ORIGINAL RESEARCH

Anti-Inflammatory and Analgesic Effects and Potential Targets of Shenzhu Jiedu Granule Against Novel Coronavirus Pneumonia Based on Network Pharmacology

Yin Zhang, PhD; Wen-Sheng Qi, PhD

ABSTRACT

Objective • The objective of this study was to investigate the preventive and therapeutic effects of Shenzhu Jiedu Granule on COVID-19 using network pharmacology and animal experiments.

Methods • Obtain the chemical components of Shenshu Jiedu Granule from the online pharmacology database and analysis platform (ETCM) of the Chinese traditional medicine system, obtain the potential target of the compound through the UniProt database, and obtain the related target of COVID-19 from GeneCards and OMIM databases; Construct a component target network diagram using Cytascape 3.7.0 software, import the protein interaction (PPI) of intersection targets into Cytascape software through STRING database, and use the Metascape platform to conduct gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomics (KEGG) enrichment analysis on intersection targets.To explore its anti-inflammatory and analgesic effects through animal ear swelling, hot plate and torsion experiments.

Results • Analysis revealed 72 key target proteins associated with the effects of Shenzhu Jiedu Granule demonstrated that mainly interleukin-6 (IL-6), interleukin-1 β (IL 1 β), B cells κ Light peptide gene enhancer nuclear factor inhibitor 1 (NFKB1), B cells κ Light peptide gene enhancer nuclear factor inhibitor 1B (NFKB1A), interferon β IFNB1, tumor necrosis factor TNF, recombinant human mitogen activated protein kinase 12 (MAPK12), serine/threonine kinase 1 (AKT1), B cells κ Light peptide gene enhancer inhibitor kinase β (IKBKB), etc.The analysis found that it is mainly related to multiple biological processes such as intercellular immune regulation, inflammatory cytokines, and ion channels in the microenvironment; KEGG analysis showed that COVID-19 pathway, influenza virus pathway and multiple immune inflammatory response pathways were mainly involved. Obtained 91 effective ingredients of Shenshu Jiedu Granule, 10 anti-inflammatory, bactericidal, and antiviral compounds, and 4 immune enhancing compounds. Shenzhu Jiedu Granule demonstrated an inhibitory effect on xylene-induced ear swelling in mice and significantly enhanced the anti-inflammatory and analgesic effects by reducing body twists and prolonging the time mice licked their feet.

Conclusions • It is suggested that Shenzhu Jiedu Granule has anti COVID-19, influenza virus, antibacterial and anti-inflammatory effects, and can significantly enhance the anti-inflammatory and analgesic effects of mice, which highlight the significance of the study in the context of current global health concerns. (*Altern Ther Health Med.* 2024;30(4):172-179)

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INTRODUCTION

A combination of remdesivir with baricitinib worked better in reducing recovery time of hospitalized patients with COVID-19 pneumonia. In 2019, the novel coronavirus Disease 2019 (COVID-19, referred to as "COVID-19" for short) swept the world, seriously affecting public health and health. After the outbreak of the epidemic, Chinese medicine has had a definite effect on preventing and treating COVID-19. Traditional Chinese medicine, including Sang Ju Yin and Yu Ping Feng San among others, have been used in the prevention and treatment of SARS and H1N1, and was explored in the treatment of COVID-19. In 2022, the World Health Organization (WHO) officially released the Report of the World Health Organization (WHO) Expert Assessment Meeting on Traditional Chinese Medicine for the Treatment of COVID-19, which clarifies the role of Chinese medicine in the effective prevention and treatment of COVID-19 mild symptoms and in reducing the incidence of severe incidence rate with western medicine, It is proposed and encouraged that the combination of traditional Chinese medicine and western medicine is the development trend of preventing and controlling the spread and pathogenicity of COVID-19.[1]

Professor Qi Wensheng, from SAS[2] to the prevalence of the COVID-19 epidemic,[3] has personally visited the outbreak area, used traditional Chinese medicine to prevent and control epidemic viruses, and accumulated rich experience in epidemic prevention and control. Professor Qi Wensheng believes that the epidemic is still a "damp poison epidemic", and the mild syndrome is that the damp poison invades the lungs, spleen, and stomach.[4] The method of dispelling filth and detoxification and its empirical formula, Shenshu Jiedu Granule, are still the most effective way to fight against COVID-19. The combination of this prescription and western medicine can effectively inhibit the severe incidence and mortality of light and medium COVID-19 patients. According to anti-epidemic experience, a mature theoretical system has been formed for the transmission characteristics and prevention of COVID-19,[5] and has participated in the preparation of the Diagnosis and Treatment Plan for novel coronavirus Pneumonia for many times, especially the trial implementation of the ninth edition has played a guiding role in the clinical treatment and prevention of the infection of Omikjon mutant strain.[6] In 2020, its experience Fangshenshu Jiedu Granule was specially approved by the "Beijing Municipal Drug and Food Administration" as a hospital preparation in Guang'anmen Hospital and was approved by the Administration of Traditional Chinese Medicine as a research and development variety of new anti-COVID-19 drugs.

Shenshu Jiedu Granule refers to the addition and subtraction of Shenshu Powder recorded in Taiping Huimin Heji Jufang. It is composed of atractylodes macrocephala and bran, tangerine peel, honeysuckle, baimao root, mulberry leaf, of which atractylodes macrocephala can eliminate evil spirits, diseases at all times and in all countries. People often burn atractylodes macrocephala at home to dispel evil spirits, so they often use epidemics". This prescription is mainly made of Atractylodes macrocephala, which removes dirt, promotes dampness, and strengthens the spleen. Honeysuckle and mulberry leaves are courtiers of anti-COVID-19 ingredients.[7] The latest research found that Atractylodes macrocephala can inhibit the replication of COVID-19 in vivo and the expression of inflammatory factors.[8] Tangerine peel can regulate qi turbidity and relieves asthma, cough, and anti-allergic inflammation.[9] Honeysuckle has spectral antibacterial, antiviral, antipyretic, anti-inflammatory properties that regulate immunity,[10] and can alleviate symptoms such as throat discomfort and cough. Baimao root has the effect of cooling blood and diuresis,[11] and contains various mucus and polysaccharide substances, which can play a role in moistening the throat and relieving cough.[12] Mulberry leaves can promote lung heat dissipation, nourish yin, and moisten the lungs.[13] The combination of five drugs can remove dirt, detoxify, clear heat, promote lung harmony, and is used to treat fever, myalgia, sore throat, cough, and fatigue in COVID-19 syndrome. Network pharmacology has been one of the academic frontiers of traditional Chinese medicine research. This project applies network pharmacology to draw a "Shenshu Jiedu Granule Traditional Chinese Medicine Component Target Interaction Model", through component target mapping, gene ontology (GO), enrichment analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG), and construction of protein-protein interaction (PPI) networks, To explore the key target and biological pathway of Shenzhu Jiedu Granule in the prevention and treatment of COVID-19, and verify its anti-inflammatory and analgesic effects through mouse ear swelling, hot plate, and body twisting experiments, to provide theoretical data support for the research and development of COVID-19 products. This study aims to explore the preventive and therapeutic effects of Shenshu Jiedu Granule on COVID-19 using network pharmacology analysis and animal experiments.

MATERIALS AND METHODS

Obtain TCM information

Collection of effective chemical components and mapping targets of Shenshu Jiedu Formula: using the Encyclopedia of Traditional Chinese Medicine (ETCM) database, http://www.tcmip.cn/ETCM/ Index. php/Home/ Index/), to detect the main components and target proteins corresponding to the main components of five traditional Chinese medicines composed of Shenzhu Jiedu Granules, namely, bran fried Atractylodes lanceolate, honeysuckle, mulberry leaves, tangerine peel, and baimao root, and to build a chemical composition database of Shenzhu Jiedu Granules. Through the UniProt database, https://www.uniprot.org/, uniformize target names and draw a "traditional Chinese medicine component target" model based on Cytascape 3.8.2 software.

Obtain the target of COVID-19

COVID-19 mapping targets are collected on the genome annotation database platform (GeneCard), https://www.genecards.com In the online Mendelian Inheritance in Man, OMIM, https://omim.org/, COVID-19 targets were searched using the keyword "Corona Virus Disease 2019" all data were summarized, and duplicate values were deleted to obtain all disease targets. Intersect the mapping target of Shenshu Jiedu Granule and the action target of COVID-19, and draw a Wayne diagram.

Constructing a "Traditional Chinese Medicine Compound Target" Network

Use R language to intersect the drug target of Shenshu Jiedu Formula and the COVID-19 disease target to obtain a common target, and draw a Wayne diagram. Import the obtained target data into Cytascape 3.7.0, and construct a "traditional Chinese medicine compound target" network to visually display the relationship between the active components of drugs and diseases.

Predictive target PPI network analysis (gene co-expression analysis)

Based on the STRING11.5 database (http://string-db.org), Introduce the predictive target of Shenshu Jiedu Granule for COVID-19 treatment, define the species as "Homo sapiens" set the maximum confidence parameter score>0.4, and the remaining parameters remain unchanged. Construct a PPI core network of predictive targets of Shenshu Jiedu Granule for COVID-19 treatment.

Target enrichment analysis

With Metascape (http://metascape.org/) Analysis of candidate target gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). GO includes cell component (CC), molecular function (MF), and biological process (BP) to interpret candidate target therapy COVID-19 biological process; KEGG focuses on studying the main therapeutic COVID-19 signaling pathway associated with candidate targets. Based on the above understanding, 20 GO, and KEGG pathways with significant differences were selected, and the results were visually analyzed using R software. Relying on the relevant targets mapped by KEGG, conduct GO function enrichment analysis and KEGG pathway enrichment analysis on the intersection targets of Shenshu Jiedu Formula in treating COVID-19 and draw bubble charts, respectively.

Animal experimental materials

100 healthy Kunming male and female mice, half male and half female, SPF grade, weighing 18-20 g, purchased from a limited company with an animal production license number of (). The experiment was conducted in accordance with the Guiding Opinions on the Treatment of Experimental Animals issued by the Ministry of Science and Technology of the People's Republic of China. The Ethics Clerk Committee of Guang'anmen Hospital approved the experiment. It was raised in the animal room of Guang'anmen Hospital, with a room temperature of 22°C to 25°C, relative humidity of 40% to 50%, free access to food and water, and adaptive feeding for 3 days under 12 hours of light.

Drug Preparation

Shenshu Jiedu Granules (produced by Chunfeng Pharmaceutical entrusted by Guang'anmen Hospital, (production batch number: W2209002); Ganmao Qingre Granule (Pharmaceutical Factory of Beijing Tongrentang Technology Development Co., Ltd., Batch No.: Guoyao Zhunzi Z11020361); Aspirin enteric coated tablets (Bayer S.p.A., batch number: GYZZ HJ20160685).

Reagents and instruments

Glacial acetic acid (AR, 99.5%, Sinopharm Chemical Reagents Co., Ltd., LOT #: c14276200); Puncher (diameter 8 mm); RB-200 Intelligent Hot Plate Instrument (Chengdu Taimeng Technology Co., Ltd.); Xylene (AR, Shanghai Wokai Biotechnology Co., Ltd., batch number: 20220128).

Experimental grouping and administration

Before the test, the pain threshold of mice was evaluated, and a constant temperature hot plate instrument at (55.0 ± 0.1)°C was used to detect that the pain threshold of mice was<5 s or>30 s, and the jumping mice were eliminated. The remaining mice were randomly divided into 6 groups, with a total of 16 mice in each group, half male and half female. (1) Control group (0.9% physiological saline); (2) Aspirin control group (aspirin 100mg · kg-1, referred to as aspirin group); (3) Control group of Ganmao Granule (2.0g · kg-1, referred to as Ganmao Granule group); (4) Shenshu Jiedu Granule small dose group (5 times, 11g · kg-1, referred to as Shenshu small dose group); (5) Shenshu Jiedu Granule medium dose group (10 times, 22g · kg-1, referred to as Shenshu medium dose group); (6) Shenshu Jiedu Granule high-dose group (20 times, 44g · kg-1, referred to as Shenshu high-dose group), with a total of 16 rats in each group. The control group was given an equal volume of physiological saline by gavage. The drug was continuously administered by gavage for 15 days.

Experimental methods

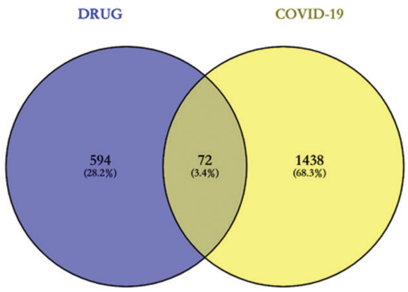
Hot plate method experiment. After 30 minutes, 50 minutes, and 70 minutes of the last administration, one mouse was placed into a thermostatic hot plate instrument preheated to (55.0 ± 0.1)°C each time. The time for the mouse to lick its feet due to thermal stimulation was the pain threshold of the mouse. After licking its feet, the mouse was quickly removed from the hot plate instrument and repeated every 5 minutes 3 times. The time for licking the feet was recorded and averaged.

Acetic acid-induced pain experiment. One hour after the last administration of acetic acid, the mice were injected intraperitoneally with 0.6% glacial acetic acid (0.1ml/10g). The latency of body twisting in the mice (the time of the first occurrence of body twisting after the injection of acetic acid) was recorded, as well as the number of body twisting reactions within 20 minutes (based on the abdominal indentation, extension of hind limbs, and hip elevation of the mice). Finally, calculate the writhing inhibition rate: writhing inhibition rate (%) = (control group writhing average - administration group writhing average)/control group writhing average × 100%.

Experimental study on xylene-induced ear swelling in mice. One hour after the last administration, 20μL xylene (100%) was evenly applied to both sides of the right ear of each group of mice (10% each μ 50) Inflammation left ear of mice not coated as control. After 30 minutes, the eyeball was removed, blood was taken, and the cervical vertebra was removed for death. Two ears of the mouse were cut along the auricle of the mouse. A circular earpiece was drilled at the same position in the left and right ears using an 8mm diameter punch and weighed separately. The mass difference between the two earpieces was used as the degree of ear swelling, and the swelling inhibition rate was calculated.

Swelling degree (mass of right earpiece - mass of left earpiece)/mass of left ear piece; Swelling inhibition rate

Figure 1. Venn diagram of the intersection target of Shenshu Jiedu Granule and COVID-19



Abbreviation: DRUG, Shenshu Jiedu Formula.

(swelling rate in each group - swelling rate in the model group)/swelling rate in the model group.

Statistical analysis

SPSS 25.0 statistical software was used for analysis, and GraphPad Prism 8.0 software was used for mapping. The data was expressed using mean ± standard deviation ($\overline{x} \pm s$), and the difference between groups was statistically significant using a *t* test ($P < .05$), considered statistically significant.

RESULTS

Active Components of Shenzhu Jiedu Granules

Searching for the traditional Chinese medicine ingredients of Shenzhu Jiedu Granule in ETCM, we obtained compounds with a moderate or higher drug resistance grade: 29 Atractylodes lanceolata (CZ), 30 Honeysuckle (JYH), 53 mulberry leaves (SY), 19 tangerine peel (CP), and 14 Baimao root (BMG).

Shenzhu Jiedu Granule - Target Acquisition of COVID-19

The keyword "COVID-19" was used to search and collect related targets of COVID-19, and 1511 COVID-19 targets were obtained in Genecards. Using the online software Venny 2.1 (https://bioinfogp. cnb. csic. es/tools/venny/index. html),[14] intersect the COVID-19 target with the Shenshu Jiedu granule, obtain 72 common targets, and draw a Venn diagram, as shown in Figure 1. After target mapping, 91 effective compounds were obtained, as shown in Table 1.

Construction of "Traditional Chinese Medicine Component Target" Network

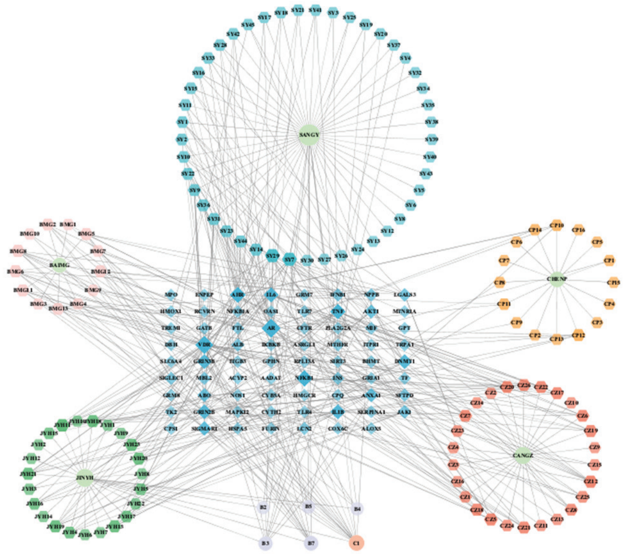
With the help of Cytascape 3.7.0, select 5 effective components of Shenshu Jiedu Granules and COVID-19 prediction targets, and construct a network diagram of "effective components of Shenzhu Jiedu Granules - potential targets". It represents the interaction between effective components of Shenshu Jiedu Granules and potential COVID-19 targets, with nodes representing compounds and disease targets. The network includes 205 nodes and 492 edges, with an average degree of 4.819, as shown in Figure 2. The diamond node represents the disease target, while the hexagon node represents the effective ingredients of traditional Chinese medicine. Each edge represents the

Table 1. Drug Target of Shenshu Jiedu Granule

		Assigned components of traditional Chinese medicine (91)
JYH, SY	B2	3'-Caffeoylquinic Acid,5'-Caffeoylquinic Acid,ChlorogenicAcid,Heriguard
JYH, SY	B3	Alexandrin, Daucosterol, CaproicAcid, EleutherosideA, Sitogluside, Strumaroside, Î'-Sitosterol-Î'-D-Glucoside
JYH, SY	B4	Ethyl Palmitate
JYH, SY	B5	Eugenol,Guaiacol
JYH, SY	B7	Sitosterol,Î'-Sitosterol
BMG	BMG1	Arundoin
BMG	BMG2	Campesterol
BMG	BMG3	Citric Acid
BMG	BMG4	Cylindrin
BMG	BMG5	D-Glucose, Glucose
BMG	BMG6	Fernenol
BMG	BMG7	Fructose
BMG	BMG8	Isoarborinol
BMG	BMG9	Malic Acid
BMG	BMG10	Oxalic Acid
BMG	BMG11	Simiarenol
JYH, SY, BMG	C1	Stigmasterol
BMG	BMG12	Sucrose
BMG	BMG13	Xylose
CP	CP1	3-O-trans ferulylquinic acid
CP	CP8	Citronellol,Menthol
CP	CP9	Decanal
CP	CP10	Hesperetin-7-O-Rutinoside
CP	CP11	Hesperetin-7-O-Rutinoside,Hesperidin
CP	CP12	Î'-Sitosterol
CP	CP14	Neohesperidin
CZ	CZ1	(+)-Eudesma-4(15),7(11)-Dien-8-One
CZ	CZ4	(2E)-2-Decene-4,6-Diyne-1,8-Diol 8-O-Î'-D-Apiofuranosyl-(1â†'6)-Î'-D-Glucopyranoside
CZ	CZ5	(2E,8E)-2,8-Decadiene-4,6-Diyne-1,10-Diol 1-O-Î'-D-Glucopyranoside
CZ	CZ6	(2R,3R,5R,7R,10S)-Atractyloside G 2-O-Î'-D-Glucopyranoside
CZ	CZ7	(5R,7R,10S)-Isopterocarpolon Î'-D-Glucopyranoside
CZ	CZ8	(X{2212})-Epicatechin,Epicatechin,Epicatechin,Epicatechol,Epigallocatechin
CZ	CZ11	3Î'-Hydroxyatractylone
CZ	CZ12	Adenosine, Adenine Nucleoside
CZ	CZ13	AtractylenolideII,Butenolide â…¡
CZ	CZ15	Atractyloside A 14-O-Î'-D-Fructofuranoside
CZ	CZ17	Atractyloside C
CZ	CZ18	Atractyloside D
CZ	CZ19	Atractyloside E
CZ	CZ20	Atractyloside G
CZ	CZ21	Atractyloside I
CZ	CZ22	Cis-Atractyloside I
CZ	CZ23	Icariside F2
CZ	CZ24	Scopoletin Î'-D-Xylopyranosyl-(1â†'6)-Î'-D-Glucopyranoside
CZ	CZ25	Syringin
CZ	CZ26	Uridine
JYH	JYH1	(E)-Aldosecologanin
JYH	JYH3	(Z)-Aldosecologanin
JYH	JYH4	3'-methoxyluteolin, Chrysoeriol
JYH	JYH5	3'-O-Methyl Loniflavone
JYH	JYH11	Hederagenin
JYH	JYH13	Ioniceroside C
JYH	JYH15	Lonicerin
JYH	JYH16	Loniflavone
JYH	JYH17	Luteolin
JYH	JYH18	Macranthoidin A
JYH	JYH19	Macranthoidin B
JYH	JYH20	Macranthoside A
JYH	JYH21	Macranthoside B
JYH	JYH22	Methyl Linoleate
JYH	JYH23	New Triterpennoid Glycoside
SY	SY1	2',4'-Dihydroxy-7-Methoxy-8-Prenylflavan,5,7-Dihydroxychromone
SY	SY2	3-Hydroxycoumarin, Folic Acid
SY	SY3	4-Hydroxycoumarin, Folinic Acid
SY	SY4	5-Hydroxycoumarin, Guaiacol
SY	SY7	Acetic Acid
SY	SY9	Ascorbic Acid,Vitamin C
SY	SY10	Aspartate, Asparagic Acid, Asparaginic Acid,Aspartic Acid, Skimmin
SY	SY11	Astragalin
SY	SY14	Campesterol, M-Cresol
SY	SY15	Choline
SY	SY16	Cudranin
SY	SY17	Cudranin, M-Cresol
SY	SY18	Ecdysterone, Folic Acid
SY	SY19	Folinic Acid
SY	SY20	Fumaric Acid
SY	SY21	Gamma-Aminobutyric Acid
SY	SY22	Glutamine
SY	SY23	Î'-Amyrin
SY	SY25	Inokosterone, Isoquercitrin
SY	SY28	Isoquercitrin, Isoquercetrin, Kuwanon H
SY	SY29	Isovaleric Acid,Lupeol
SY	SY31	Lupeol,Moracetin
SY	SY33	Moracetin,Moracin D
SY	SY36	Moran A, Morusin, Paeonol, Quercetin
SY	SY37	Morin
SY	SY41	P-Cresol
SY	SY42	PentanicAcid,Scopolin
SY	SY44	Quercetin-3-O-Glucoside, Quercetin-3-O-Glucoside
SY	SY45	Rutin,Rutoside,Vitamin P

Abbreviations: JYH, Honeysuckle; SY, Folium Mori; BMG, Baimao Root; CP, Orange Peel; CZ, Atractylodes Atractylodes)

Figure 2. Network Diagram of Active Constituents and Action Targets of Shenzhu Jiedu Granules.



Note: Diamond nodes represent disease targets, while hexagon nodes represent effective traditional Chinese medicine ingredients.

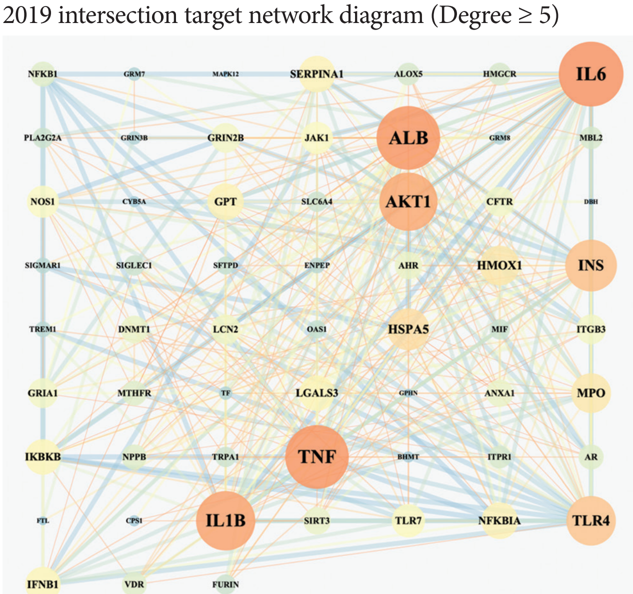
Abbreviations: JYH, Honeysuckle; SY, Folium Mori; BMG, Baimao Root; CP, Orange Peel; CZ, Atractylodes Atractylodes.

Table 2. Topological parameters of traditional Chinese medicine component target network

Number	Drugs	Degree	Betweenness	Closeness
1	SANGY	51	18535.75	0.39458415
2	Isoarborinol	35	7136.609	0.36624774
3	VDR	31	4462.516	0.3523316
4	JINYH	29	5862.2114	0.32535884
6	NFKB1	23	2031.4003	0.3306321
7	AHR	21	2544.1064	0.3222749
8	TNF	17	535.5315	0.29955947
12	BAIMG	14	3847.6113	0.30493274
13	SY29	14	4430.528	0.3109756
14	Sitosterol	14	2140.0066	0.31627908
15	SY7	13	3199.6035	0.3655914
16	GRIN3B	11	984.8837	0.30676693
17	GRIN2B	11	984.8837	0.30676693
18	Simiarenol	10	210.75145	0.29608127
19	ANXA1	10	229.53244	0.28854313
20	JYH11	9	879.2917	0.3044776
21	AKT1	9	743.2592	0.28936172
22	C1	9	2785.2336	0.38202247
23	ABO	9	956.09894	0.3114504
25	BMG8	8	560.88477	0.30722892
26	BMG6	8	560.88477	0.30722892
27	B3	8	1315.1849	0.36298934
28	SY14	7	680.6362	0.34343433
29	JYH23	7	198.99011	0.28895184
30	JYH21	7	198.99011	0.28895184
31	JYH20	7	198.99011	0.28895184
32	JYH19	7	198.99011	0.28895184
33	JYH18	7	198.99011	0.28895184
34	JYH13	7	198.99011	0.28895184
38	LGALS3	7	593.8299	0.29868227
40	BMG11	7	509.67432	0.3063063
41	B7	7	1024.5123	0.3617021

interaction between the compound and the disease target. The number of connecting routes between nodes is expressed in degrees. The larger the degree value, the more routes, and the larger the node, the greater the role it plays. CytoNCA was an excellent tool for calculating centrality, evaluating and visualizing biological networks. Using the CytoNCA plug-in to perform topology analysis on this network, we obtained a median of degree of 3, a median of betweenness centrality (BC) of 156.14, and a median of proximity centrality (CC) of 0.28. Consistent with a DC value greater than 2 times the median, i.e., Degree>6, while BC>156.14 and CC>0.28 nodes are considered key pharmaceutical biological components of the network, as shown in Table 1. Including, Sitosterol, Î'-Sitosterol, Simiarenol, Fernenol, Isoarborinol, Hederagenin, Ioniceroside C, Macranthoidin A, Macranthoidin B, Macranthoside A, Macranthoside B, New Triterpennoid Glycoside.The major genes with a larger degree are IL-6 and IL-1 β, AKT1, NFκBIA, NFκBI, TNF, GRIN2B, GRIN3B, VDR, ANXA1, LGALS3, AR, SIGMAR1, DNMT1, etc. It can be seen in the figure that the compounds and targets interact with each other, and they may be the main active ingredients and potential targets of traditional Chinese medicine to be explored in this experiment.

Figure 3. Active ingredient of Shenzhu Jiedu Granule - COVID-2019 intersection target network diagram (Degree ≥ 5)



Note: The size of the dot represents the degree value, the color from cold to warm represents the centrality value, and the color of the line represents the confidence score.

Construction of PPI network

In order to further screen the more important network nodes of COVID-2019, 72 candidate genes will be obtained and imported into the STRING11.5 database. The target PPI network and node relationship data of Shenshu Jiedu Granule for COVID-2019 prevention and treatment will be exported to Cytoscape 3.9.0. The PPI network (Figure 3) will be obtained. Free nodes will be removed based on Drgree ≥ 5, with a total of 59 nodes and 358 edges. Using the CytoNCA plug-in to perform topology analysis on the network, the median of degree is 9, the median of BC is 6.92, and the median of CC is 0.51. Nodes that meet the median of Degree values greater than 2 times, i.e., Degree>18, and nodes with BC>6.92, and CC>0.51 are considered key nodes of the network (see Table 3),They are IL-6 and IL-1β, ALB, TNF, AKT1, INS, TLR4, HSPA5, HMOX1, MPO, etc.

Table 3. Intersection target PPI network topology parameters

Number	Target	Degree	Betweenness	Closeness
1	IL6	42	362.26987	0.74358976
2	ALB	41	624.0515	0.7733333
3	TNF	41	290.20325	0.725
4	IL1B	37	312.2323	0.725
5	AKT1	36	426.84003	0.7160494
6	INS	30	115.64986	0.63736266
7	TLR4	28	83.15081	0.61702126
8	HSPA5	21	85.72379	0.5979381
9	HMOX1	19	136.41925	0.5631068
10	MPO	19	29.121687	0.5631068

Figure 4. PPI network diagram of key proteins

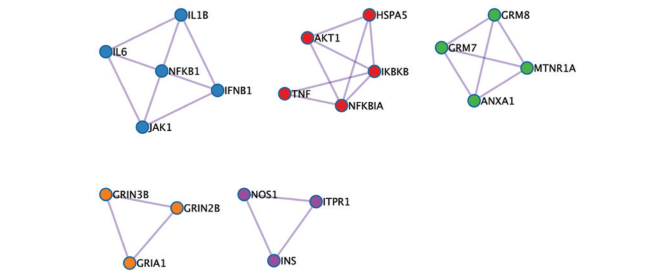
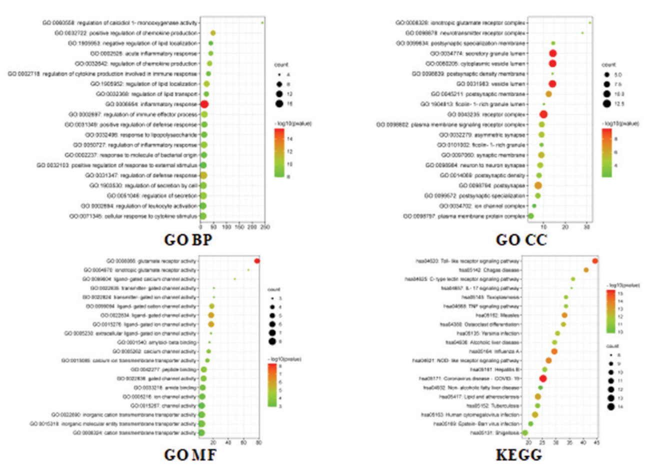
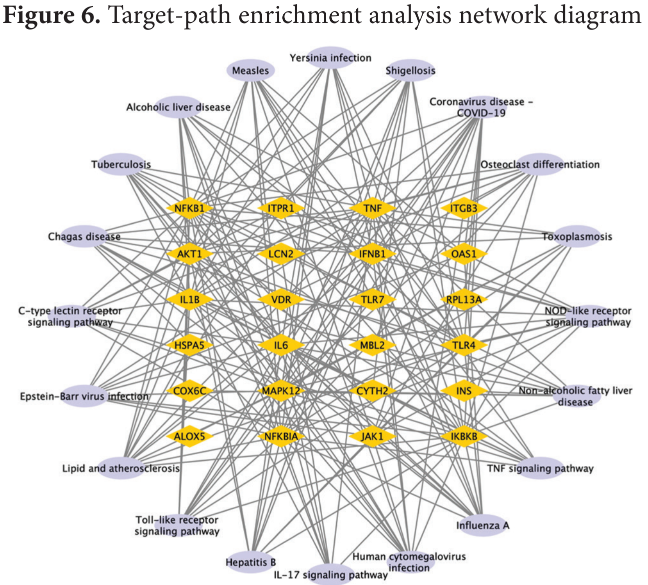


Figure 5. GO and KEGG Analysis of the Intersection Target of Shenzhu Jiedu Granule COVID-19



Note: The size of the circle represents the number of targets; The color represents the size of the *P* value.

Figure 6. Target-path enrichment analysis network diagram



PPI network analysis of key proteins

The modules with higher density in the complex PPI network are potential subnets of the PPI network, which can more accurately analyze the mechanism of Shenshu Jiedu Granule in preventing COVID-19. Through MCODE analysis fivesub-modules are obtained, as shown in Figure 4.

GO and KEGG enrichment analysis

Conduct GO and KEGG analysis on 72 candidate targets using the Metascape platform. There are 789 GO analysis results, including 673 biological processes, 54 cell components, and 62 molecular functions. Further similarity clustering, taking Kappa similarity>0.3 to obtain 20 Biological Processes (BP), Mainly involving acute inflammatory response, regulation of cytokine production involved in immune response, regulation of immune effector process, response to molecule of bacterial origin, response to lipopolysaccharide, negative regulation of lipid localization, regulation of lipid localization, regulation of lipid transport, etc. GO Molecular Functions (MF) 20, mainly involving glutamate receptor activity, calcium channel activity, transmitter-gated-channel activity, ligand-gated channel activity, ion channel activity, etc.; 20 cellular components (CC), mainly involving neurotransmitter receptor complex, plasma membrane protein complex, ion channel complex, synaptic membrane, cytoplasmic vesicle lumen, etc. It can be seen that the tissue microenvironment, such as the plasma membrane and cell vesicles, are the sites of their occurrence, and immune-inflammatory reactions, calcium ions, glutamate plasma, and neurotransmitters participate in the pathogenesis.

There are 126 KEGG enrichment pathways. The pathways related to COVID-19 infection, inflammatory immune response, and signal regulation are selected. According to the negLog10 (*P*) value, 20 pathways are selected to draw bubble diagrams in R language, as shown in Figure 5. The horizontal axis of each graph represents the ratio of the pathway gene to the total input gene, the vertical axis represents the enrichment name, and the size of the bubble area represents the number of pathway gene enrichment. The larger the bubble, the more enriched the number of genes, the more relevant the enrichment is. The color of the bubble represents the size of the *P* value, and the redder the color, the smaller the *P* value. There is one Coronavirus disease COVID-19 pathway, one influenza pathway, and six pathways related to immune inflammation, including IL-17 signaling pathway, HIF-1, Toll-like receptor signaling pathway, TNF signaling pathway, C-type lectin receptor signaling pathway, NOD-like receptor signaling pathway, etc. It is suggested that Shenzhu Jiedu Granule can prevent and treat COVID-19 through the COVID-19 pathway, influenza virus pathway and immune-inflammatory pathway.

Potential target screening

Based on KEGG, the top 20 pathways with significant differences were selected to further explore the potential targets of Shenshu Jiedu Granule in preventing COVID-19 and draw a network diagram, as shown in Figure 6. Visible

IL-6, IL-1β, IFNB1, TNF, AKT1, NFkB1, NFkB1A, IKBKB, MAPK12 are potential key target genes for Shenshu Jiedu Granule to intervene in COVID-19.

Experimental research results: Effect of Shenzhu Jiedu Granule on acetic acid writhing in mice

Table 4 shows that compared with the control group, the number of acetic acid writhing in the small, medium, and high dose groups of Shenzhu Jiedu Granule significantly decreased and the incubation period prolonged, with statistical significance ($P < .05$, $P < .01$). The incubation period in the small dose group was the longest, while the number of writhing in the medium dose group was the lowest; The inhibition rate in the medium dose Shenshu Jiedu group was the highest, 35.38%.

Effect of Shenzhu Jiedu Granule on Pain Caused by Hot Plate

Table 5 shows that there is no statistical difference in the pain threshold of mice in each group before grouping. Compared with the control group, the pain threshold in the large, medium, and small groups of Shenzhu Jiedu Granule significantly increased 30 minutes after administration ($P < .01$) 50 min and 70 min were still statistically significant ($P < .05$).

Effect of Shenzhu Jiedu Granule on Xylene Induced Ear Swelling in Mice

The degree of swelling reflects the degree of mouse ear swelling after xylene inflammation, and the swelling inhibition rate reflects the degree of regression of mouse ear swelling under the action of drugs. See Table 6: After 30 minutes of xylene-induced inflammation, compared with the control group, the ear swelling degree of mice in the high, medium, and low dose groups of Shenzhu Jiedu Granule significantly decreased ($P < .05$, $P < .01$), while the ear swelling degree of mice in the low dose group was the lowest; The inhibition rates of body twisting in the three groups of mice were 9.61%, 25.4%, and 36.94%, respectively, with the highest in the low dose group.

Table 4. Effect of Shenzhu Jiedu Granule on acetic acid writhing in mice ($\bar{x} \pm s$)

Group	Sample	Incubation (min)	Number of times of twisting within 20 minutes(times)	Torsion inhibition rate (%)
Control	16	5.65±0.84	26.25±1.53	—
Aspirin	16	7.48±1.02[b]	15.81±1.38[b]	39.66
Ganmao Granule	16	6.92±1.14[b]	17.75±1.81[b]	32.25
Shenshu detoxification high-dose	16	6.66±1.17[a]	19.25±1.69[b]	30.15
Shenshu detoxification medium dose	16	6.51±1.0[a]	16.81±1.60[b]	35.38
Shenshu detoxification small dose	16	7.04±1.16[b]	20.06±2.21[b]	23.28

[a]Compared with the control group, $P < .05$
[b]Compared with the control group, $P < .01$

Table 5. Effect of Shenzhu Jiedu Granule on Pain Caused by Hot Plate ($\bar{x} \pm s$)

Group	Sample	Pain threshold in mice before administration	Pain thresholds in mice at different times after administration		
			30 min	50 min	70 min
Control	16	10.56±1.97	10.86±1.93	10.88±3.65	10.93±2.90
Aspirin	16	10.63±2.25	16.38±2.75[b]	15.31±2.44[b]	14.06±2.52[b]
Ganmao Granule	16	10.75±1.95	15.56±3.03[b]	13.69±3.09[a]	13.56±2.97[a]
Shenshu detoxification high-dose	16	10.69±2.02	14.5±3.6[b]	13.25±3.02[a]	13.50±2.63[a]
Shenshu detoxification medium dose	16	11.19±1.96	14.31±2.63[b]	13.50±3.43[a]	13.38±2.96[a]
Shenshu detoxification small dose	16	10.72±1.98	15.31±3.53[b]	13.44±2.53[a]	13.13±3.22[a]

[a]Compared with the control group, $P < .05$
[b]Compared with the control group, $P < .01$

Table 6. Effect of Shenzhu Jiedu Granule on Xylene-Induced Ear Swelling in Mice ($\bar{x} \pm s$)

Group	Sample	Swelling degree (mg)	Swelling inhibition rate (%)
Control	16	6.0±1.05	—
Aspirin	16	1.65±0.27[b]	75.37
Ganmao Granule	16	4.45±0.99[b]	33.58
Shenshu detoxification high-dose	16	6.06±0.84[a]	9.61
Shenshu detoxification medium dose	16	5.00±0.96[b]	25.4
Shenshu detoxification small dose	16	4.23±0.95[b]	36.94

[a]Compared with the control group, $P < .05$
[b]Compared with the control group, $P < .01$

DISCUSSION

The treatment of infectious diseases with traditional Chinese medicine has dual effects of inhibiting pathogens, i.e., antiviral, and improving the natural and specific immunity of the body.[15] Professor Qi Wensheng proposed the theoretical basis of traditional Chinese medicine for regulating the organism's internal environment, resisting the invasion of dampness and heat, and enhancing the immune function of the organism in response to this epidemic.In this study, the network pharmacology "drug component target network" topology analysis was used to obtain 17 main active components of Shenshu Jiedu Formula, including 10 antiviral, anti-inflammatory, and bactericidal compounds such as Lonicera macranthoides subsaponin A (anti-viral, antibacterial, antipyretic), and neotriterpenoid glycosides (anti-inflammatory, antibacterial, and antiviral). Lonicera macranthoides saponin B (regulating immunity, anti-tumor, liver protection), Lonicera macranthoides subsaponin B (regulating immunity, anti-tumor, liver protection) and other four anti immune compounds. Shenzhu Jiedu Granule has anti COVID-19, influenza virus, antibacterial and anti-inflammatory effects, and can significantly enhance the anti-inflammatory and analgesic effects of mice, which highlight the significance of the study in the context of current global health concerns.

Key targets derived from the PPI network include Inflammatory cytokines including interleukin-6 (IL-6), interleukin-1β (IL-1β), NFκB1, NFκBIA, IFNB1, TNF, MAPK12, AKT1, IKBKB, etc. Inflammatory cytokines including IL-6, IL-1β, induced protein 10 (IP10) and monocyte chemoattractant protein-1 (MCP-1) were significantly elevated in COVID-19 patients. Both IL-6 and IL-10 belong to the interleukin family and are important inflammatory factors. Research has found that[16-17] Chinese medicine has a definite effect in inhibiting inflammatory reactions to prevent COVID-19. The activation of NF-κB gene-related signaling pathway indicates that the immune system is activated, which can induce the release of inflammatory factors, activate inflammatory cells, and trigger cytokine storm, which may be the turning point of mild

COVID-19 to severe disease.[18] There are clinical data suggests that a cytokine storm is associated with COVID-19 severity and is also a crucial cause of death from COVID-19. IFN has an antiviral effect, and TNF-α have similar functions, both of which have the effect of inhibiting viral replication, blocking viral protein synthesis, and the production of viral particles.[19] MAPK12 is a mitogen-activated protein kinase family that is activated by inflammatory factors and viral infections that stimulate phosphorylation of MAPK family proteins.[20.] The study found that[21] the MAPK family is involved in the pathogenesis of COVID-19, in which MAPK1 and MAPK3 are the main targets of COVID-19. The high expression of MAPK3 can reduce the proinflammatory cytokine TNF in lung injury TNF-α and IL-1β. Akt protein is involved in the pathogenesis of COVID-19,[22] and its related pathway PI3K Akt is the key protein and pathway of influenza virus pathogenesis.[23] Akt signaling pathway components have distinct roles in inflammatory disease regulation through controlling inflammatory cytokines.

The increase of inflammatory indicators increases the risk of becoming severe.[24] Inflammatory factor storms can cause liver and kidney damage and abnormal energy metabolism.[25] Further GO enrichment analysis shows that the biological process of Shenzhu Jiedu Granule against COVID-19 is mostly related to inflammatory reaction and immune regulation. The pathways identified by KEGG enrichment analysis include COVID-19, influenza, and multiple immunoinflammatory related pathways such as IL-17, HIF-1, and C-type lectin receptor signaling pathways. Based on this analysis, further animal experiments found that Shenzhu Jiedu Granule has anti-inflammatory and analgesic effects on mice. It can be seen that this formula has a soothing effect on the pain and inflammation caused by COVID-19. Biological processes include antiviral, anti-inflammatory, immune regulation, improving the inflammatory environment, affecting neuroendocrine metabolism, and other multi-angle, multi-ion pathway biological processes. It is further confirmed that Shenshu Jiedu Granule can inhibit the entry of COVID-19 into host cells, interfere with virus replication and enhance the body's immune function.

CONCLUSIONS

The analysis of multiple ingredients, multiple targets, and multiple pathways, as well as their possible cross-linking effects discovered by network pharmacology provides an opportunity for the advantages of TCM in overall syndrome differentiation and treatment. Further research and development of the main effective ingredients and targets in TCM are needed for the prevention and treatment of COVID-19, of which will provide ideas, data, and theoretical support for the development of traditional Chinese medicine.

DATA AVAILABILITY

The data could be obtained by contacting the corresponding author.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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