanism, dominant components, applicable patients, clinical cautions and so on. As regarding to some types of CPMs without accessible published evidence for treating 2019-nCoV, we introduced the relevant results of infectious or health-threatening diseases. The aim of present review was to provide the scientific basis and share clinical experiences for promising choices of CPMs against COVID-19.

2. The profiles of current evidence

First, the comprehensive retrieval of electronic databases in both Chinese and English (the China National Knowledge Infrastructure Database, WangFang Database, Sinomed Database, PubMed and Embase) was performed for collecting the published research from inception to Apr 10, 2020. On the one hand, the following terms of COVID-19 were adopted: "COVID-19 [Supplementary Concept]," "2019 novel coronavirus", "COVID19", "SARS-CoV-2", "2019-nCoV", "coronavirus disease 2019", "coronavirus disease-19". On the other hand, the searching terms of CPMs mainly included their Mandarin Chinese and trade name. The results of literature search were displayed in Fig. 1A, a total of 28 citations were yielded initially for eight CPMs. Only one eligible study was in English, focused on the anti-viral and anti-inflammatory activities of *Lianhuaqingwen* Capsule for treating COVID-19, which published by the team of distinguished Academician Nanshan Zhong.

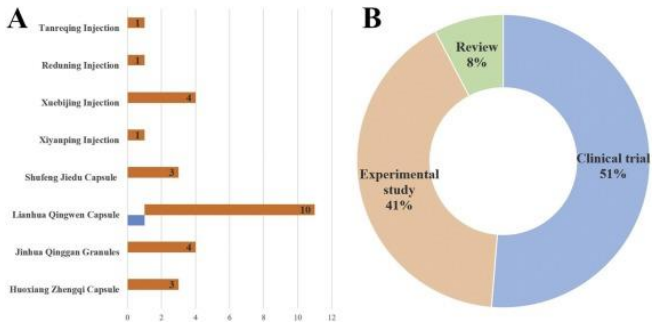

Fig. 1. The profiles of current evidence for CPMs against COVID-19.

Note: A: The retrieval results for eligible studies. B: The distribution of research types.

In addition, the on-going clinical trials concerning on CPMs anagist COVID-19 were supplemented to identify the potential evidence by utilizing the platform of Chinese Clinical Trial Register (www.chictr.org.cn/). Ultimately, there were 11 undergoing clinical trials that used CPMs for the treatment of 2019-nCoV infection, namely, *Xiyanping* injection (Registration Number: ChiCTR2000030218, ChiCTR2000030117, ChiCTR2000029756), *Lianhua Qingwen* Capsule/ Granules (ChiCTR2000029434, ChiCTR2000029433), *Xuebijing* Injection (ChiCTR2000030388, ChiCTR2000029381), *Tanreqing* Injection/Capsule (ChiCTR2000029432, ChiCTR2000029813), *Reduning* Injection (ChiCTR2000029589), Shenfu Injection (ChiCTR2000030043). Taken together, the research type of clinical trials occupied approximately half proportion in current evidence (Fig. 1B).

3. Basic information of recommended CPMs against COVID-19

According to aforementioned guidelines of COVID-2019 in China, totally 14 types of CPMs were officially issued to prevent and treatment COVID-19, 57.14 % (8 types) of them were Chinese herbal injection, and others were oral dosage forms of TCM. As illustrated in Fig. 2, there were different optimal choice among CPMs corresponding to infected individuals with different stages or TCM syndromes, for example, the suspected cases under medical observation could receive the CPMs including *Huoxiang Zhengqi* Capsule, *Jinhua Qinggan* Granules, *Lianhua Qingwen* Capsule, *Shufeng Jiedu* Capsule according to their specific clinical manifestations and syndrome differentiation in the theory of TCM. Remarkably, CPMs were often composed by many kinds of Chinese herbal materials, there was a vast difference between their compositions among enrolled CPMs, for instance, *Xiyanping* Injection contained sole active ingredient, whereas *Suhexiang* Pill was processed from 15 crude herbs. The relationship of CPMs and their compositions was depicted in Fig. 3. Further, the details involving compositions, therapeutic effects, clinical indications for recommended CPMs were summarized in Table 1, to provide the relative references for clinician and specialists to control the spread of this fatal disease.

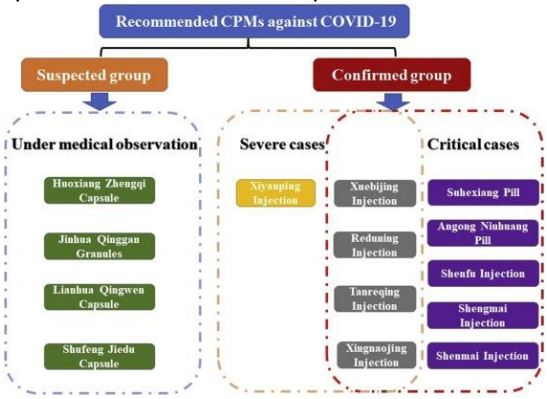

Fig. 2. Recommended CPMs and corresponding applicable patients with COVID-19.

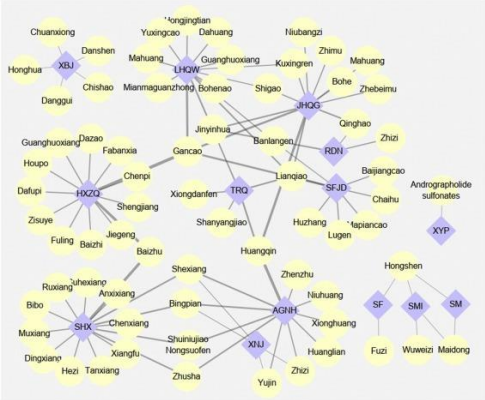

Fig. 3. The relationship of recommended CPMs and compositions.

Note: The purple nodes represented different CPMs, and compositions were labelling yellow. AGNH: *Angong Niuhuang* Pill;

HXZQ: *Huoxiang Zhengqi* Capsule; JHQG: *Jinhua Qinggan* Granules; LHQW: *Lianhua Qingwen* Capsule; RDN: *Reduning* Injection;

SF: *Shenfu* Injection; SFJD: *Shufeng Jiedu* Capsule; SHX: *Suhexiang* Pill; SM: *Shenmai* Injection; SMI: *Shengmai* Injection;

TRQ: *Tanreqing* Injection; XBJ: *Xuebijing* Injection; XNJ: *Xingnaojing* Injection; XYP: *Xiyanping* Injection

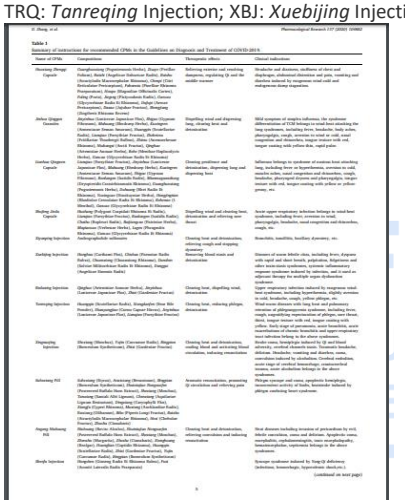

Table 1 (*continued*)

Name of CPMs	Compositions	Therapeutic effects	Clinical indications
		Reviving yang for resuscitation, tonifying *Qi* and preventing exhaustion	Palpitation, asthma, cough, stomachache, diarrhea, arthralgia induced by Yang deficiency (*Qi* deficiency).
Shengmai Injection	*Hongshen* (Ginseng Radix Et Rhizoma Rubra), *Maidong* (Ophiopogonis Radix), *Wuweizi* (Schisandra Chinensis Fructus)	Tonifying *Qi* and nourishing *Yin*, recovering pulse and preventing exhaustion	Palpitation, shortness of breath, limbs frosty, cold sweat, barely palpable pulse induced by deficiency of *Qi* and *Yin*. Myocardial infarction, cardiogenic shock, septic shock and other belongs to the above syndromes.
Shenmai Injection	*Hongshen* (Ginseng Radix Et Rhizoma Rubra), *Maidong* (Ophiopogonis Radix)	Tonifying *Qi* and preventing exhaustion, nourishing *Yin* and generating body fluid, activating pulse	Shock, coronary heart disease, viral myocarditis, chronic cor pulmonale, granulocytopenia induced by deficiency of *Qi* and *Yin*. It can improve the immune function of patients with tumors. When combined with chemotherapeutic drugs, it has a certain synergistic effect and can reduce the side effects caused by chemotherapeutic drugs.

4. Preferable benefits of CPMs for cases under medical observation

Based on the findings of related review, the CPMs including *Huoxiang Zhengqi* Capsule, *Jinhua Qinggan* Granules, *Lianhua Qingwen* Capsule, *Shufeng Jiedu* Capsule could be presumably as preventive measure for the patients in the medical observation period [34]. It was noteworthy that early treatment was essential for suspected or mild cases, accumulated evidence of bioinformatics, pharmacodynamics and clinical findings suggested that these multiple component Chinese herbal products also exerted effects on immune regulation, symptom improvement, anti-inflammation and so on during the treatment of COVID-19. The results of previously pharmacological research indicated that *Huoxiang Zhengqi* Capsule or other dosage forms could improve gastrointestinal dysfunction, modulate immune responses, and anti-inflammation [35]. With regard to the national and provincial guidelines against COVID-19 in China, *Huoxiang Zhengqi* Capsule was recommended for patients with fatigue and gastrointestinal discomfort [36]. Through the techniques of network pharmacology and molecular docking, *Huoxiang Zhengqi* Oral Liquid could regulate multiple signaling pathways involving Hepatitis B, small cell lung cancer, non-small cell lung cancer and inhibit the replication of 2019-nCoV to exhibit the preventive or therapeutic effects on COVID-19, and its active compounds had definite affinity with angiotensin converting enzyme II (ACE2) and 3- chymotrypsin-like protease (3CLpro) [37,38].

Compared with other recommended CPMs, *Jinhua Qinggan* Granules posed the shorter period of medication history, nevertheless, it was proved that the application of *Jinhua Qinggan* Granules could significantly alleviate clinical symptoms such as fever, cough, fatigue, expectoration, and relieve psychological anxiety of mild cases suffered from COVID-19 [39]. The results of systems biology and bioinformatics revealed that the mechanism of *Jinhua Qinggan* granules in the treatment of COVID-19 involving multiple targets, namely MAPK1, CASP3, TP53, ALB, TNF, IL6, and multiple pathways, which might be related to antiviral, immune

regulation, inflammation inhibition and apoptosis regulation via PI3K-Akt, HIF-1, TNF, MAPK, NF-κB pathways, and dominant principles including kaempferol, baicalein and oroxylin A could take participate in multiple signal pathways (such as PTGS2, HSP90AB1, PTGS2, BCL2 and CASP3) by binding with ACE2 [[40], [41], [42]].

Relieving typical symptoms and representative complications, diminishing inflammation and infection, the Chinese herbal products of *Lianhua Qingwen* Capsule was recognized as an excellent antidote in this anti-epidemic by adequate clinical and fundamental research [34,43]. According to preliminary clinical evidence of retrospective, multicenter study and cases reports, for general type patients and suspected cases with COVID-19, the scheme of *Lianhua Qingwen* Capsule combined with western medicine had considerable effective rate without obvious adverse reactions (ADRs), and it could not only improve clinical symptoms including fatigue, fever, cough, expectoration, shortness of breath, chest tightness, poor appetite, etc., but also control the progression of upper respiratory tract infection, reduce the rate of conversion from mild into serious status, shorten the duration of hospital stay [[44], [45], [46], [47], [48]]. Similarly, accumulated evidence displayed that its pharmacodynamic mechanisms were associated with the biological functions involving anti-inflammation, inhibition of 2019-nCoV replication, affection of virus morphology, activation of T-cell, molecular response to bacterial origin, broad-spectrum antivirus, immune regulation and so on [[49], [50], [51], [52]].

Meanwhile, compared with the control group that only receiving arbidol, *Shufeng Jiedu* Capsule combined with arbidol could achieve favorable effects in the treatment of COVID-19 and without reported drug-related adverse events, there were significant improvements in CD^{4+} and CD^{8+} T cell subpopulations, white blood cell and CT imaging, antipyretic time, the disappearance time of dry cough, nasal congestion, pharyngeal pain, fatigue, diarrhea, and the negative conversion of nucleic acid examination for 2019-nCoV [53,54]. The latest research investigated the potential targets and mechanisms of *Shufeng Jiedu* Capsule for treating COVID-19 via constructing the "drug-component-disease-target" network, the results demonstrated that therapeutic mechanism involved a variety of biological processes such as the interaction of viral proteins with cytokines, with critical proteins as IL-6, ALB, MAPK3 [55]. For patients infected 2019-nCoV, the large amounts of cytokines might be associated with rapidly provoking of acute respiratory distress syndrome (ARDS), single or multiple organ failure, and eventually death.

5. Therapeutic potentials for severe or critical patients

Depending on the differentiation of clinical syndromes, the severe or critical subjects could receive the Chinese herbal injections that prescribed by Chinese herbalists or doctors,
including *Xiyanping* Injection, *Xuebijing* Injection, *Reduning* Injection, *Tanreqing* Injection, *Xingnaojing* Injecti on [56]. Among them, only *Xiyanping* Injection was applied for treating severe patients, whereas others were also recognized as the complementary choices for critical cases with COVID-19. Compared with oral administration of TCM, the Chinese herbal injections possessed the benefits of rapid onset, high bioavailability, and content accuracy [57], therefore, they were more suitable for the severe or critical patients with COVID-19.

Some scholars concluded that *Xiyanping* Injection was reputed as effective alternative to antibiotics in clinical practice [58]. Modern pharmacological studies showed that its active ingredient, sulfonated andrographolide had notable effects of antipyretic, anti-inflammation to treat various infectious diseases [59]. In addition, prevenient Chinese research pointed out its clinical advantages that were related to improve respiratory symptoms, inhibit concurrent bacterial infection, and regulate immune function, superior clinical safety, especially certain hepatoprotective effects, suggesting it might have potentials to relieve some drug-induced liver injury during the treatment of COVID-19 for serious cases [60]. Remarkably, it was reported that andrographolide sulfonate could ameliorate sepsis in mice through suppressing MAPK, STAT3 and NF-κB pathways, these pathways also played the important role in pulmonary diseases [[61], [62], [63]].

The prescription of *Xuebijing* Injection originated from therapeutic principles of TCM, that was proposed by famous integrative medicine emergency experts, Professor Jin-Da Wang, it was approved as second grade national new medicine for treating sepsis in China over 15 years [64,65]. In this clinical fight against COVID-19, considerable effects of *Xuebijing* injection had been displayed by retrospective study and relevant review, the results revealed that *Xuebijing* injection could promote the absorption of lung infection, and improve clinical efficacy and negative rate of nucleic acid [66,67]. Besides, some research demonstrated its

characteristics of multi-target and multi-pathway in treating COVID-19 based on the approaches of network pharmacology and molecular docking. Among a series of involved signaling pathways, such as HIF-1 and PI3K-Akt were represented pathways against COVID-19 in terms of lung inflammation, virus infection and lung injury. Besides, core targets including TNF, MAPK1, JUN, IL6, STAT3, EGFR, etc. were closely correlation with the inhibition of cytokine storm in severe cases, and fatal risk of cytokine storms in the immune system of serious patients might result in organ failure and even death [[68], [69], [70]].

Reduning Injection was widely utilized to treat upper respiratory tract infection with multiple functions such as clearing heat, dispelling wind, and detoxification, and previous pharmacological research proposed that it could ameliorate paraquat-induced acute lung injury involved in regulating AMPK/MAPK/NF-κB signaling pathways [71]. In this regard to treating COVID-19, *Reduning* injection might be related to anti-inflammatory, immunoregulation of active compounds through multi-target and multi-pathway. On the one hand, the critical targets were namely PTGS2, PTSG1, CCL2, RELA, NOS2, HMOX1, CASP3, IL6 and MAPK1, some of them belonged to chemokines, which posed the immune activation profiles of 2019-nCoV. On the other hand, KEGG pathway enrichment analysis revealed 45 related pathways, mainly IL-17, C-type lectin receptor, HIF-1 and NF-κB signaling pathways [72,73].

A large number of clinical data had accumulated to confirm superior efficacy of *Tanreqing* Injection in the treatment of acute bronchitis disease, tuberculosis and so on [74,75]. In particular, the severe patients with COVID-19 suffered similar clinical manifestations of its dominant diseases. Meanwhile, the underlying mechanism and binding activity of *Tanreqing* Injection were elucidated, the results in molecular level showed that it might be potential as antiviral agent due to critical components (kaempferol, quercetin, baicalein luteolin, wogonin, etc.) had good affinity with 3CLpro of 2019-nCoV. The enrichment analysis of biological process indicated that the target of *Tanreqing* injection involved in inflammatory response, immune system, signal pathway and apoptosis process. Interesting, the core targets involved IL6, IL1B, MAPK1, IL10, IL4, CXCL8, IP10, etc. [76]. This finding was consistent with former clinical report regarding severe patients with higher level of IL2, IL7, IL10, GSCF, IP10 in the plasma [77].

The commercialized injectable product of *Xingnaojing* Injection was extracted and refined scientifically from classic Chinese emergency prescription "*Angong Niuhuang* Pill", it had the functions of clearing heat and detoxication, cooling blood and activating blood circulation, inducing resuscitation and widely used in the treatment of intracerebral haemorrhage, cerebral ischemia, and nervous system disorders in China [[78], [79], [80]]. In view of critical cases with COVID-19 might suffer from consciousness disturbance, *Xingnaojing* Injection had major biological effects to relax the cerebral vascular and protect the mature neuron, in vivo and in vitro research, it was confirmed that the mechanism of cerebrovascular protection might be relevant to the activation of PI3K/Akt/eNOS signaling pathways and the suppression of NLRP3 inflammasomes [81,82].

6. Only adjuvant rescue for critical infection

In aforementioned guidelines, five CPMs were recommended as adjuvant rescue just for critical infections with COVID-19, namely *Suhexiang* Pill, *Angong Niuhuang* Pill, *Shenfu* Injection, *Shengmai* Injection, *Shenmai* Injection. Currently, there were paucity of accessibly published evidence concerning on these CPMs and 2019-nCoV simultaneously, it was urgent and essential that subsequently clinical trials or pharmacological research to provide sufficient references for clinical recommendation. Herein, we brief summarized the findings in the field of infectious or health-threatening diseases to supplement the correlative knowledge.

Although both of them were famous Chinese emergency prescription and contained same resuscitation-inducing aromatic herbs as *Bingpian* (Borneolum Syntheticum), *Suhexiang* Pill and *Angong Niuhuang* Pill were used for patients with opposite syndromes. The former was adopted to seizures, infantile convulsions and stroke with cold syndromes [83], therefore, *Suhexiang* Pill might achieve therapeutic effectiveness for COVID-19 cases with critical conditions such as delirium, phlegm syncope, central nervous depression, and coma or worsen. Its neuroprotective, anticonvulsant and antioxidative effects had been proven by fundamental research both in vitro and in vivo, suggesting its pharmacological mechanism involved the suppression of JNK hyperactivation and apoptosis, inhibition of EGFR/ERK pathways and glial cell proliferation, decreasing ROS formation and restoring mitochondrial function [[84], [85], [86], [87]]. As a recipe of "*Liangkai Sanbao*" in TCM theory, the latter was recognized to treat heat diseases only, including acute ischemic stroke, viral encephalitis, acute hemorrhagic stroke, and trauma brain injury, *Angong*

Niuhuang Pill could play desirable role for treating critical infections with 2019-nCoV in attenuating the negative symptoms including hyperthermia, stupor, coma, etc. [88]. Meanwhile, based on the research was conducted on a high-fat and vitamin D3-induced rodent model of atherosclerosis, the results presented that *Angong Niuhuang* Pill had antiplatelet aggregation, lipid regulatory, antioxidant, anti-inflammatory and anti-apoptotic properties contributing to robust ant-atherosclerosis and cardio-protective effects [89], which might be beneficial for critical cases with cardiovascular and cerebrovascular diseases. The mechanisms of *Angong Niuhuang* Pill exhibited the neuroprotection was related to depressed Bax/Bcl-2 ratio and caspase-3 level, resulting the inhibition of apoptotic cell [90].

Three ginseng containing formulations, *Shenfu* Injection, *Shengmai* Injection and *Shenmai* Injection had similar therapeutic effects of tonifying *Qi* and preventing exhaustion, they could be adjuvant rescue and alternative treatment of COVID-19 patients with septic shock, viral myocarditis, and cardiogenic shock in clinical practice [[91], [92], [93]]. For example, in rabbits with LPS-induced septic shock, *Shenfu* injection could increase mean arterial pressure, decrease the serum lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and glutamate transaminase (ALT) levels, improve the tissue morphology of heart, liver and kidney, and increase the contents of ATP and taurine in the heart tissue during septic shock [94]. Besides, previous studies demonstrated that *Shenfu* Injection, *Shengmai* Injection and *Shenmai* Injection were associated with protective effects on lung ischemia-reperfusion injury, reducing chemotherapy-induced adverse effects, and promoting cellular immunity and cognitive dysfunction and so on, presumably, these clinical functions might improve symptoms of critical patients with COVID-19 in terms of lung inflammation, virus infection, drug-induced disease lung injury [[95], [96], [97], [98]].

7. Discussion

Since the globally health-care-associated outbreaks of 2019-nCoV, in China, the implementing “attach equal importance to TCM and Western medicine” policy in clinical practice exerted essential effects for treating COVID-19 [99,100]. Based on unique guiding principles of syndrome differentiation and treatment in TCM, its clinical superiority had considerable recognition and public acclaim in the furious battle for 2019-nCoV, the cumulative number of research revealed that its curative advantages involved in whole treatment process, namely prevent disease progression, alleviate clinical symptoms, improve the hospital stay and negative results of nucleic acid detection, and promote the physical recovery [21,22,101]. Notably, the majority of enrolled CPMs possessed the medical history approximately 20 years in clinical practice among Chinese citizens, therefore, the clinical applications of CPMs to tackle the epidemic were with definite therapeutic effects, clinical basis, social recognition and other superior factors. For example, *Huoxiang Zhengqi* Capsule and *Lianhua Qingwen* Capsule was over-the-counter medicines, and some CPMs were covered by national lists of essential medicine and basic insurance program in China.

Regarding suspected and mild patients with COVID-19, we calculated the frequency of compositions for each CPMs, the results revealed that the crude herbs with the higher frequency of recommended CPMs were *Jinyinhua* (Lonicerae Japonicae Flos), *Lianqiao* (Forsythiae Fructus) and *Gancao* (Glycyrrrhizae Radix Et Rhizoma), and they all had therapeutic effects of clearing heat and detoxication, which could bring potential for the prevention and treatment of 2019-nCoV. For instance, phillyrin, the natural lignan of *Lianqiao*, had antibacterial, anti-quorum sensing and anti-inflammatory activities for the control of infectious pathogens via the regulation the MyD88/IκBα/NF-κB signalling pathway, decrease the virus titers and IκBα, IL-1β, IL-6, TNF-α levels, reduction the expression of influenza hemagglutinin protein, and attenuation of lung tissue damage [[102], [103], [104]]. With regard to severe cases, the Chinses herbal injections could obviously alleviate clinical symptoms and correct complications. In particular, *Xuebijing* injection was widely used for treating patients with critical diseases including sepsis, severe pneumonia, severe acute pancreatitis, infection-induced systemic inflammatory response syndrome, chronic obstructive pulmonary disease (COPD), ARDS, multiple organ dysfunction syndrome (MODS) and so on [104,105]. Moreover, our study highlighted that its clinical beneficial effects in severe cases was closely associated with the inhibition of cytokine storm, improve virus infection and lung injury, superior binding activities with 3CLpro and ACE2. In addition, three types of recommended herbal injectable dosage forms contained *Hongshen* (Ginseng Radix Et Rhizoma Rubra), ginseng was generally known for its tonic properties, and previous findings also suggested it might be a promising supplemental remedy against infectious diseases, this administration of ginseng could fast and accurate adjust excess or deficiency between *Yin* or *Yang* and restore their balance among critical patients [[106], [107], [108]].

When great attention was paid to the application of TCM in real-world, there were growing concerns related to the potential toxicity or inevitable ADRs of herbal medicines. The results of pharmacovigilance pointed out the risk factors of ADRs triggered by TCM including responsible Chinese materia medica, susceptible patients and clinical administration [109]. In this regard, our research should emphasize the following aspects, including poisonous composition, the safety status of Chinese herbal injection, special population, and irrational drug use. First, *Angong Niuhuang* Pill contained cinnabar (HgS) and realgar (As_2S_2) which were correlated to hepatorenal toxicity [110]. *Fuzi* (Aconiti Lateralis Radix Praeparaia) in *Shenfu* Injection was associated with narrow therapeutic window, its cardiac ADRs has been frequently observed that mainly manifested as palpitations, hypotension, arrhythmia, ventricular fibrillation, and even shock [111,112]. Second, compared with other dosage forms of TCM medications, Chinese herbal injections were associated with the higher risk of ADRs, especially serious side effects [113,114]. Therefore, during the treatment of COVID-19, corresponding measures including drug safety monitoring and risk management for CPMs should be strengthened to achieve optimal benefits and minimal hazards. Third, the available evidence highlighted that special population including children, gravida and elderly people were particularly vulnerable to unfavorable drug responses, because their pharmacokinetic and pharmacodynamic profiles differed from the general population [115]. Indeed, clinical medication was complicated, the irrational uses reflected in misuse or abuse application of CPMs, overdose, repeated medication, dissolvant mismatch, pharmacologic antagonism, pharmaceutical incompatibility, herb-drug interaction and inappropriate syndrome differentiations and so on, these factors all would be culminating in drug-induced risk. The selection of treatments for individuals infected 2019-nCoV should be consistent with the theory of TCM. For example, it was not suitable to receive tonic herbs and *Lianhua Qiwen* Capsule for cases with COVID-19 simultaneously. Besides, *Huoxiang Zhengqi* Liquid contained ethanol, which could cause disulfiram-like reaction combined with cephazolin, cefoperazone, cefoperazone and sulbactam, latamoxef and so on [116]. To overall contain the COVID-19 pandemic, except for support medication and equipment supply, we suggested that pharmacists should play important role in the guarantee of medication safety including prescription checking and the rational use of CPMs.

Several limitations of our study should be noted. Unsatisfactory, there were lack of published research concerning on both COVID-19 and some recommended CPMs, such as those were only adjuvant rescue for critical infection (*Suhexiang* Pill, *Angong Niuhuang* Pill, *Shenfu* Injection, *Shengmai* Injection, *Shenmai* Injection). Thus, clinical benefits and therapeutic mechanism of CPMs against COVID-19 should be substantiated further confirmed and illustrated by the well-designed fundamental studies. Present review only focused on the recommended CPMs for treating COVID-19, except for the enrolled CPMs, it was noteworthy that plenty of prescriptions in TCM that were crucial to the prevention and management of COVID-2019, such as *QingFei Paidu* decoction, the relevant retrospective study showed that *Qingfei Paidu* decoction combined with western medicine in the treatment of COVID-19 was more effective than patients receiving western medicine alone, which can significantly shorten the hospitalization, improve clinical symptom and imaging results [117].

In conclusion, the present study was devoted to summarize the comprehensive information and updated evidence of recommended CPMs for different suitable patients with COVID-19. As accessible, efficient and modern products of TCM, CPMs played an indispensable role in the prevention and treatment of this epidemic diseases, it could be an alternative approach against COVID-19 in both suspected cases and high-risk population. In order to share Chinese experiences in clinical practice and provide scientific references for the international health systems, the continued evidence was still needed to support existing therapies of CPMs for 2019-nCoV.

Declaration of Competing Interest

The authors of the manuscript that titled “The clinical benefits of Chinese patent medicines against COVID-19 based on current evidence” declared that there are no conflicts of no financial/personal interest.

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Recently, there have been many controversies around the world about the treatment of coronavirus disease 2019 (COVID-19). As a traditional Chinese medicine (TCM) practitioner, I think that the TCM treatment of COVID-19 is ignored. On Feb 6, 2020, the guideline for rapid advice on diagnosis and treatment of 2019-nCoV pneumonia has been released in China [1]. For the treatment of COVID-19, the guideline recommended using routine treatment plus TCM. Routine treatment mainly includes the use of antiviral and antibiotic drugs, providing nutritional support, and giving mechanical ventilation when necessary and so on. As far as I know, since December 2019, the majority of COVID-19 patients in China have been treated with integrated Chinese and Western medicine. Hundreds of herbs have been used throughout the country. Most Chinese herbal medicines can be divided into three categories: clearing heat, eliminating dampness and detoxification. At the Eighth People's Hospital of Guangzhou, China, doctors found that 50 patients with mild COVID-19 were treated with Toujie Quwen granule, and all patients returned to normal temperature after 1 week, their overall symptoms improved significantly, without any serious symptoms. It was found that this drug significantly improved the clinical symptoms caused by COVID-19 and had a tendency to reduce the occurrence of severe pneumonia [2].Recently, 4 provincial hospitals in China used Qingfei Paidu decoction to treat 214 patients with COVID19, taking 3 days as a course of treatment, and the total effective rate reached more than 90%. Among them, the

symptoms and imaging performance of 60% of patients improved significantly. 30% of patients have smooth symptoms without exacerbation [3]. Despite there are some methodological problems in these studies, such as no control groups, no mention of randomization, lack of blinding and allocation concealment designs, unclear evaluation indicators, and lack of long-term efficacy and follow-up, but considering the urgency of patients' treatment need, large-scale randomized controlled studies are almost impossible and involve ethical issues. There is no high-quality evidence for the safety of some Chinese herbs, but when used correctly, it is generally believed that there are no serious adverse reactions [4]. In veiw of the current evidence for the treatment of COVID-19 mainly comes from in vitro cell tests, animal experiments, and data mining, the level of evidence for the effectiveness of TCM research is relatively high, because it is clinical and real-world studies.

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Abstract

The outbreak caused by COVID-19 is causing a major challenge to clinical management and a worldwide threat to public health. So far, there is no specific anti-coronavirus therapy approved for the treatment of COVID-19. Recently, as the efficacy and safety of traditional Chinese medicine (TCM) is widely acknowledged, it has been brought to a crucial status by the public, governments, and World Health Organization (WHO). For a better popularization of TCM, governments have made several advances in regulations and policies for treatment and measures of novel coronavirus pneumonia (NCP). Therefore, on the basis of epidemiology and virology information, we reviewed relevant meta-analysis and clinical studies of anti-coronavirus therapeutics by TCM, in the aspect of mortality, symptom improvement, duration and dosage of corticosteroid, incidence of complications and the like. In addition, we also summarized preclinical rationale for anti-coronavirus activity by TCM in terms of virion assembly and release, as well as viral entry and replication, which could be a useful contribution for figuring out effective Chinese herbal medicine (CHM) for coronavirus, including ingredients from single monomeric compounds, Chinese herbs, Chinese herb extracts and Chinese herb formulas, or potential targets for medicine. We would like to see these relevant studies, ranging from basic researches to clinical application, could provide some idea on effects of CHM to combat COVID-19 or other coronaviruses, and also offer new thinking for the exploration of therapeutic strategies under the guidance of TCM.

115. Zhang Q, Cao F, Wang Y, Xu X, Sun Y, Li J, Qi X, Sun S, Ji G, Song B. The efficacy and safety of Jinhua Qinggan granule (JHQG) in the treatment of coronavirus disease 2019 (COVID-19): A protocol for systematic review and meta analysis. Medicine (Baltimore). 2020 Jun 12;99(24):e20531. doi: 10.1097/MD.0000000000020531.

Abstract

Background:

Currently, the global number of infected novel coronavirus has exceeded 2.6 million and the death toll has exceeded 170,000, but the specific drug for the treatment of COVID-19 has been not appears. In the process of fighting COVID-19 in China, JHQG has been promoted by the Chinese government and widely used in the treatment of COVID-19. The purpose of this study is to systematically evaluate the efficacy and safety of JHQG for COVID-19.

Methods:

We are going to search the electronic databases: PubMed, EMBASE, Cochrane library, Web of Science (WOS), Google scholar, China National Knowledge Infrastructure (CNKI), Chinese Biomedical literature Database

(CBM), Chinese Scientific and Journal Database (VIP), Wan Fang database (Wanfang) for published clinical trails and search clinical trials register platforms of Chinese Clinical Trial Registry (ChiCTR) and ClinicalTrials.gov (www.ClinicalTrials.gov/) for ongoing trials of Jinhua Qinggan granule for COVID-19. The primary outcomes of the included studies contain Clinical symptom disappearance rate and the secondary outcomes obtain: TCM syndrome scale score, Hamilton anxiety scale score, and adverse events. We will use RevMan V5.3 software to perform the calculations. PRISMA-P checklist was used in writing this report.

Results:

The study results will be submitted to a peer-reviewed journal for publication.

Conclusion:

This study will provide a high-quality evidence of the efficacy and safety of Jinhua Qinggan granule on patients with COVID-19.

PROSPERO registration number:

CRD42020181919.

1 Introduction

In December 2019, COVID-19 caused by severe acute respiratory syndrome coronavirus 2(sars-cov-2) was first discovered in Wuhan China.[1–3] The virus was found to be highly infectious and susceptible to infection in all kinds of people. At present, the epidemic has spread rapidly, with more than 2.6 million COVID-19 cases confirmed globally,[4] which is seriously threatening human life and health. On March 12, 2020, the WHO declared the COVID 19 outbreak to be characteristically a pandemic. Symptoms of COVID-19[5–8] mainly include cough, fever, expectoration, dyspnea, chest pain, fatigue, loss of appetite, headache, myalgia, hemoptysis, and diarrhea. As a new viral infectious disease, COVID-19 still can not be treated with specific therapeutic drugs.[9] A recent study[10] found that among the confirmed patients, 69.9% of patients were diagnosed with mild pneumonia, 25.5% with severe pneumonia, patients with severe condition always to be the old male. Another study[11] of 41 confirmed COVID 19 inpatients showed similar results: the majority of infections were male (30 out of 41 cases, 73%).

During the fight of COVID-19 epidemic in China, it was found that Chinese herbal medicine had a good effect on COVID-19. Chinese central government had formulated series of TCM diagnosis and treatment plans for COVID-19,[12–16] and recommended a batch of Chinese herbal medicines for the prevention and treatment of COVID-19.[15,16] JHQG is one of them. However, there has been no systematic review of JHQG for COVID-19. Therefore, in this study, the efficacy and safety of the application of JHQG to COVID-19 will be systematically evaluated.

2 Methods and analysis

We used Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement[17] in writing this report.

2.1 Study inclusion criteria

Studies of Randomized controlled trials (RCTs) with JHQG for COVID-19 will be included. There won't be any restriction on publishing language and publishing status. Patients diagnosed with COVID-19 of all ages. Genders and racial groups will be included. The intervention of the experimental group should contain JHQG alone or with other interventions. While, the control group could use any other treatments (e.g., External therapy, usual or standard care, placebo, or no treatment) except JHQG. Primary outcomes of the included studies contain Clinical symptom disappearance rate. The secondary outcomes obtain: TCM syndrome scale score, Hamilton anxiety scale score and adverse events.

2.2 Search methods

We will search the following electronic databases of PubMed, EMBASE, Cochrane library, Web of Science (WOS),Google scholar, China National Knowledge Infrastructure (CNKI), Chinese Biomedical literature Database (CBM), Chinese Scientific and Journal Database (VIP), and Wan Fang database (Wanfang) for identifying literature of studies of JHQG for COVID-19, the search strategy of Pbumed will be shown as Table 1, we would also change the search strategy properly according to exact database. Unpublished data of Ongoing trials will be searched from Chinese Clinical Trial Registry (ChiCTR) and ClinicalTrials.gov (www.ClinicalTrials.gov/). All the databases and online registration platforms will be searched from inception to May 30, 2020 and there will be no language restrictions.

Search strategy for PubMed.

2.3 Data collection and management

2.3.1 Selections of studies

Two reviewers (CF and XQ) will import the retrieved literature into Endnote software (V. X9.0), they will screen literature by inclusion and exclusion criteria independently. Firstly, duplicate literature will be eliminated by Endnote, and then the obviously inappropriate ones will be eliminated by reading the title and abstract. Finally, the last inappropriate literatures will be eliminated by reading the full article. (Fig. 1 shows the screening process) If it comes with disagreements, which will be arbitrated by a third reviewer (JNL)

The screening process.

2.3.2 Data extraction

Two reviewers (CF and XQ) will review all the included studies and extract data contain items of title, first author, publication year, country, publication language, journal, information of participants: gender, age, study design, sample size, intervention, controls, type of measures, primary and second outcomes and other detail information. All the data, which will be cross-checked by the 2 reviewers, would be extracted with Excel (V.2019) software. The results will be cross-checked by the 2 reviewers.

2.4 Risk of bias assessment

Two reviewers (QSZ and FC) will assess the methodological quality of the included studies with the Cochran collaboration tool. The bias of sequence generation, allocation concealment, blinding of participants personnel and outcome assessment, incomplete outcome data, selective outcome reporting, and other bias will be accessed by the 2 authors. A third reviewer (BLS) will arbitrate the disagreement which appears in the evaluation process. The risk of bias would be classified[18] as low, high, or unclear.

2.5 Measures of treatment effect

To assess JHQG in the treatment of COVID-19. We will use relative risk (RR) for dichotomous data. Mean differences (MD) or the standard mean differences (SMD) for continuous data. Using 95% confidence intervals (CI) to show the effect sizes.

2.6 Dealing with missing data

Two reviewers (GCJ and XQ) will try to contact the corresponding author for missing or insufficient by e-mail or telephone. We will perform the analysis based on the available studies, if we failed to get the data missed. We would also evaluate the potential impact of missing information on the outcome.

2.7 Assessment of heterogeneity

Statistical heterogeneity of included studies will be assessed with a standard I^2 test, if $I^2 < 50\%$, the heterogeneity of the texts may be ignored, the fixed-effect model will be applied. While if $I^2 \geq 50\%$, statistical heterogeneity will be regarded as significant, the random-effects model will be used.

2.8 Assessment of reporting bias

We will make use of funnel plot to assess the reporting bias with more than 10 studies are included.[19] If the funnel is symmetrical, which indicates there is no publishing bias, otherwise, there is. But if the included studies are less than 10, *P* value will be used.

2.9 Data syntheses

We will make use of RevMan (version 5.3) software to conduct the meta-analysis. Fix-effect model will be applied with the condition that there is no heterogeneity of the results. Otherwise, we will turn to the random effects model after the clinical heterogeneity has been taken out.

2.10 Analysis of subgroups or subsets

Subgroup analysis will be conducted based on gender, age, hospitalization time, or other conditions of participants, if potential heterogeneity exists in the included studies.

2.11 Sensitivity analysis

We would conduct a sensitivity analysis to assess the robustness of the study results. We will focus on the processing method of missing data, sample size, and methodological quality.

2.12 Grading the quality of evidence

We will take advantage of Grading of Recommendations Assessment, Development and Evaluation Reliability Study (GRADE) to assess the quality of evidence. The grades are very low, low, moderate, and high.

2.13 Ethics and dissemination

Ethical approval is not needed for this systematic review. For nothing of the data in this review is related to an individual patient.

3 Discussion

COVID-19 is wreaking havoc around the world. JHQG is a traditional Chinese herbal medicine prescription, which was recommended by the National Health Commission of China in the Plan of Diagnosis and Treatment for COVID-19.[13–16] JHQG has been made extensive use in the treatment of COVID-19 in China, recent studies shows that JHQG has good effect on the treatment of COVID-19, However, the mechanism is not clear, health authorities in countries other than China may have doubts about the effectiveness and safety of JHQG. Thus, a systematic review about it is urgently needed. This study could provide evidence of JHQG used in the treatment of COVID-19 and help the clinicians to make decisions.

Author contributions

QSZ, YFW, BLS and FC designed this review. JNL, XQ, and XHX contributed to developing the search strategy and drawing the figure of the study selection process. QSZ, YHS, GCJ, and SQS wrote the manuscript. All authors approved the final version of the manuscript.

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Abstract

The outbreak caused by 2019 novel coronavirus(2019-nCoV) is still spreading, posing a great threat to the safety and health of general population. However, there have not been any effective drugs for treatment, with symptomatic treatment and prevention prevailing. The treatment plans of severe acute respiratory syndrome(SARS) and Middle East respiratory syndrome(MERS) are often used for reference in clinic. The advantages of traditional Chinese medicine(TCM) in treating SARS and MERS are that it can intervene and block the progression of disease in early stage, significantly reduce symptoms, shorten the treatment duration of patients, reduce complications and side effects caused by hormone therapy. The coronavirus disease 2019(COVID-19) belongs to the category of TCM epidemic diseases. Chinese patent medicines and prescriptions in medical observation and clinical treatment were recommended in the "pneumonia diagnosis and treatment plan for new coronavirus infection"(trial version fifth) of the National Health Commission of the People's Republic of China. Qingfei Paidu Decotion was recommended for the treatment of COVID-19 by the National Health Commission of the People's Republic of China and National Administration of Traditional Chinese Medicine. TCM shows good clinical efficacy and great potential in the treatment of COVID-19. Previous studies of TCM have shown broad-spectrum antiviral activity, providing a variety of sources for the discovery of new antiviral drugs. In this paper, we reviewed traditional Chinese medicines and its active ingredients in the hope of bringing novel inspirations to the drug screening and clinical treatment for COVID-19.

Keywords: 2019-nCoV; COVID-19; Chinese herbal medicine; Middle East respiratory syndrome; human coronavirus; intervention effect; severe acute respiratory syndrome.

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118. **Zhou Z, Zhu CS, Zhang B. Study on medication regularity of traditional Chinese medicine in treatment of COVID-19 based on data mining. Zhongguo Zhong Yao Za Zhi. 2020 Mar;45(6):1248-1252. doi: 10.19540/j.cnki.cjcmm.20200220.502.**

Abstract

The coronavirus disease 2019(COVID-19) is developing rapidly and posing great threat to public health. There is no specific medicine available for treating the disease. Luckily, traditional Chinese medicine has played a positive role in the fighting against COVID-19. In this paper, We collected and sorted the prescriptions of modern Chinese medicine for COVID-19 released by national government, different provinces, autonomous regions and municipalities, as well as online databases, such as CNKI, WanFang medical network, and VIP database. These prescriptions were combined with the inheritance of traditional Chinese medicine auxiliary V2.5, and the complex system entropy clustering method was used to determine the association rules and frequency of single drug and drug combination in the prescription. In the end, 96 effective prescriptions were included. Among them, the four properties were mainly concentrated in temperature, cold and level, the five tastes were mainly concentrated in bitter, hot and sweet, and the meridians were mainly concentrated in lung, stomach and spleen. The high-frequency drugs were Glycyrrhizae Radix et Rhizoma, Armeniacae Semen Amarum, Gypsum Fibrosum, etc., and the high-frequency combinations are Gypsum Fibrosum-Armeniacae Semen Amarum, Gypsum Fibrosum-Glycyrrhizae Radix et Rhizoma, Armeniacae Semen Amarum-Glycyrrhizae Radix et Rhizoma, the core combinations are Lepidii Semen-Armeniacae Semen Amarum-Gypsum Fibrosum, Pogostemonis Herba-Zingiberis Rhizoma Recens-Magnoliae Officinalis Cortex, Ophiopogonis Radix-Armeniacae Semen Amarum-Scutellariae Radix and so on. Form new prescriptions Lepidii Semen, Armeniacae Semen Amarum, Gypsum Fibrosum, Pogostemonis Herba, Zingiberis Rhizoma Recens, Magnoliae Officinalis Cortex. Ophiopogonis Radix, Armeniacae Semen Amarum, Scutellariae Radix, Schisandrae Sphenantherae Fructus, Panacis Quinquefolii Radix. From the medicinal properties to high-frequency drugs and new prescriptions, it could be seen that the overall treatment of COVID-19 by traditional Chinese medicine was to strengthen body resistance, eliminate pathogenic factors, and give attention to Qi and Yin.

119. **Zhu Y, Jiang Z, Zhang Y, Zhang Q, Li W, Ren C, Yao R, Feng J, Ren Y, Jin L, Wang Y, Du B, Li W, Huang H, Xi X. Assessment of Chinese medicine for coronavirus-related pneumonia: A protocol for systematic review and meta-analysis. Medicine (Baltimore). 2020 Jun 12;99(24):e20613. doi: 10.1097/MD.0000000000020613.**

Abstract

Background: The 2019 novel coronavirus disease has caused a global pandemic with substantial morbidity and mortality. Chinese medicine has been extensively employed in the coronavirus-related pandemic in China. We aim to assess the efficacy and safety of Chinese medicine in treatment of coronavirus-related pneumonia with the updated results of relevant clinical trials.

Methods: Six electronic databases including PubMed, EMBASE, Cochrane Library, China National Knowledge Infrastructure, Chongqing VIP, and SinoMed will be searched to identify randomized controlled trials up to May 2020. Patients diagnosed with coronavirus-related pneumonia including severe acute respiratory syndrome, Middle East respiratory syndrome, and 2019 novel coronavirus disease and administrated with Chinese medicine will be included. The primary outcome is the all cause mortality at the longest follow up available. The second outcomes include the length of stay in hospital and intensive care units, the duration of mechanical ventilation, and adverse events. The pooled effects will be analyzed and reported as risk ratios for dichotomous data using the Mantel-Haenszel method or mean differences for continuous data using the inverse-variance method. Sensitivity and subgroup analyses will be performed to test the robustness of the results and to explore the potential sources of heterogeneities. The Egger test and/or funnel plots will be used for the examination of publication bias. The grades of recommendation assessment, development, and evaluation methodology will be used to summarize the quality of evidence. The trial sequential analysis will

be conducted to test whether the meta-analysis has a sufficient sample size after adjustment of the increased type I and II error risks.

Results: The evidence to date of Chinese medicine in treatment of coronavirus-related pneumonia will be systematically reviewed and meta-analyzed.

Conclusion: The relevant studies will be summarized and further evidence will be provided.PROSPERO registration number: CRD42020178879.