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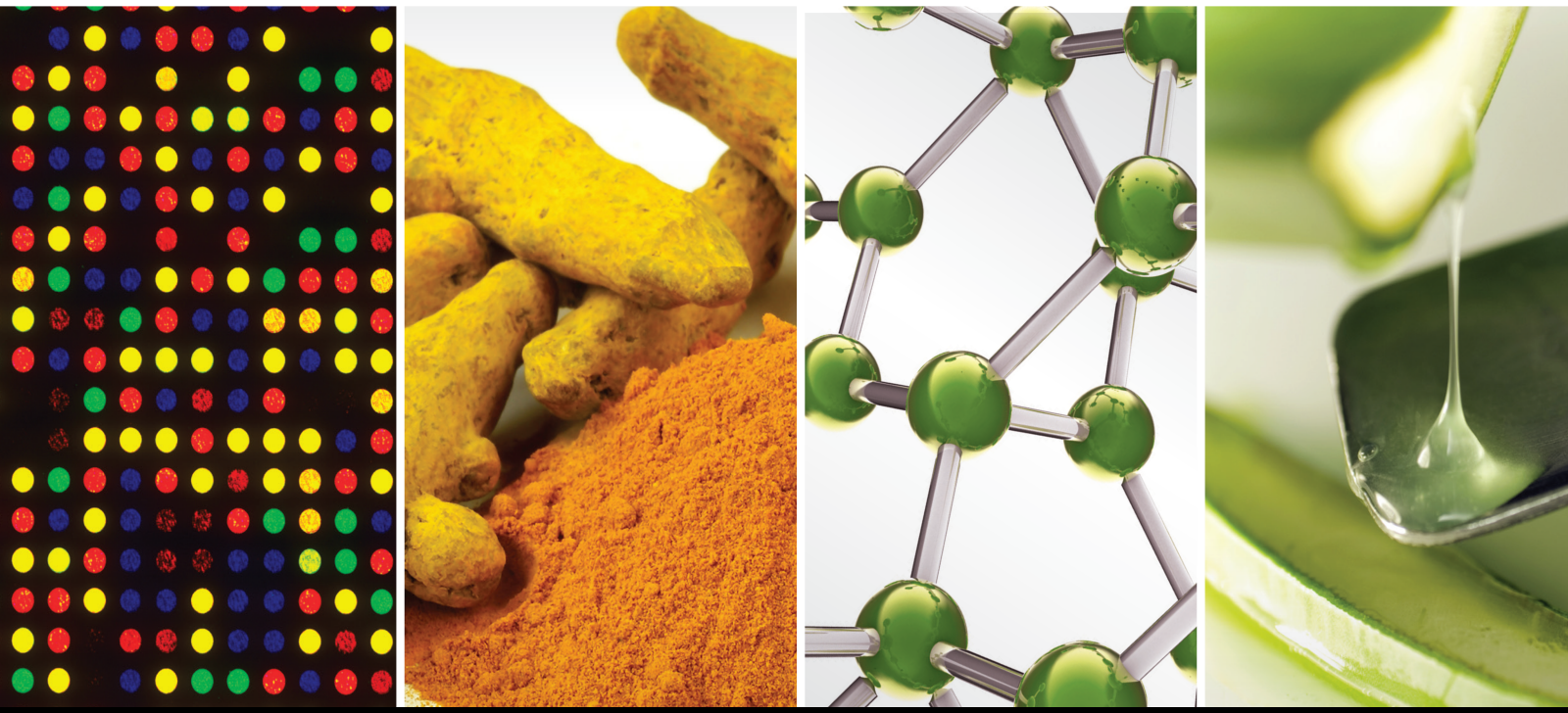

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 5992394, 12 pages
https://doi.org/10.1155/2022/5992394

Research Article

Molecular Mechanism of *Salvia miltiorrhiza* Bunge in Treating Cerebral Infarction

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Received 10 July 2021; Accepted 22 December 2021; Published 29 March 2022

Academic Editor: Junqing Huang

Background. Cerebral infarction (CI) is a common brain disease in clinical practice, which is mainly due to the pathological environment of ischemia and hypoxia caused by difficult cerebral circulation perfusion function, resulting in ischemic necrosis of local brain tissue and neurological impairment. In traditional Chinese medicine (TCM) theory, CI is mainly due to blood stasis in the brain. Therefore, blood-activating and stasis-dissipating drugs are often used to treat CI in clinical practice. *Salvia miltiorrhiza* Bunge (SMB) is a kind of traditional Chinese medicine with good efficacy in promoting blood circulation and removing blood stasis, and treatment of CI with it is a feasible strategy. Based on the above analysis, we chose network pharmacology to investigate the feasibility of SMB in the treatment of CI and to study the possible molecular mechanisms by providing some reference for the treatment of CI with TCM. *Methods*. The active ingredients and related targets of SMB were obtained through the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, and CI-related targets were obtained from the GeneCards and DisGeNET databases. The target of SMB for the treatment of CI was obtained using Cytoscape software and visualized. GO and KEGG enrichment analysis was performed based on "clusterProfiler" within R, and the prediction results were validated by molecular docking technique. *Results*. By constructing a compound-target (C-T) network, it was found that the active components in SMB mainly treated CI by regulating key proteins such as AKT1, IL-6, and EGFR. These key proteins mainly involve in pathways such as immune regulation, cancer and lipid metabolism, such as lipid and atherosclerosis, chemical carcinogenesis-receptor activation pathways, and IL-17 signaling pathway. In the GO term, it mainly regulates the response to steroid hormones, membrane rafts, and G protein-amine receptor coupled activity. Eventually, we verified that the luteolin and tanshinone IIA components in SMB have a good possibility of action with AKT1 and IL-6 by in silico techniques, indicating that SMB has some scientificity in the treatment of CI. *Conclusion*. SMB mainly treats CI by regulating 94 proteins involved in lipid and atherosclerosis, chemical carcinogenesis-receptor activation, and IL-17 signaling pathway. Our research strategy provided a template for the drug development of TCM for the treatment of CI.

1. Introduction

Cerebral infarction (CI), also known as ischemic stroke, is very common in cerebrovascular diseases, and the number of patients with this disease accounts for about 70% of patients with acute cerebrovascular diseases, which indicates that reasonable treatment is important for patients with this disease. The disease is mainly attributed to local tissue ischemia and hypoxia caused by cerebral blood flow disorders, followed by brain tissue necrosis and, in severe cases, neurological impairment [1]. At the same time, CI has the characteristics of complex pathogenesis, high mortality as

well as recurrence rate, and an effective treatment plan is urgently needed for its treatment in clinical practice. Thrombolytic therapy is a feasible treatment for CI, but this method has the problem of short half-life of drugs, which may cause body toxicity if the preparation is used in large amounts. Therefore, we wanted to find a new drug to replace thrombolytic therapy for CI.

With an in-depth understanding of traditional Chinese medicine, people focus on finding appropriate traditional Chinese medicine (TCM) for the treatment of CI. In TCM theory, CI belongs to the category of "stroke," and the common symptom is blood stasis. Therefore, drugs promoting blood circulation to remove blood stasis can be used to treat CI [1].

Salvia miltiorrhiza Bunge (SMB), a traditional Chinese medicine with the effect of promoting blood circulation and removing blood stasis, is also used as a botanical drug for the treatment of diseases in many Asian countries in addition to its widespread use in China [2]. At the same time, modern pharmacological studies have shown that SMB can inhibit thrombosis by improving blood circulation [3].

Compound *Salvia miltiorrhiza* (CSM) injection prepared with SMB as the main raw material is a commonly used drug for promoting blood circulation and removing blood stasis, which has been authorized by the China Food and Drug Administration. CSM is available to treat CI [1]. However, due to the complex mechanism of TCM in the treatment of diseases, it is difficult for ordinary research methods to systematically elaborate on diverse mechanisms.

Network pharmacology, as an emerging research tool, can be used to analyze the complex mechanisms of TCM in the treatment of diseases and reveal the route of drug action at the protein level [4]. Therefore, in this study, we selected network pharmacology to investigate the research means of SMB in the treatment of CI (Figure 1).

2. Materials and Methods

2.1. Screening Active Components of SMB. In general, oral bioavailability (OB) is an important measure of drug efficacy and drug-like properties (DL) represent the potential of compounds to become drugs [5]. We first collected all compounds contained in SMB in the TCM Systems Pharmacology (TCMSP) database (https://tcmspw.com/tcmspsearc-h.php), using "Radix Salviae" as the keyword. Then, we used (OB) ≥30% and (DL) ≥0.18 as the screening criteria to determine the potential active ingredients that may exist.

2.2. Predicting the Targets of the Compounds. We predicted possible targets associated with SMB compounds by searching the TCMSP database for "relevant targets" and normalized the target names by UniProt (https://www.uniprot.org/).

2.3. Screening of Disease Targets. CI related targets were searched in GeneCards and DisGeNET databases using the keyword "cerebral infarction."

2.4. Construction of a Compound-Target (C-T) Network. Firstly, we constructed a network of drug compound targets interacting with disease targets by using Venny (https://bioinfogp.cnb.csic.es/tools/venny/index.html). Then, using Cytoscape V3.7.1 (https://www.cytoscape.org/) software, we performed a visual analysis of the C-T network.

2.5. Construction of a Protein-Protein Interaction (PPI) Network. Interaction networks between disease proteins were constructed by the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (https://string-db.org/) [6].

2.6. GO (Gene Ontology) and KEGG Pathway Enrichment. Enrichment analysis of GO and KEGG for therapeutic proteins was performed by the "clusterProfiler" package in R (R Project for Statistical Computing, Vienna, Austria) [7].

2.7. Expression of Targets in Organs. The drugs refer to TCM and are used under the guidance of the TCM theory, while the medication law of SMB also follows the TCM theory. According to TCM theory, the occurrence of diseases is often due to the imbalance of the overall state of the body, and the lesions of a certain part or organ are often accompanied by abnormalities in other parts of the body. Therefore, during treatment, we need to implement the concept of combining global with local and multidimensional to gain insight into the development and changes of the disease [8]. We want to know whether SMB can modulate different organs to treat CI. Therefore, we obtained the expression of the top 20 proteins of the PPI network in different organs by BioGPS (https://biogps.org/).

2.8. Computational Validation of C–T Interactions. To verify the reliability of the previous prediction results, protein structures of AKT1 and IL-6 proteins with PDB codes 4EKL and 1ALU were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) https://www.rcsb.org/), respectively. We performed in silico docking studies by AutoDock Vina software for the two predicted specific compounds and the two targets described above, respectively. We used the fraction of binding energy as a criterion to evaluate the possibility of binding of components to proteins. It is generally accepted that the smaller the binding energy score, the greater the possibility that components act on proteins [9].

3. Results

3.1. Screening of Active Components and Targets of SMB. In the TCMSP database, 202 possible chemical constituents of SMB were obtained. According to the screening parameters of OB ≥30% and DL ≥0.18, a total of 59 potential active ingredients corresponding to 113 drug targets were obtained by excluding components without corresponding targets (Table 1). The 113 potential drug targets are detailed in Supplementary Table S1.

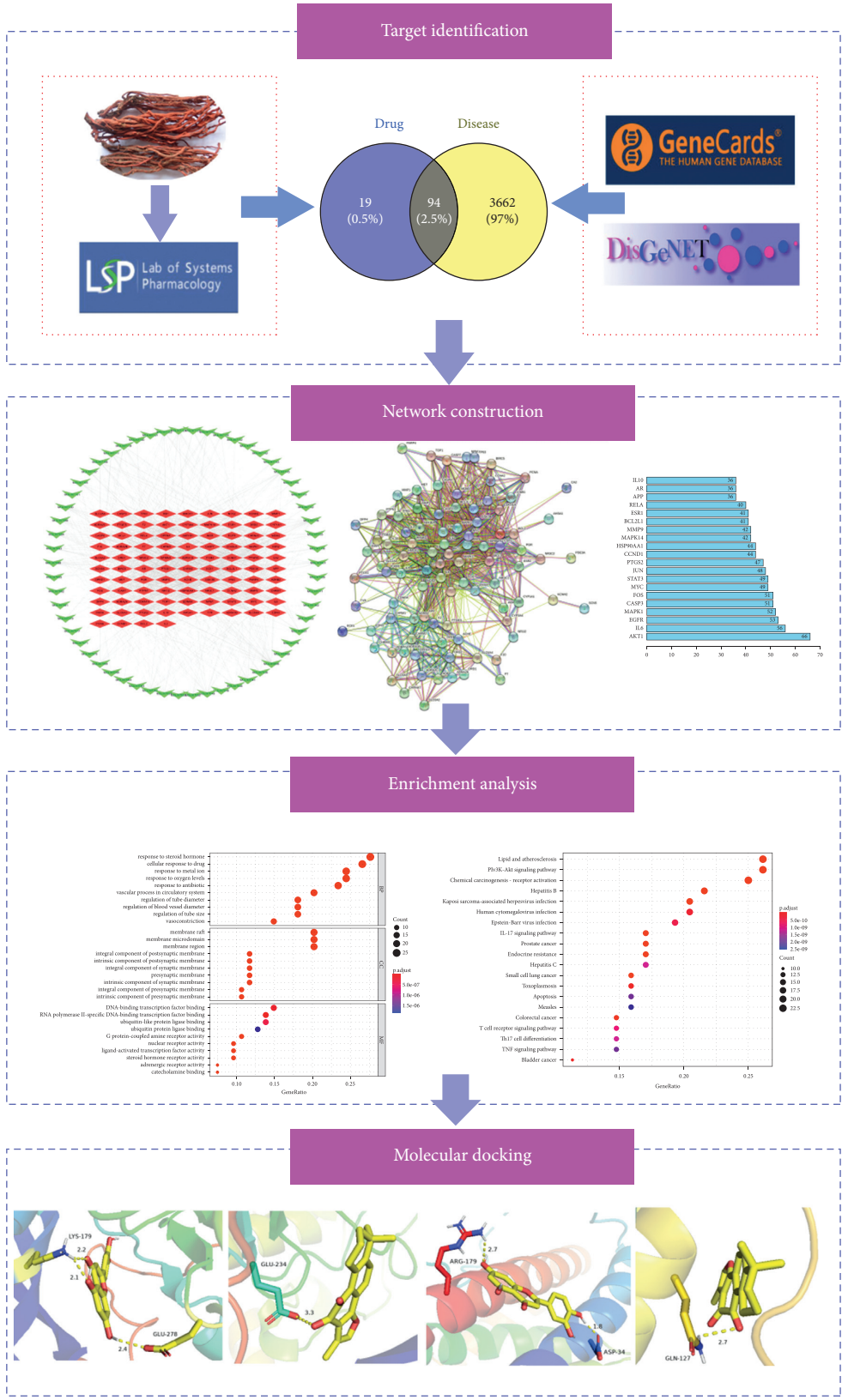

Figure 1: Molecular mechanism of *Salvia miltiorrhiza* Bunge in treating cerebral infarction.

Table 1: A total of fifty-nine ingredients were selected as the details of the active ingredients of SMB in this study.

No.	Mol ID	Molecule name	OB	DL	Reference
1	MOL001601	Trijuganone B	38.74	0.35	[10]
2	MOL001659	Poriferasterol	43.82	0.75	[11]
3	MOL001771	Poriferast-5-en-3beta-ol	36.91	0.75	[12]
4	MOL001942	Isoimperatorin	45.46	0.22	[13]
5	MOL002222	Sugiol	36.11	0.27	[14]
6	MOL002651	Dihydrotanshinone IIA	43.76	0.40	[15]
7	MOL002776	Baicalin	40.12	0.75	[16]
8	MOL000569	Digallate	61.84	0.25	
9	MOL000006	Luteolin	36.16	0.24	[17]
10	MOL007036	5,6-Dihydroxy-7-isopropyl-1, 1-dimethyl-2,3-dihydrophenanthren-4-one	33.76	0.28	[18]
11	MOL007041	2-Isopropyl-8-methylphenanthrene-3,4-dione	40.86	0.22	[19]
12	MOL007045	3α-Hydroxytanshinone IIA	44.92	0.44	[20]
13	MOL007048	(E)-3-[2-(3,4-Dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl]acrylic acid	48.24	0.31	[21]
14	MOL007049	4-Methylenemiltirone	34.34	0.22	[22]
15	MOL007050	2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	62.78	0.39	[23]
16	MOL007058	Formyltanshinone	73.44	0.41	
17	MOL007059	3-Beta-hydroxymethyllenetanshiquinone	32.16	0.40	
18	MOL007061	Methylenetanshinquinone	37.07	0.36	[24]
19	MOL007063	Przewalskin A	37.10	0.64	[25]
20	MOL007064	Przewalskin B	110.32	0.43	[26]
21	MOL007068	Przewaquinone B	62.24	0.41	[27]
22	MOL007069	Przewaquinone C	55.74	0.40	[28]
23	MOL007070	(6S, 7R)-6,7-Dihydroxy-1,6-dimethyl-8,9-dihydro-7h-naphtho[8, 7-g]benzofuran-10,11-dione	41.31	0.45	
24	MOL007071	Przewaquinone F	40.30	0.45	[28]
25	MOL007077	Sclareol	43.67	0.20	[29]
26	MOL007079	Tanshinaldehyde	52.47	0.45	[30]
27	MOL007081	Danshenol B	57.95	0.55	[31]
28	MOL007082	Danshenol A	56.96	0.52	[32]
29	MOL007085	Salvilenone	30.38	0.37	[33]
30	MOL007088	Cryptotanshinone	52.34	0.39	[34]
31	MOL007093	Dan-shexinkum D	38.88	0.55	[35]
32	MOL007094	Danshenspiroketallactone	50.43	0.30	[36]
33	MOL007098	Deoxyneocryptotanshinone	49.40	0.28	[37]
34	MOL007100	Dihydrotanshinlactone	38.68	0.32	
35	MOL007101	Dihydrotanshinone I	45.04	0.36	[38]
36	MOL007105	Epidanshenspiroketallactone	68.27	0.30	[36]
37	MOL007107	Ferruginol	36.06	0.24	[39]
38	MOL007108	Isocryptotanshi-none	54.98	0.39	[38]
39	MOL007111	Isotanshinone II	49.91	0.39	[40]
40	MOL007115	Manool	45.04	0.20	[41]
41	MOL007119	Miltionone I	49.68	0.32	[42]
42	MOL007120	Miltionone II	71.02	0.43	[43]
43	MOL007121	Miltipolone	36.55	0.36	[44]
44	MOL007122	Miltirone	38.75	0.25	[45]
45	MOL007124	Neocryptotanshinone II	39.46	0.23	[46]
46	MOL007125	Neocryptotanshinone	52.48	0.32	[47]
47	MOL007127	1-Methyl-8,9-dihydro-7h-naphtho[5,6-g]benzofuran-6,10,11-trione	34.72	0.36	[48]
48	MOL007130	Prolithospermic acid	64.37	0.31	[49]
49	MOL007132	(2R)-3-(3,4-Dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid	109.38	0.35	
50	MOL007141	Salvianolic acid G	45.56	0.60	[50]
51	MOL007142	Salvianolic acid J	43.37	0.72	[51]
52	MOL007143	Salvilenone I	32.43	0.22	[33]
53	MOL007145	Salviolone	31.72	0.23	[52]
54	MOL007150	(6S)-6-Hydroxy-1-methyl-6-methylol-8,9-dihydro-7h-naphtho[8,7-g]benzofuran-10,11-quinone	75.38	0.45	[51]
55	MOL007151	Tanshindiol B	42.66	0.45	[52]
56	MOL007152	Przewaquinone E	42.85	0.45	[28]
57	MOL007154	Tanshinone IIA	49.88	0.39	[53]
58	MOL007155	(6S)-6-(Hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7h-naphtho[8,7-g]benzofuran-10,11-dione	65.25	0.44	[54]
59	MOL007156	Tanshinone VI	45.63	0.29	[55]

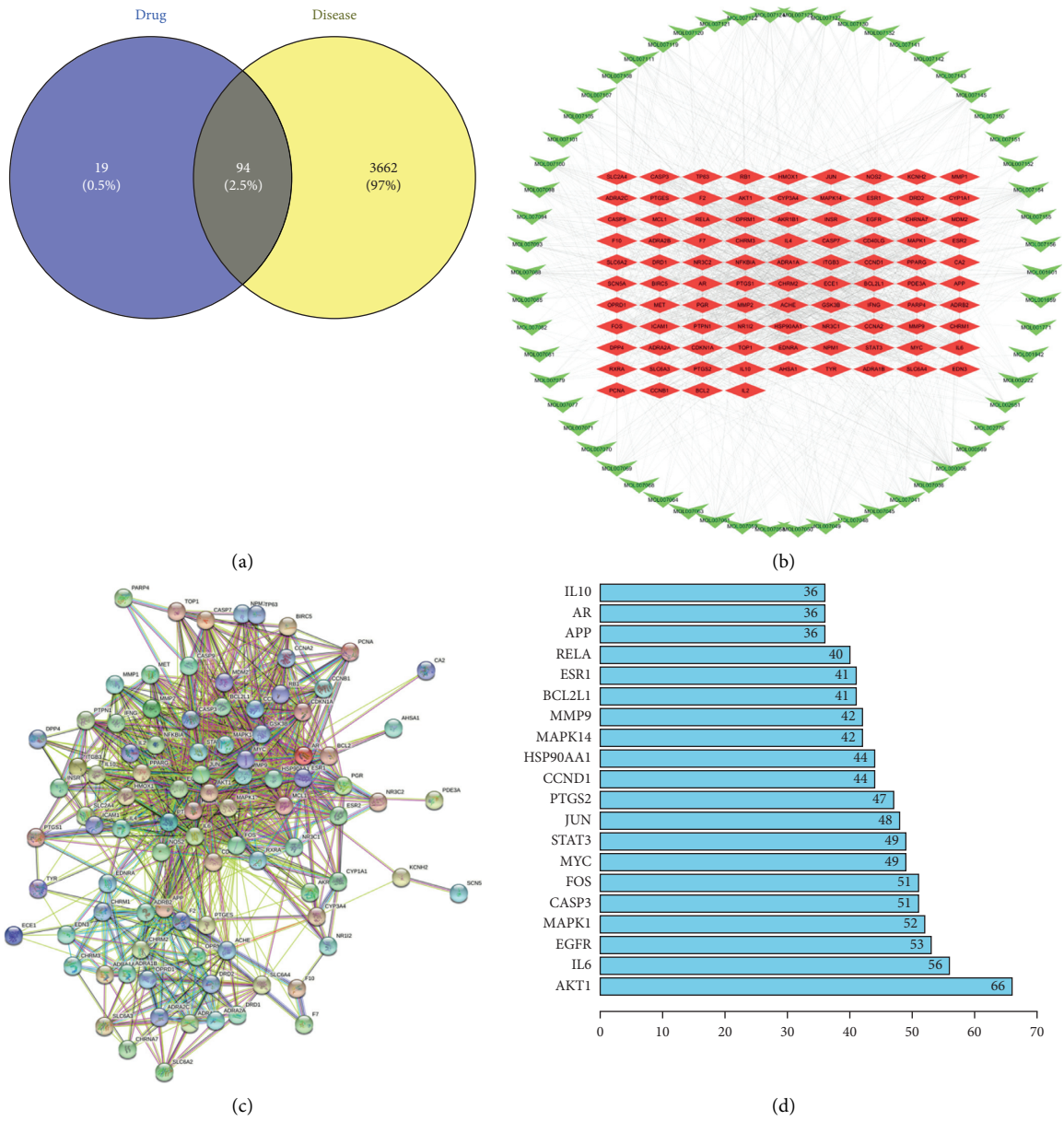

Figure 2: (a) Overlapping targets between diseases and drugs. (b) C-T network. (c) PPI network. (d) Bar plot of the PPI network.

3.2. Acquisition of Known Therapeutic Targets for CI. A total of 3,756 therapeutic targets of CI were obtained from GeneCards and DisGeNET after the removal of duplicated targets (Supplementary Table S2).

3.3. Analyses of the C-T Network. There were 94 overlapping targets between 58 active component-related targets and disease targets in SMB (Figures 2(a) and 2(b)), indicating that these 94 proteins were target proteins for the treatment of CI by SMB. The active ingredients in SMB were indicated by green arrows; therapeutic targets were indicated by red squares.

Further analysis of the C-T network revealed that the node degrees of the components luteolin, tanshinone IIA, and dihydrotanshinone were 43, 35, and 26, respectively. These components have a high node degree, indicating that these components may be key to CI therapy.

Modern studies have shown that some of the components in SMB have a role in treating CI. For example, luteolin can activate the AMPK/mTOR signaling pathway and improve neurological impairment due to CI [56]. Tanshinone IIA can prevent CI development by inhibiting neuronal apoptosis and inflammatory responses [57]. This also justifies to some extent the predictions based on network pharmacology.

3.4. Analyses of the PPI Network. As shown in Figure 2(c), we constructed the regulatory relationship between the above 94 therapeutic targets into a PPI network for presentation (Supplementary Table S3). Then, the top 20 ranked proteins

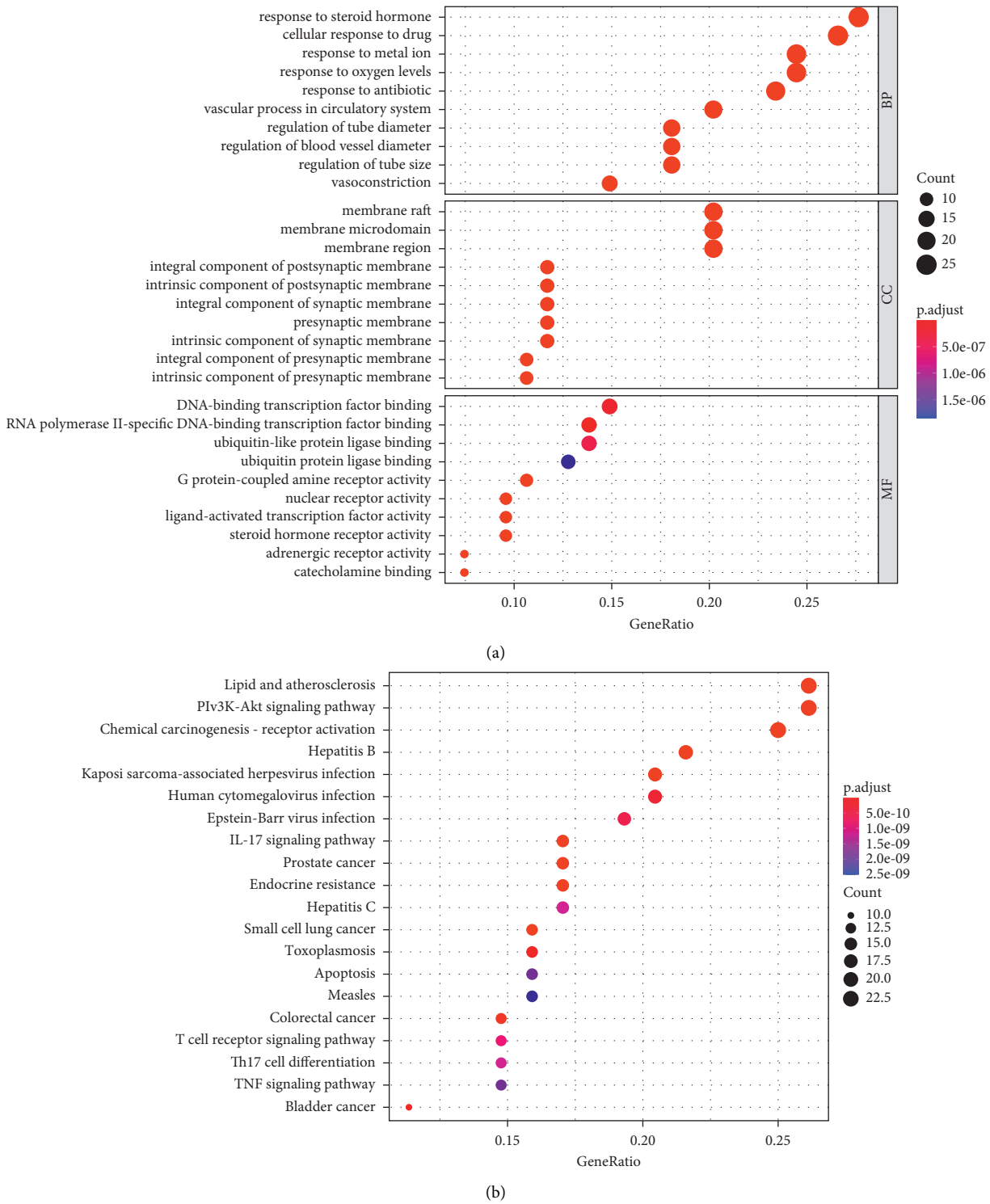

Figure 3: (a) GO process enrichment results. (b) KEGG pathway enrichment results.

are selected according to the node degree (Figure 2(d)). It can be seen that the node degrees of AKT1, IL-6, and EGFR are 66, 56, and 53, respectively. They belong to the top three targets ranked by degree, indicating that they may play a key role in therapy and are the focus of subsequent studies.

3.5. Pathway-Enrichment Analyses Using GO and KEGG Databases. We selected the top 10 biological processes (BP), cellular components (CC), and molecular functions (MF) of 94 therapeutic targets for analysis (Figure 3(a)). BP mainly includes response to steroid hormone (GO:0048545),

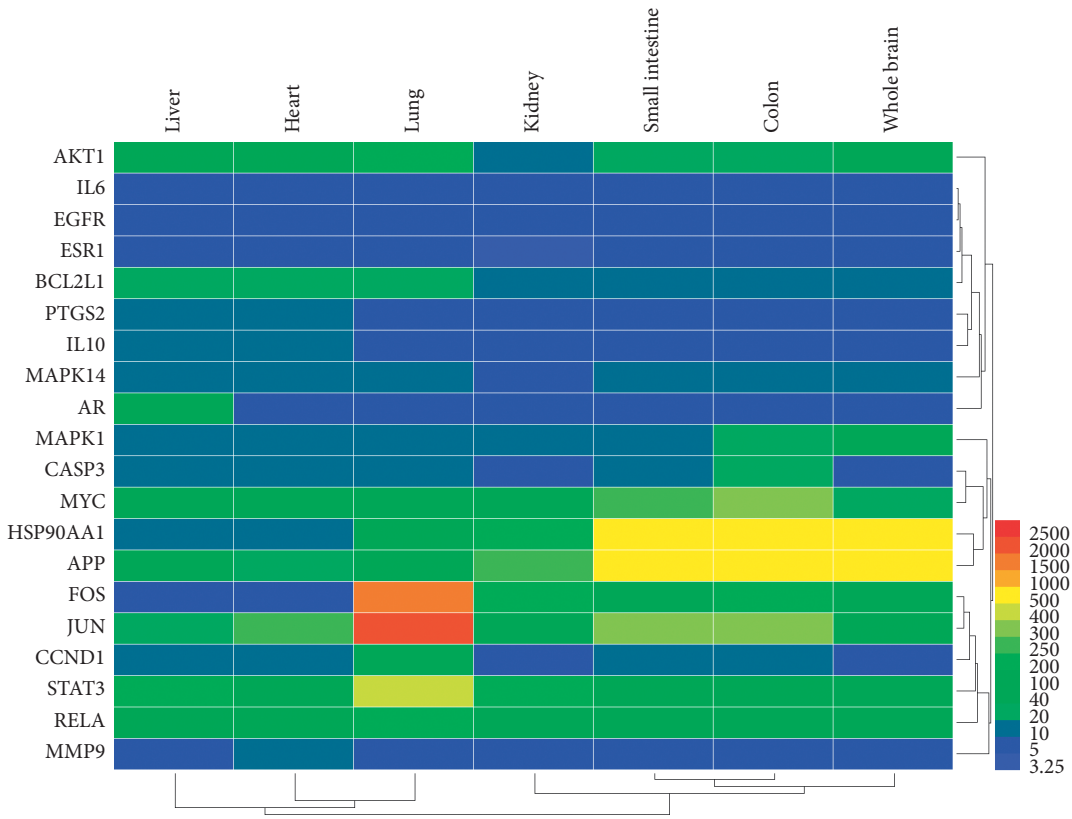

Figure 4: Heatmap of target expression in different organs. The *x*-axis indicates the organ name. The *y*-axis indicates the target name; from left to right, the liver, heart, kidney, lung, small intestine, and whole brain.

vascular process in circulatory system (GO:0003018), and cellular response to drug (GO:0035690). CC mainly includes membrane raft (GO:0045121), membrane microdomain (GO:0098857), and membrane region (GO:0098589). MF mainly includes G protein-coupled amine receptor activity (GO:0008227), adrenergic receptor activity (GO:0004935), and nuclear receptor activity (GO:0004879). The details of the GO process enrichment results are shown in Supplementary Table S4.

Analysis of the KEGG pathway enrichment, results revealed that SMB may treat CI by regulating 138 pathways. We selected the top 20 pathways for analysis (Figure 3(b)). The results showed that SMB treatment of CI mainly involved lipid and atherosclerosis, chemical carcinogenesis-receptor activation, IL-17 signaling pathway, and other pathways (Supplementary Table S5).

3.6. Expression of Targets in Different Organs. According to TCM theory, when lesions occur in local organs or tissues, they often cause reactions in other parts of the body. Therefore, we selected the top 20 proteins of the PPI network to observe their expression in different organs (Figure 4).

We can see that most proteins are also highly expressed in the lungs, in addition to higher expression in the brain. This suggests that it may be possible that SMB may also be linked to physiological processes in the lungs when treating CI through these targets. Studies have also shown that lobectomy may cause serious cardioembolic cerebral infarction complications [58]. This also illustrates to some extent that SMB may play a therapeutic role by regulating the organs associated with CI and allowing them to reach homeostasis.

3.7. Computational Assessment of Selected C-T Interactions. We selected AKT1, IL-6 and luteolin, and tanshinone IIA, respectively, for docking activity validation by in silico techniques. When the binding energy of protein and active ingredient is <0, it indicates that the active ingredient has the possibility of acting on protein. The lower the binding energy, the more reliable the possibility of action [9].

First, we selected luteolin and AKT1 and IL-6, respectively, for binding validation. The calculated score between luteolin and AKT1 was −8.5 kcal/mol. The calculated score of luteolin with IL-6 was −7.1 kcal/mol, and hydrogen bonds were formed between luteolin and multiple amino acid residues of AKT1 and IL-6, respectively (Figures 5(a) and 5(b)), indicating that luteolin may act on the above two proteins.

Next, tanshinone IIA was docked with AKT1 and IL-6, respectively. The docking scores were −9.1 and −7.3 kcal/mol, respectively. Tanshinone IIA can also form hydrogen bonds with multiple amino acid residues of AKT1 and IL-6, respectively (Figures 5(c) and 5(d)). The validation results illustrate that tanshinone IIA had a good possibility of binding to two CI targets.

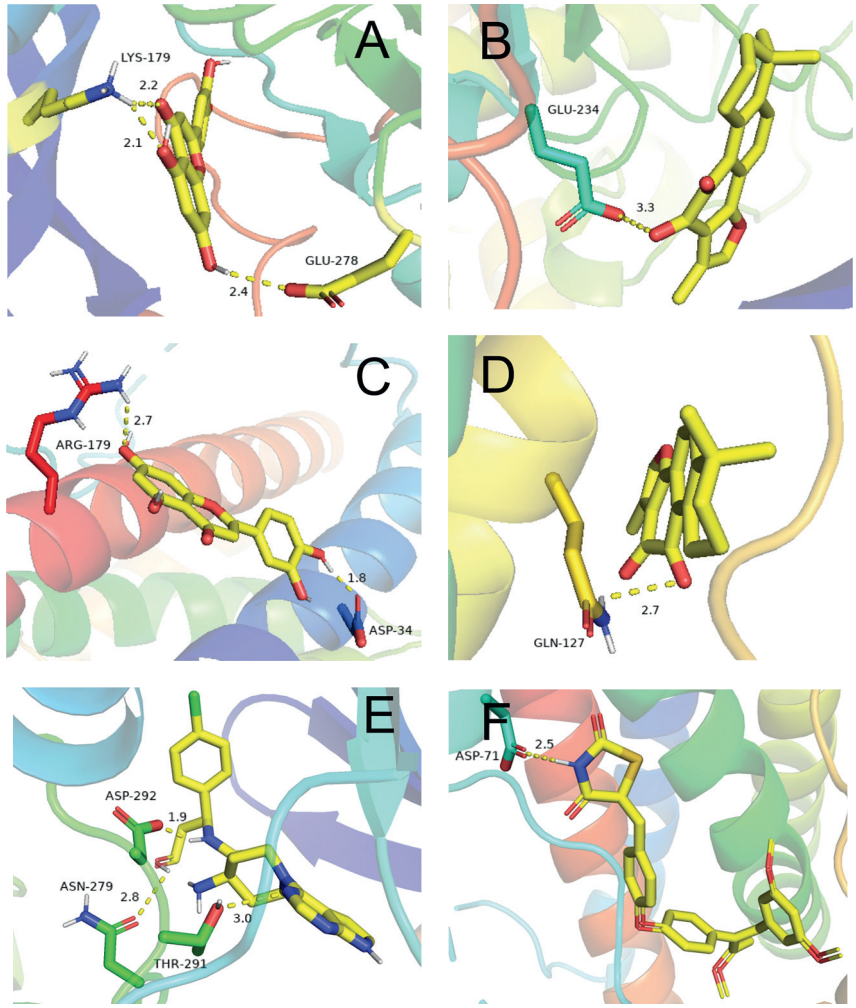

Figure 5: Binding studies of selected compound-target interactions. (a) Luteolin with AKT1. (b) Luteolin with IL-6. (c) Tanshinone IIA with AKT1. (d) Tanshinone IIA with IL-6. (e) AKT1 and small molecule inhibitors. (f) IL-6 and small molecule inhibitors.

Finally, we performed activity validation of AKT1 and IL-6 proteins with their small molecule inhibitors, respectively, for judging the reliability of predicting active components. The calculated scores for the two were -9.1 and -6.8 kcal/mol, respectively. At the same time, both small molecule inhibitors and predicted active components bind to the same active pocket of the corresponding protein, except that the amino acid residues that form hydrogen bonds are different (Figures 5(e) and 5(f)), which was determined by the different chemical properties between the components. Overall, the above results suggest some plausibility of the previously predicted active ingredient.

4. Discussion

It is reported that patients who die due to CI account for about 40% of patients with cardiovascular disease each year in China, with the number of people affected being about 1.2 million [59]. For the health of patients, the development of an effective treatment for CI is currently the primary problem. CI is generally a brain function impairment caused by blood circulation dysfunction. According to TCM theory, CI is caused by blood stasis and venation obstruction, which is consistent with the clinical treatment symptoms of SMB, indicating that SMB may be a potential drug for the treatment of CI. Therefore, we hope to explore the scientificity and mechanism of action of SMB in the treatment of CI using network pharmacology.

Luteolin, tanshinone IIA, miltirone, dihydrotanshinlactone, cryptotanshinone, and isocryptotanshinone and other components may be the main material basis for CI treatment. Modern studies have shown that luteolin can regulate oxidative stress and related proteins on apoptosis pathways (such as TLR4, TLR5, p38 MAPK, etc.), thereby alleviating CI [60]. Tanshinone IIA can treat CI by various mechanisms such as promoting cerebral blood circulation and inhibiting inflammatory processes [61]. Supercritical CO_2 extract of SMB with a cryptotanshinone content of 4.55% can alleviate cerebral ischemic injury by reducing thrombosis and platelet aggregation [62]. Miltirone is considered as a potential drug for the treatment of CI through antiplatelet drugs [63]. Based on the above evidence, it is scientific that we use network pharmacology to screen the active ingredients of SMB for the treatment of CI.

Luteolin, tanshinone IIA, miltirone and other components may play a key role in the treatment of CI.

Proteins such as AKT1, IL-6, EGFR, and MAPK1 occupy an important position in PPI networks. AKT protein plays a key role in the occurrence and development of CI, and AKT protein phosphorylation can play a protective role when brain tissue is ischemic. At the same time, AKT can also regulate the biological activity of many nerve cells [63]. Inflammatory factors can not only indicate the physiological status of CI patients but also regulate the progression of CI itself. In general, at the onset of CI, the cerebral blood flow circulation is interrupted, causing the release of a large number of inflammatory factors including IL-6 and recruiting a variety of immune cells (such as macrophages, T lymphocytes, and natural killer cells) to accumulate, resulting in an excessive immune response [64]. Therefore, reasonable control of the occurrence of inflammatory response can effectively reduce the harm of CI to the body. Neurogenesis is an important mechanism for the treatment of CI, and EGFR can activate and regulate neurogenesis during CI development and is a key protein in CI treatment [65]. In addition, the phosphorylation level of MAPK1 is also closely related to neuronal survival [66].

GO enrichment results indicate that CI treatment mainly regulates processes such as response to steroid hormone, membrane raft, and G protein-coupled amine receptor activity. The signaling pathway enrichment results suggest that pathways such as lipid and atherosclerosis, chemical carcinogenesis-receptor activation, and IL-17 signaling pathway are of higher importance in CI therapy.

5. Conclusions

SMB is a commonly used drug in TCM clinical practice and has great application prospects. We predicted the mechanism of action of SMB in the treatment of CI by network pharmacology and validated some of our prediction results using in silico docking techniques. Of course, more evidence is needed to show the effectiveness of SMB in the treatment of CI, which will also be the focus of our later study. Our study provides a reference for TCM treatment of CI.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request. The authors obtained the composition of rhubarb and its potential target from the TCMSP database (https://tcmsp-e.com/). They also obtained the potential target of cancer according to the DisGeNET database (https://www.disgenet.org/search) and GeneCards database (https://www.genecards.org) and, subsequently, PPI analysis (STRING database, https://string-db.org/), 343 KEGG pathways analysis, and GO biological processes (clusterProfiler package).

Additional Points

Traditional Chinese medicine (TCM) is a complicated system containing hundreds of constituents responsible for their therapeutic effects. As a brand-new area of pharmacology, network pharmacology provides new approaches of drug discovery for complex diseases and offers new methods for elucidating the multiple action mechanisms of drugs. In TCM theory, cerebral infarction (CI) belongs to the category of "stroke" and the common symptom is blood stasis, which can be treated by blood-activating and stasis-dissipating drugs. This is consistent with the therapeutic symptoms of *Salvia miltiorrhiza* Bunge (SMB), but its specific active components and mechanism of action are not clear. In this study, network pharmacology approaches were used to investigate the possible mechanism underlying the effectiveness of SMB in the treatment of CI. Firstly, our work was to demonstrate the efficiency of SMB in the treatment of CI. Our findings did not only provide scientific proofs for SMB in the treatment of CI but also indicate the feasibility of studying the mechanism of action of TCM in treating diseases based on network pharmacology. Our work provides a direction for elucidating the scientific connotation of traditional Chinese medicine in the treatment of diseases and drug discovery.

Disclosure

XY and JL share first authorship.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

YC and YH conceived and designed the study. XY was responsible for the writing of the paper. All authors participated in the drafting of the manuscript and revising it before final submission. Ye Xietao and Liu Jiali equally contributed to this work.

Acknowledgments

This work was supported by the Special Project for the Development of Traditional Chinese Medicine Science and Technology in Jiangsu Province (2020ZX15).

Supplementary Materials

Table S1: details of active compounds and targets. Table S2: known therapeutic targets in cerebral infarction. Table S3: the details of the PPI network. Table S4: the details of the GO analysis. Table S5: the KEGG pathway enrichment analysis. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 6847917, 17 pages
https://doi.org/10.1155/2022/6847917

Review Article

Combination of Stem Cells with Chinese Herbs for Secondary Depression in Neurodegenerative Diseases Based on Traditional Chinese Medicine Theories

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Received 12 July 2021; Revised 15 November 2021; Accepted 30 January 2022; Published 3 March 2022

Academic Editor: Mozaniel Oliveira

Depression is a common secondary symptom in neurodegenerative diseases (NDs) caused by the loss of neurons and glial cells. Recent research focuses on stem cell therapy to replace dead nerve cells, but the low efficiency of stem cell differentiation and short survival time are obstacles limiting the therapy's effectiveness. Clinically, patients with different diseases cannot obtain the same effect by using the same cell therapy. However, traditional Chinese medicine (TCM) often uses syndrome differentiation to determine the treatment plan for NDs. Based on TCM syndrome differentiation and treatment, this article summarizes the advantages of Chinese herbal medicine combined with stem cell therapy, mainly for the effects of various herbs on diseases and stem cells, including prolonging the survival time of stem cells, resisting inflammation, and antidepressant-like effects. In particular, it analyzes the unique pathways of the influence of drugs and acupuncture on different therapies, seeking to clarify the scientific TCM system. This review mainly elaborates on the treatment of secondary depression in TCM and the advantages of a herbal combined stem cell therapy in various methods. We believe it can provide a new clinical concept for secondary depression to obtain good clinical effects and reduce the risks borne by patients.

1. Introduction

Depression is a more common secondary symptom related to neurodegenerative diseases (NDs) than sleep disturbances and psychosis [1]. Moreover, neuropsychiatric disturbances are often more problematic and distressing than aspects of NDs to both patients and their families [2]. Zhao et al. [3] reported that depression is present in approximately 40% of people with Alzheimer's disease (AD). Furthermore, many studies have shown that 10–45% of people with Parkinson's disease (PD) have suffered from clinically significant depression [4–6]. Recent studies have explored the relationship between NDs and depression and mechanisms underlying the association between depression and NDs, or co-therapy for both diseases.

NDs are the collective term for AD, PD, Huntington's disease, amyotrophic lateral sclerosis, frontotemporal dementia, and spinocerebellar ataxias [7–9]. NDs are a major threat to human health and are becoming increasingly prevalent due to the growing aging population [10]. The most common NDs, AD and PD, are predominantly observed in elderly individuals, and the risk of these diseases increases with age. NDs have many factors, which include age [11], inflammation [12], reactive oxygen species (ROS) [13], and DNA damage [14]. It is known that aging is a major risk factor for neurodegeneration [9]. Adila Elobeid revealed that the brain tissue from older individuals contains abnormal deposits of aggregated proteins which contain hyperphosphorylated tau (p-tau), amyloid-β (Aβ), and α-synuclein [15]. However, it remains unclear whether the

levels of these deposits are linked with the degree of cognitive impairment and disease progression. When C-X Gong used pseudophosphorylation or the phosphomimicking method to imitate permanent tau phosphorylation, they revealed the structural and functional aspects of the pathologically p-tau in AD's brain and p-tau neurotoxic effects, including caspase activation and initiation of apoptosis [16]. Tau phosphorylation in the proline-rich [17] and C-terminal regions [18] regulates microtubule binding and tau aggregation differentially. Therefore, site-specific tau phosphorylation is one of the culprits of the disease due to p-tau inducing neuronal death. Patients with NDs or secondary depression present with both extracellular Aβ plaques and intracellular tau-containing neurofibrillary tangles in the brain [19]. Many studies have reported that the pathogenesis of NDs relates to changes in Aβ that precipitate the disease process and initiate a deleterious cascade involving p-tau [20]. Therefore, the source of Aβ should be explored. Olige Aβ accumulated in the brains of patients with NDs is thought to be the more toxic species for cells [21, 22] because they can permeabilize cellular membranes, thus resulting in a series of events leading to cell dysfunction and death [23]. Furthermore, amyloid aggregates (low molecular weight) derived from Aβ deposits with redox-active metal ions are considered more toxic since they can produce ROS, deleterious for the normal Aβ peptide and the surrounding biomolecules [24]. The phenomenon will promote the disease progression because the toxic effect constantly causes plaque enlargement and cell autophagy. Increased induction of autophagy involving Aβ is relatively common in NDs, including some disorders in which autolysosome clearance mechanisms are impaired [25]. This increases the neuronal apoptosis rate and accelerates NDs progression. However, many preclinical and clinical studies suggest that a common feature of NDs and secondary depression is dysregulation of the hypothalamic-pituitary-adrenal axis (HPA). Dysregulation of the HPA is caused by Aβ accumulation and tau hyperphosphorylation [26]. Previous studies have shown that Aβ administration can induce dysregulation of the HPA axis [27] and depressive-like behavior in animals [28].

Another common feature of depression and AD is the occurrence of neuroinflammation. Neuroinflammation exists in microglial activation and concentrations of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in both neurological conditions [29]. The phenomenon can influence the pathogenesis of both depression and AD by interfering with neuronal growth, differentiation, survival, and synaptic activity [30]. This may explain why depression is a secondary symptom in NDs. In a study of AD, microglial cells could remove Aβ plaques [31], but microglial cells also released inflammatory mediators that could contribute to Aβ deposition and further development of plaques due to cell death. In addition, products from dead neurons can further amplify this response by activating toll-like and inducing receptors for advanced glycation end products (RAGE)-dependent activation of p38 mitogen-activated protein kinase (MAPK), thus contributing to Aβ-mediated cortical synaptic dysfunction [32]. In summary, neuronal death is a serious consequence that results from a number of risk factors [33] and is commonly believed to lead to depression [34]. Secondary depression in NDs represents a major threat to human health, so it is necessary to seek treatments to reduce its prevalence.

Nowadays, there is a mainstream view that depression secondary to NDs can be treated by treating the underlying NDs. The treatment of NDs includes drug therapy, gene therapy, and cell therapy. Vinpocetine, a common drug, can decrease the expression levels of serum inflammatory cytokines, TNF-$\alpha$and MCP-1; and increase TLR3 mRNA levels, as well as protein levels of the downstream signaling molecules TRIF-$\beta$and IRF-3, and serum levels of the anti-inflammatory cytokines IL-10 and IL-8. Meanwhile, it also alleviates cognitive impairment [35] or depression. Furthermore, gene therapy, the delivery of genetic material to supply gene products that will permanently restore abnormal function or bring in target tissues/cells a therapeutic gene [36], has the potential to cure NDs and depression. Nevertheless, drug therapy and gene therapy can only delay the course of the disease or have many limitations. For example, patients are still at risk of NDs because dead nerve cells are rarely replaced by new cells. However, stem cell therapy can avoid this adverse outcome because stem cells will differentiate into nerve cells or other cells. However, differentiations need specific conditions.

The stem cell therapy is widely used and can delay the progression of NDs, plus it appears efficacious for depression. In the treatment of NDs, mesenchymal stem cells (MSCs) are the main stem cell therapy cells used, as they have high differentiation efficiency and safety in patients. Recently, several studies reported that bone-marrow-derived MSCs suppressed T-cell proliferation [37, 38], and the phenomenon may help combat immune rejection during treatment. Inflammation, which plays an important role in NDs and secondary depression in NDs [39], can be relieved by the immunomodulatory effect of MSCs and slows down disease progression. The MSC-induced immunosuppressive mechanism is as follows: (1) MSCs engage the inhibitory molecule PD-1 by its ligands to inhibit T-cell proliferation [40]; (2) MSCs release indoleamine 2,3-dioxygenase (IDO), which inhibits the proliferation of IFNγ-producing TH1 cells [41] and blocks NK-cell activity together with prostaglandin e2 (pGe2) [42]; (3) MSCs produce soluble HLA-G5 which can suppress T-cell proliferation, as well as NK-cell and T-cell cytotoxicity, and promote the generation of regulatory T cells [43, 44]. In clinical applications of MSCs, stem cells for tissue repair should easily access the target organ to exert their therapeutic effect through *in situ* administration. However, the method can be hampered by the anatomical location of the damaged tissue or multiple diseased sites [45]. Therefore, the ideal therapy should provide both systemic and local therapeutic effects. Steven M Devine found that MSCs can spread to many tissues after intravenous administration in a study of nonhuman primates using transplantation experiments [46]. Furthermore, other clinical research studies have shown that systemically administered MSCs preferentially migrate to the injury site to support functional recovery [47]. Furthermore, MSCs migrate in response to several chemokines (vascular cell-

adhesion molecule 1 and p-selectin) that bind to cognate receptors expressed on their cell surface [48] and lead to the activation of matrix metalloproteinases that degrade the basement membrane [48], accelerate the progression of systemic delivery. In summary, the systemic delivery of MSCs could be a useful way to administer these cells in clinical practice, but we should examine how to enhance stem cell migration and differentiation efficiency.

Traditional Chinese medicine (TCM) refers to the holistic approach to diagnosis, pathophysiology, and therapy in the Chinese herbs, based on accumulated knowledge and practice over a long period of time [49]. TCM has its own theoretical system and application form in stem cell research that emphasizes holism and syndrome differentiation treatment. It has been reported that certain TCM can promote the proliferation and differentiation of stem cells. Compared with other resultants or methods, TCM is relatively safe and easy to be administered in clinical applications. TCM can induce proliferation and differentiation of stem cells and also cause changes in the microenvironment, enhancing stem cell survival and function. Studies have shown that TCM can induce MSCs to differentiate into neural-like cells and modulate immune function to reduce or eliminate immune rejection [50–52]. Using TCM, the concept of holistic medicine, syndrome differentiation, and treatment as guidance, investigation of the mechanisms of proliferation, differentiation, and damage resistance of MSCs can advance the application of MSCs in NDs.

2. Mechanism and Advantage of Stem Cells in the Treatment of Secondary Depression

As mentioned earlier, nerve cell death is an important cause of secondary depression in NDs. MSCs primarily originated from bone marrow, and subcutaneous fat can differentiate into neural cells under any conditions [53]. MSCs exist in various tissue systems such as bone marrow, umbilical cord blood, umbilical cord, placenta, amniotic fluid, and adipose tissues [54–56]. MSCs were originally found in the bone marrow, which is also the primary source [57]. They can differentiate into ectomesenchymal cells, including ossification, cartilage, and fat cells [58–60], or non-mesoderm lineages, such as Schwann-like cells that play a role in development, myelination, and regeneration in the peripheral nervous system [61–63]. Therefore, using an injection of MSCs is an effective method that clinicians can use to treat nervous system diseases. MSCs promote other cells to differentiate into neural-like cells but also differentiate into neural-like cells under certain conditions. MSCs promote other cells to differentiate into neural-like cells, but MSCs also induce a proliferation of neural cells [64]. MSCs can produce cytokines, chemokines, and growth factors, which have important roles in creating favorable microenvironments for the proliferation of neural cells at the injury site, enhancing angiogenesis, synaptogenesis, and neurogenesis in the damaged brain tissue [65, 66]. The microenvironment provided by bone marrow MSCs (BMSCs) is beneficial for neural stem cells (NSCs) to differentiate selectively into neuronal and astrocytic phenotype cells. BMSCs can induce neuronal differentiation of NSCs and also enhance neuron survival. Soluble factors secreted by BMSCs are responsible for the neuronal differentiation of NSCs [67]. BMSCs can cross the blood-brain barrier and migrate into the injured site [68] and then differentiate into mesenchymal lineage cells, including neurons and non-neuronal cells in the brain [69]. Jahani et al. [70] indicate that MSCs cultured on nanofiber scaffold can differentiate into neuronal cells. The electrospun scaffolds, particularly scaffolds with random nanofibers, can promote the differentiation of MSCs. Magnetoelectric (ME) nanofibers also comprise the scaffold for the growth of MSCs and differentiation into neural cells because the material can improve the high yield differentiation of MSCs to neural-like cells [71], which is extremely important in treating chronic disease. Furthermore, recent research found that static pressure enables rat MSCs into neural-like cells without bio-factors or chemical disbalance [72]. Studies indicate that cells derived from bone marrow survive, proliferate, migrate, and differentiate into glial and neuronal phenotypes, such as nestin, neurofilaments, MAP-2, or Neu-N [73–78]. Compared with NSCs, MSCs are easy to isolate from the small aspirates of bone marrow that can be obtained under local anesthesia, capable of rapid proliferation in culture, amenable to survival and integration in the host brain, and immunologically inert [79]. MSCs have many advantages in brain injury: (1) inhibition of apoptosis and fibrosis; (2) stimulation of angiogenesis and recovery of blood supply in the lesion [80]; and (3) attenuation of oxidative stress [81]. The MSC-mediated immune regulation mechanism includes suppression of CD4 + and CD8 + T-cell proliferation [82], promotion of regulatory T-cell quantity and enhancement of its immunosuppressive activity [83], and addition to the secretion of immunosuppressive substances [84]. Therefore, MSCs have wide beneficial applications in nerve tissue repair. In clinical practice, doctors always select adipose tissue-derived MSCs (ASCs) to replace BMSCs. Compared with BMSC, ASCs can be more easily isolated, are safer, and considerably larger amounts of ASCs can be obtained [85]. Although injection therapy with ASCs and BMSCs has become the main treatment method for serious tissue damage diseases, the treatment effect can be poor or fail due to unstable ASCs and BMSCs differentiation states. Therefore, we should aim to identify more effective ways to improve the efficiency of cell therapy from other perspectives, for example, TCM.

3. Analyzing Stem Cell Therapy of Secondary Depression in NDs Based on the Theory of TCM

"Lingshu·Sea Theory" records that "people have a sea of marrow, a sea of blood, a sea of air, and a sea of valleys. . ." and the brain is the sea of marrow. This coincides with the main mechanism of differentiation of BMSCs into neuron-like cells in modern medicine. The Lingshu says "At the beginning of generation, the essence is generated first. After the generation of the essence, the brain is formed." "YixueZhongzongCanxiLu" summarizes its theory as "The brain

is the sea of bone marrow, which is generated by the Yang Qi and Yin Qi of the kidney." The kidney is the congenital foundation, and the main bone produces marrow. The kidney essence is the foundation of the brain and the congenital source of the brain marrow. "Suwen Ni Tiao Lun" records that "if the kidney does not grow, the marrow cannot be full." The congenital essence hidden in the kidney is nourished by the essence of the water and grain after a person is born to maintain the abundance of the essence of the kidney. The enrichment of the kidney essence also depends on the nourishment of the body's Qi, blood, and body fluid, and the brain marrow is also closely related to the body's Qi, blood, and body fluid. If the Qi and blood are full, the brain marrow will grow. If the Qi and blood are unbalanced and the kidney essence is dysfunctional, the marrow sea will be empty. Insufficiency of the marrow sea can also cause insufficiency of the kidney essence, which affects the body's viscera. Therefore, if the kidney essence fails, the sea of marrow is depleted, and the sea of marrow is filled to strengthen the kidney essence. "ZhuBingYuanHouLun" records that "the kidney stores the essence, and the essence is made by the blood." Therefore, there is the saying that "essence (Jing) and blood being derived from the same source," and both essence and blood originate from the essence of the kidney. However, the kidney stores the essence, produces the marrow, and mediates the blood. "Essence and blood are homologous" can also be called "the essence and marrow being derived from the same source." Therefore, essence, Qi, blood, marrow, and brain form an interactive system. Kidney essence, Qi, and blood are the marrow sea, which is the material basis for brain growth and development. Kidney essence can correspond to embryonic stem cells and the kinds of tissues and organs differentiated from ESCs. MSCs are mainly derived from bone marrow, which has the characteristics of essence, marrow, and blood and can be transformed into other substances. Therefore, people have high expectations for the effect of MSCs in the treatment of neurological diseases. When the Yin and Yang of the Qi and blood of the viscera are unbalanced, it affects the production of innate essence, causing insufficiency of Qi and blood, dystrophy of kidney essence, dysfunction of kidney essence, insufficiency of the marrow sea, and loss of nutrition in the brain, and its physiological functions are naturally affected. This process can also cause nerve cell necrosis or apoptosis and impair the regulation mechanism of stem cell proliferation, migration, and differentiation. Therefore, starting from the theory of TCM, combined with modern medical research, it can be concluded that the regulation mechanism of bone marrow mesenchymal stem cell activation and repair is closely related to essence, marrow, Qi, and blood, and they complement each other and act synergistically. However, secondary depression in NDs, known as "Yu syndrome" in TCM, is mainly caused by emotional injury and stagnation of liver Qi, resulting in a loss of liver relief, loss of spleen health transport, displacement of the heart, and imbalance of Yin, Yang, Qi and blood in the viscera. Based on the principle of "holistic concept, syndrome differentiation, and treatment," we analyzed the etiology and pathogenesis of secondary depression in NDs. We believe that the main causes are the loss of Qi in the liver and spleen and brain orifices caused by heart displacement and nourishing. Therefore, in the treatment of secondary depression, we should also consider targeted methods based on the TCM theory.

4. Application of the TCM Theory Combined with Stem Cell Therapy on Secondary Depression in NDs

Holistic care is important in TCM and the theory emphasizes the unity of both micro- and macroenvironments. Holism has been used in the clinical diagnosis of TCM for a long time, such as syndrome differentiation (Bian Zheng) and treatment. Following the method detailed in Qiao et al. [86], we have divided the Chinese herbal medicines into five categories: tonifying Qi and reinforcing the healthy Qi; tonifying Qi and activating blood circulation; activating blood and resolving stasis; tonifying the kidney to supply essence; and inducing resuscitation by a Fu-unblocking therapy. We have examined those that could be used in the treatment of depression and NDs (Table 1).

4.1. Tonifying Qi and Reinforcing the Healthy Qi (TQRHQ). TCM believes that humans have sufficient positive Qi to resist diseases. On the one hand, positive Qi can stimulate the functions of the Zang-Fu, promote the production of Qi, blood, and other subtle substances of the human body, and improve the metabolism of the microenvironment. Adequate Qi makes it difficult to develop depression or even secondary depression because Qi is related to emotions. TCM doctors have confirmed that *Hedysarum Multijugum Maxim. (huangqi)* and *Panax Ginseng C. A. Mey. (renshen)*, TQRHQ herbs, can promote stem cell regeneration and relieve secondary depression. Astragaloside IV (AS-IV) is a Qi invigorating drug, and *huangqi* has been widely used for the treatment of nervous system diseases in China. *AS-IV* attenuated TLR4 expression through the NF-kB signaling pathway in MSCs, promoting the proliferation of MSCs [87]. In a recent study, *AS-IV* was shown to accelerate differentiation by enhancing the expression levels of nerve growth factor (NGF), which is strongly related to GSK3β/β-catenin signaling [88]. Further studies showed that the MSCs could differentiate into neurocyte-like and gliocyte-like cells *in vitro* [89]. Both Wnt-1 and Ngn-1 genes play important regulatory roles during the differentiation of rat bone-marrow-derived MSCs to neurocyte-like cells [89]. *huangqi* injection can induce the differentiation of MSCs into neuron-like cells, and the process of differentiation might be mediated by activating Wnt signaling pathways [90]. Wu et al. [91] demonstrated that *ginsenoside-Rg1* from *renshen* could strengthen the spatial learning memory ability in dementia rats with transplantation of BMSCs, which might be possibly correlated to up-regulating mRNA expression of NGF in the basal forebrain after BMSCs transplantation. Yang et al. [92]. found that *astragalus polysaccharide (APS)* effectively reduces mitochondrial ROS accumulation, which

Table 1: Function and classification of traditional Chinese medicine.

Treatment rules	Herb or patent drug	Component	Function
Tonifying Qi and reinforce the healthy Qi	Huangqi	Astragaloside IV	Promotes proliferation and differentiation of stem cells; antidepressant-like effects
		Astragalus polysaccharide	Reduces mitochondrial ROS accumulation and iron overload
	Renshen	Ginsenoside-Rg1	Upregulates mRNA expression of NGF
		Ginsenoside Rk1	Antidepressant-like effect
	Shenqi Fuzheng injection		Promotes differentiation of stem cells
Tonifying Qi and activating blood circulation	Danggui	*n*-butylidenephthalide	Maintains stem cell pluripotency; antidepressant-like effect
		Angelica sinensis polysaccharides	Promotes differentiation of stem cells
	Hongjingtian	Rhodioloside	Increases the survival of stem cells; antidepressant-like effect
	Buyang Huanwu Tang		Promotes differentiation of stem cells
	Naomai Yihao capsule		Promotes angiogenesis and neurological recovery
Activating blood and resolving stasis	Danshen	Salvianolic acid B	Promotes differentiation of stem cells; antidepressant-like effect
	Yinxingye	EGb761	Promotes differentiation of stem cells; antidepressant-like effect
	Sanqi	Total saponins of panax notoginseng (tPNS)	Promotes differentiation of stem cells
	Chuanxiong	Sodium ferulate	Promotes differentiation of stem cells
	Huzhang	Polydatin	Facilitates stem cells migration; reduce oxidative-induced apoptosis
	Xuesaitong capsules	Panax notoginseng saponins	Promotes mobilization of stem cells; regulate the immune environment
Tonifying the kidney to supply essence	Shudihuang	Rehmannia glutinosa polysaccharide	Increases the survival of stem cells; antidepressant-like effect
	Yinyanghuo	Icariin	Promotes proliferation of stem cells; regulate female hormonal imbalance; antidepressant-like effect
	Gouqizi	Lycium barbarum polysaccharide	Promotes the generation of neurons; antidepressant-like effect
	Shanzhuyu	Morroniside	Anti-apoptosis effect; antioxidative stress effect; anti-ischaemic effect; attenuate stem cells dysfunction
Inducing resuscitation by a fu-unblocking therapy	Dahuang	Rhubarb aglycone	Promotes transplantation of stem cells
	Huangqin	Baicalin	Anti-apoptosis effect; antidepressant-like effect
	Houpo	Honokiol	Anti-inflammation function

can remarkably inhibit apoptosis, senescence, and the reduction of proliferation and pluripotency of BMSCs caused by iron overload [93]. *APS* may play a critical role in the maintenance of the number of MSCs, to ensure the treatment effect when the patient receives the cell therapy. *AS-IV* is also a potential drug against depression as it increases PPARγ expression and GSK3β phosphorylation and decreases NF-κB phosphorylation [94]. In *AS-IV*'s functions, the most important mechanism is the upregulation of PPARγ expression because PPARγ expression level affects the inhibition of neuroinflammation. Moreover, as a herb of TQRHQ, *ginseng* also has antidepressant-like effects via ameliorating neuroinflammation and decreasing neuronal apoptosis [92]. Li et al. showed that *ginsenoside Rk1* acts as an antidepressant through its antioxidant activity, the inhibition of neuroinflammation, and the positive regulation of the BDNF-TrkB pathway [95]. Qi can promote the absorption of acquired essence. Acquired essence can replenish the kidney essence, and the kidney essence can be transformed into the marrow, promoting the replenishment of the marrow sea. Patients with secondary depression in NDs possess Qi deficiency, which will hinder the process of marrow replenishment. *Huangqi* and *renshen*, as representative Chinese medicines for TQRHQ, promote the circulation of Qi and the replenishment of marrow (stem cell differentiation).

4.2. Tonifying Qi and Activating Blood Circulation (TQABC). In TCM, Qi can push the blood to move, and Qi deficiency will cause blood stasis. At the same time, the stasis in vessels will further aggravate Qi deficiency, leading to Qi stagnation

and secondary depression. Therefore, *TQABC* can promote blood circulation and prevent blood stasis, which is a common method for the treatment of the acute stage of nervous system diseases such as central infarction and cerebral hemorrhage, as well as a TCM method for preventing depression. Recent Chinese medicine studies related to TQABC reported that it induces differentiation of MSCs to neurocyte-like cells. Nie et al. [96] showed the effect of transdifferentiation of MSCs into nerve cells by an ultrafiltration membrane extract mixture from *Angelicae Sinensis Radix (danggui)*, which revealed that BMSCs changed neuralmorphologically after induction through upregulation expression levels of neuron-specific enolase (NSE), nestin, NFP, MAP2, glial fibrillary acid protein (GFAP) in the positive control and ultrafiltration membrane extract mixture groups. However, *n-Butylidenephthalide*, an *danggui* extract, was found to be useful in maintaining stem cell pluripotency via the Jak2-Stat3 pathway by inducing cytokine (leukemia induced factor, IL-5, IL-11, et al.) expression levels [97]. *Angelica sinensis polysaccharides*, another component in *danggui*, can significantly up-regulate cyclin D1, RUNX2, OCN, ALP, and BMP-2 protein levels in MSCs. We also found that *Angelica sinensis polysaccharides* activated PI3K/AKT and Wnt/β-catenin signaling pathways in MSCs, to promote bone formation [98]. Ha et al. confirmed that *rhodioloside*, which is the main ingredient of *Rhodiola rosea L. (hongjingtian)*, could activate the HIF-1 pathway to promote the survival of BMSCs and repair damaged neurons [99]. Therefore, *rhodioloside* combined with MSCs could be used in the treatment of patients with NDs. We also reviewed herbs that have antidepressant-like effects in TQABC. *Danggui* also exerts antidepressant effects through increasing the level of BDNF protein and increasing the phosphorylation of its downstream targets, which contain cAMP-response element-binding protein (CREB) and extracellular signal-regulated protein kinase (ERK 1/2) in the hippocampus because the phosphorylation of ERK 1/2 and CREB was significantly decreased in the hippocampus of animals with depression [100]. Regarding research of *Rhodiola rosea L.* in depression, these results showed that its extract SHR-5 is efficacious in treating patients with depression [101] and improves depressive and anxiety symptoms [102]. At the same time, its components appear to be well-tolerated with a favorable safety profile compared with conventional antidepressants [103]. However, other herbs of TQABC can improve the symptoms of patients with depression through neurological and rehabilitation effects. Qi can preserve essence, blood can nourish organs and tissues, and blood can transform spirits, so Qi and blood are the main substances in the body's mental activities. Abundant Qi and blood, and unblocked blood vessels, are conducive to the recovery of the sea of marrow and the stability of spiritual sentiment. TCM with the dual functions of invigorating Qi and activating blood can treat secondary depression in NDs caused by Qi stagnation and blood stasis.

4.3. Activating Blood and Resolving Stasis (ARBS). From a TCM viewpoint, the prognosis and rehabilitation of patients with a reduced formation of new blood will be seriously affected due to blood stasis. In the acute stage of brain injury, blood stasis appeared, leading to neuronal necrosis and apoptosis. Blood stasis, similar to thromboses, also severely damaged the regulation mechanism of proliferation, migration, and differentiation of NSCs, increasing the incidence of secondary depression. Herbs of ARBS can usually nourish the blood, remove blood stasis, and promote blood production. Studies have shown that certain Chinese herbs have a specific function of ABRS and have roles in MSCs differentiating into nerve cells, improving central nervous system microcirculation, and assisting in the rehabilitation process after a stroke or brain injury [104]. After induction of *Radix Salviae (danshen)*, MSCs exhibited the typical form of perikaryon with a pyknic cell body and prominence projection neuron. These cells expressed NSE, NF-M, and nestin and were negatively expressed in GFAP [105]. *Salvianolic acid B (Sal B)*, a major bioactive component of the traditional Chinese herb, *danshen*, is widely used in the treatment of cardiovascular diseases [106] and exerts neuroprotective effects [107]. Xu et al. found that *Sal B* had no obvious toxic effects on hMSCs, whereas *Sal B* can promote the osteogenic differentiation of hMSCs by activating the ERK signaling pathway [108]. An extract of *Ginkgo Folium (yinxingye)* named *EGb761* increases stem cells proliferation, but *EGb761* induces stem cell to neural differentiation instead of glial cell differentiation [109]. *EGb761* also provided high levels of neuroprotection, revealing that *yinxingye* for ARBS is beneficial for NDs and secondary depression [110]. Zheng et al. [111] studied the effects of *total saponins of Panax notoginseng (tPNS)* on angiogenesis in rat BMSCs (rBMSCs), and their study showed that *tPNS* (100 μg/ml) can significantly enhance the mRNA expression level of vascular endothelial growth factor (VEGF)-A but no obvious effect on the expression of Flt-1. In Zheng's study, different concentrations of *tPNS* were found to increase the capillary network formation of rBMSCs after Matrigel endothelial differentiation *in vitro* [111]. In studies on *Panax Notoginseng (Burk.) F. H. Chen Ex C. Chow (sanqi)*, the active ingredient of *sanqi* has been reported to have an enhancing effect on osteogenic differentiation of MSCs *in vitro* by upregulating the gene expression level of TGF-β1 [112]. *Sodium Ferulate*, as the main active constituent of *Chuanxiong Rhizoma (chuanxiong)*, combined with BMSCs could not only promote expression of glucose transporter 1 (Glut1) and neuron-specific class III beta-tubulin (Tuj1) in the peri-infarct area but also improve BMSCs expression of Glut1, GFAP, and Tuj1, due to up-regulation of stromal cell-derived factor-1 alpha (SDF-1α)/chemokine (CXC motif) receptor-4 axis [113]. Similarly, *Polygoni Cuspidati Rhizoma Et Radix (huzhang)* also has the effect of ABRS, which contains a key component *Polydatin* [114]. *Polydatin* can facilitate BMSC migration [115], protect BMSCs from oxidative stress-induced apoptosis [116], promote the neuronal differentiation of BMSCs via Nrf2 activation, and improve neurons functional recovery [117]. This type of herb can also be used in the treatment of depression. *Danshen* positively affects stem cells and the antidepressant-like effect mediated by ERK-CREB-BDNF in the hippocampus [118]. *Yinxingye*

and its extracts also have a good effect on depression. Results from both clinical practice [119] and animal models [120] showed that it has an antidepressant-like effect and can effectively improve depressive symptoms by reducing the expression of serum S100B, which is a marker of brain injury. As mentioned earlier, NDs are accompanied by blood stasis, making it difficult for blood to flow smoothly. Blood stasis is blocked in the brain, which further increases the burden of brain tissue; that is, the brain loses nourishment from the blood, and blood stasis will also prevent the essence from becoming marrow to replenish the marrow sea. The lack of marrow in the marrow sea will lead to secondary depression, suggesting that we can avoid secondary depression by promptly using Chinese medicine for ABRS in the early stage of the disease.

4.4. Tonifying the Kidney to Supply Essence (TKSE). According to the liver and kidney homology theory, the liver stores blood, the kidney stores essence, essence, and blood can breed and transform each other, showing that the liver and kidney are fundamental to each other. Liver Qi stagnation can lead to depression because the liver is related to emotions. The kidney stores the kidney essence, which is related to the production of brain marrow, and its essence is equivalent to the function of stem cells. Secondary depression in NDs is caused by nerve cell death and a lack of stem cells. This is similar to the liver abnormalities caused by kidney deficiency and then emotional disorders. The method of TKSE may play an important role in the prevention and treatment of secondary depression. *Rehmannia glutinosa polysaccharide (RGP)* is one of the effective components of *Rehmanniae Radix Praeparata (shudihuang)*, with the effect of TKSE, which can improve the survival rate of stem cells via increasing the p18 expression and decreasing cellular senescence-associated protein p53 and p16 [121]. Furthermore, in mouse behavioral despair depression models, *shudihuang* produced antidepressant-like activities by decreasing serum corticosterone levels, enhancing monoaminergic nervous systems, and upregulating the expression of BDNF or TrkB [122]. It has recently been reported that *Icariin (ICA)*, a major constituent of flavonoids from the Chinese medical herb *Epimrdii Herba (yinyanghuo)*, promotes the proliferation of various types of differentiated cells [123–125]. In the treatment of osteoporosis, *ICA* can promote osteogenic differentiation of MSCs and suppress the formation of adipocyte-like cells. Increased mRNA expression for osteogenic differentiation marker Runx2, osteocalcin, and bone sialoprotein (BSP), and decreased mRNA expression for adipogenic differentiation markers peroxisome proliferator-activated receptor gamma (PPARγ), lipoprotein lipase (LPL), adipocyte fatty acid-binding protein (aP2) occurs. *ICA* inactivated glycogen synthase kinase-3beta (GSK3β) and suppressed PPARγ expression is the main mechanism of function [126, 127]. Shuyan Qin's results demonstrate that *ICA* promotes the proliferation of BMSCs through activating ERK and p38 MAPK signaling, which further leads to the up-regulation of their downstream transcription factors Elk1 and c-Myc [128]. *Yinyanghuo* is often suggested as an antidepressant and health product because it is an anxiolytic medicine and is effective in female hormonal disorders [129]. *Lycium barbarum polysaccharide*, from *Lycii Fructus (gouqizi)*, also promotes the generation and development of new neurons and inhibits the MeHg-induced abnormal differentiation of astrocytes [130]. Zhou et al. reported that *Lycium barbarum polysaccharide* might be a potential therapeutic agent for the treatment of NDs against multiple targets that include synaptic plasticity and Aβ pathology due to enhancing neurogenesis [131]. Similarly, *gouqizi* also has the same effect in depression due to its antioxidative properties and decreasing the apoptosis of striatum neuro [132]. *Morroniside*, the main active component of *Cornus Officinalis Sieb. Et Zucc. (shanzhuyu)*, shows abundant biological activities, including anti-apoptosis [133], antioxidative stress [134], and anti-ischemic effects [135]. *Shanzhuyu* can also attenuate hydrargyrum-induced BMSC dysfunction by inhibiting AGE-RAGE signaling and activating Glo1 [136]. Essence is the carrier of sentiment and the foundation of marrowization. The essence of the human body is based on an innate essence, and acquired essence is constantly replenishing and generating. The kidney stores the innate essence and can transform the acquired essence into innate essence. Abundant innate essence can be transformed into marrow to replenish the damaged marrow sea. TCM of TKSE can nourish the kidney and replenish essence, promote the transformation of kidney essence into the marrow, and replenish the marrow of the marrow sea so that the damaged brain tissue can be restored.

4.5. Induce Resuscitation by Fu-Unblocking Therapy (IRFT). In TCM, depression is often related to the internal "heat" of the Fu, such as the stomach, gallbladder, and large intestine. IRFT can remove the "heat" from Fu. The removal of "heat" is equivalent to alleviating inflammation, which can alleviate local inflammation of the brain and slow down the apoptosis of nerves. Therefore, IRFT is useful in the treatment and prevention of secondary depression in NDs. Rhubarb aglycone, a component of *Radix Rhei Et Rhizome (dahuang)*, can decrease the degradation of basal lamina Col IV and the permeability of brain micrangium in cerebral ischemic rats with BMSCs transplantation by regulating the balance of matrix metalloproteinase-9 (MMP-9) and increasing the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) [137]. *Baicalin*, from *Scutellariae Radix (huangqin)*, has a potent antidepressant activity because of its ability to suppress apoptosis via preventing apoptotic protease-activating factor-1 expression and effectively suppressing caspase-mediated apoptosis signaling cascades [138]. *Honokiol* derived from *Magnolia Officinalis Rehd Et Wils. (houpo)*, a famous traditional herb for IRFT, has an anti-inflammation function [139] and fewer adverse effects [140] compared with antibiotics. Importantly, IL-1β, a target of *honokiol*, activates inflammatory pathways resulting in a vicious circle of MSCs transplanting and cell survival [141]. In a study of *honokiol* and human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) [142], it was found that *honokiol*

relieved these negative impacts induced by IL-1β and suppressed the nuclear factor-κB (NF-κB) pathway by downregulating the expression of p-IKKα/β, p-IκBα, and p-p65 in a dose-dependent and time-dependent manner. In Asian countries, *houpo*, used to treat mental disorders, including depression, can increase GFAP mRNA and protein levels to reverse the glial atrophy in the rat brain [143]. Therefore, the combination of anti-inflammation therapy and stem cells could be a novel strategy for better tissue repair. Fu refers to the large intestine, which can relieve patients' accumulated heat and turbidity, makes the blood flow smoothly, and stabilizes the mind. Patients with secondary depression in NDs are often accompanied by dysfunction of Fu and abnormal balance of the middle energizer due to the mutual influence of wind, phlegm, fire, and blood stasis. If the Qi in Fu is not smooth, the heat cannot be evacuated, and the heat will disturb the brain orifice, leading to obstruction of the brain orifice and abnormal emotions. The Fu's Qi is unblocked, and the filthy Qi descends, so the brain orifice opens, and the consciousness is clear. Herbs of IRFT can also purge heat and clear the middle energizer, restoring the circulation of Qi and the blood, removing the heat of the internal organs, and relieving the blockage of the brain.

4.6. Chinese Herbal Compound Prescription Agents. Single herbs can be effective in treating diseases, but their side effects can also be more apparent. Therefore, TCM doctors are more inclined to use a range of herbs to treat diseases. Chinese doctors believe that TCM prescriptions can integrate the therapeutic advantages of various herbs and decrease the toxic and side effects. In addition, prescriptions composed of multiple herbs are safer because lower dosages of single herbs contribute to their clinical safety. The TCM prescriptions mentioned below have benefits in preventing and treating secondary depression in NDs. *Shenqi Fuzheng injection (shenqifuzhengzhusheye)*, composed of *Astragalus* and *ginseng*, was proven to induce MSCs to differentiate into neurons *in vivo* in rats with middle cerebral artery occlusion (mcao). Immunohistochemical staining showed that *Shenqi Fuzheng injection* significantly increased the differentiation of MSCs to human NSE, neurofilament (NF), and GFAP [92]. *Buyang Huanwu Tang (buyanghuanwutang)* combined with MSCs transplantation could repair the injured blood vessels and lesion tissues. A study showed that VEGF and Ki-67 expressions were significantly upregulated in the MSCs and combination groups compared to the control and sham operation groups. Moreover, the combination group showed the strongest effect among these groups [144]. In another study of *Buyang Huanwu Tang* [145], it was found that mRNA and protein expression of the neuronal marker, NSE, and neural stem cell marker, nestin, were decreased in BMSCs by treatment with *Buyang Huanwu Tang*, and ERK and p38 in the MAPK signaling pathway were induced to participate in BMSCs differentiation into neuron-like cells. *Naomai Yihao Capsule (naomaiyihaojiaonang)* has the function of TQABC and can resolve phlegm to regulate the "sea of blood in the brain." The observation of *Naomai Yihao Capsule* combined with BMSCs transplantation showed that *Naomai Yihao Capsule* could promote angiogenesis and neurological impairment recovery by increasing the expression of CD31 in the brain tissue in focal cerebral ischemia rats, which underwent BMSCs transplantation, and the effect was reinforced with extended treatment time [146]. *Xuesaitong capsules (xuesaitongjiaonang)* is one of the main patent drugs used for ABRS, and *Panax notoginseng saponins* is the main ingredient. In rats with cerebral infarction [147], researchers have demonstrated that middle- and high-dosages of *Xuesaitong capsules* can promote and increase the level and mobilization of BMSCs in peripheral blood, which increases the level of stem cell factors and the number of CD117-positive cells and decreases the number of CD54- and CD106-positive cells in the plasma and bone marrow. Despite the long history of TCM prescriptions, few studies have been conducted on the use of stem cells combined with TCM prescriptions. This area is worthy of further investigation.

4.7. Acupuncture Combined with MSCs' Therapy. Acupuncture, originating from China, involves the insertion of a fine needle into the skin or deeper tissues at specific locations of the body (acupoints) to prevent and treat diseases [148]. Many clinical studies have shown that acupuncture can effectively promote the functional recovery of neurons after various types of central nervous system injuries (CNSIs) [149]. Its potential mechanisms include the prevention of inflammatory and oxidant stress, suppression of apoptosis, and stimulation of proliferation and differentiation of endogenous NSCs [150, 151]. However, there is an insufficient number of endogenous NSCs capable of differentiating into functional neurons, and the therapeutic effect of acupuncture can be inadequate. In spinal cord injuries, acupuncture promotes neural regeneration and axon sprouting by activating multiple cellular signal transduction pathways, such as the Wnt, Notch, and Rho/Rho kinase (ROCK) pathways [152, 153]. Z. Liu found that the Governor Vessel electro-acupuncture (GV-EA) may activate the process of cell metabolism and initiate synthesis and secretion of endogenous neurotrophic factors in the ambient tissues at the lesion site of the spinal cord [154]. Moreover, based on modified neurological severity scores and immunohistochemistry, a study regarding a cerebral ischemia/reperfusion injury revealed that electro-acupuncture and mesenchymal stem cell transplantation interventions are better than MSC transplantation alone as they improve neurological impairment and upregulate VEGF expression around the ischemic focus [155]. However, acupuncture is also used to treat depression. Based on the results of a meta-analysis, acupuncture may be a suitable adjunct to usual care and standard antidepressant medication [156].

5. Views and Prospects

For the treatment of NDs and secondary depression, the stem cell therapy has become a major treatment method, but

the low survival rate and low differentiation rate of stem cells are still an issue. However, recent studies have shown that Chinese herbal medicine has a positive effect on the survival and differentiation of stem cells and can adjust the inflammatory immune microenvironment and restore dopaminergic nerve function. In particular, the mechanism of action of single or compound components in herbs, inducing MSCs to differentiate into neuron-like cells and its antidepressant effect, is worthy of a more in-depth study. Based on the TCM syndrome differentiation and treatment system, we summarize the mechanism of action of Chinese herbal medicines on disease and the classification of Chinese herbs.

In TCM clinics, patients are defined with a certain type of syndrome, and currently, physicians will prescribe according to the type of syndrome.

The arachidonic acid pathway is involved in neuroinflammatory processes and has protective and detrimental effects in NDs or secondary depression (Figure 1) [157, 158]. This pathway is believed to be related to the excessive activation of microglia, and the excessive activation of microglia is the cause of NDs. Saliba et al. [159] reported that coumarin compounds and their structural analogs can inhibit the cascade effect of the arachidonic acid pathway from slowing down neuroinflammation without involving the endocannabinoid system. These findings show that the combination of TCM components and stem cell therapy can maintain the number of stem cells to ensure efficacy, improve the neuroimmune microenvironment, and reduce the death of nerve cells in regulating the local inflammatory response in patients. The neuroactive ligand-receptor interaction pathway is also a highly relevant pathway for each of our treatment principles. The disorder of this pathway has been shown to be significantly related to NDs and secondary depression [160], and α-synuclein can induce miRNA disorders, and miRNA targeting neuroactive ligand-receptor interaction pathways *in vivo* [161]. Among them, miR-133b targets the paired-like homeodomain transcription factor (Pitx3) and regulates neuronal differentiation and activity [162]. MiR-128 can inhibit the transcription factor EB (TFEB) in A9 and A10 neurons, thereby further inhibiting mTOR activation and defense against α-synuclein toxicity [163]. The efficacy of drugs with different effects on this pathway can alleviate the toxic effects of α-synuclein and affect the differentiation of stem cells into neuron-like cells [164]. This is extremely beneficial for *in situ* stem cell injection therapy of cholinergic synapses, dopaminergic synapses, and retrograde endocannabinoid signaling. Amphetamine addiction is also closely related to the treatment of NDs and secondary depression. These common pathways support the stem cell therapy and focus on eliminating high-risk factors of disease, including inflammation and neuronal cell death, which are beneficial for NDs and secondary depression.

However, there are special pathways for Chinese medicines of different treatment methods (Figure 2 and Table 2). In TQABC, there is an apoptosis and neurotrophin signaling pathway. We found the clinical phenomena that the use of

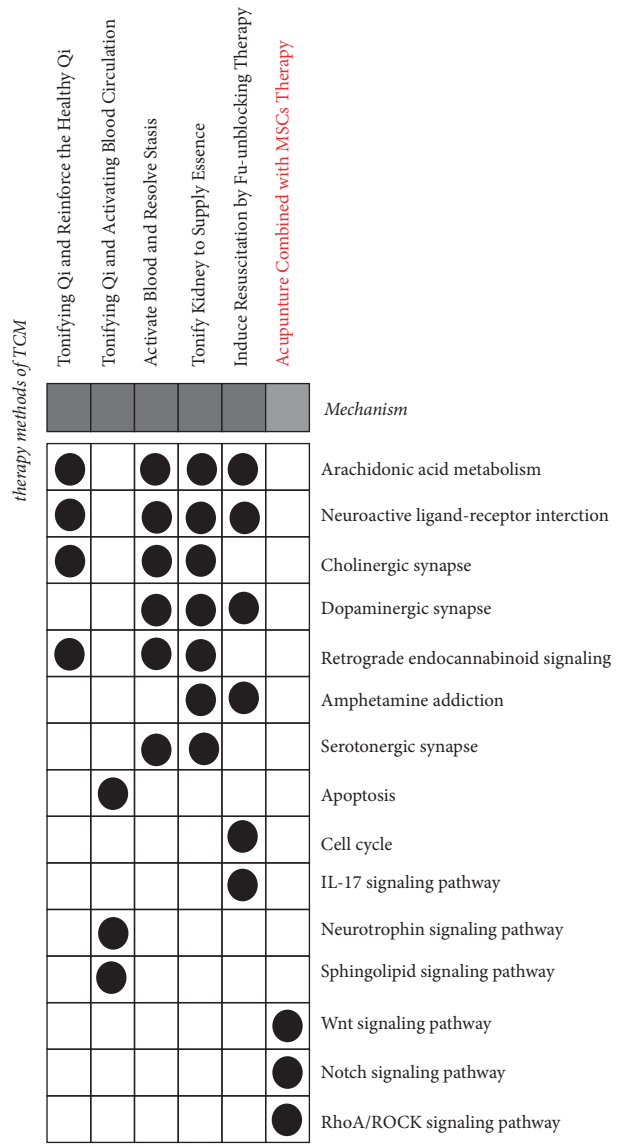

Figure 1: Similarities and differences of pathway enrichment among different treatments. The black dot represents that the treatment method affects the pathway, the right side is the entry of the drug-influenced pathway, and the name of the treatment method is on the top.

blood-activating drugs in patients with cerebral infarction can protect the brain nerves of the patients and promote the effects of the patients' volatilization [165]. IRFT is related to the cell cycle pathway and IL-17 signaling pathway. The cell cycle pathway can promote cell proliferation and constant number [166]. IL-17 is one of the working cytokines of the inflammatory storm [167]. Its level is significantly related to apoptosis. This is not available in other syndrome types. Therefore, this type of TCM is extremely beneficial to the survival of the stem cells and the suppression of the secretion of local inflammatory factors after stem cell therapy. Thermogenesis is a unique pathway for TQRHQ treatment, which is related to the formation of NDs because this pathway is related to the activity of mitochondria, and changes in mitochondrial redox affect cell proliferation and

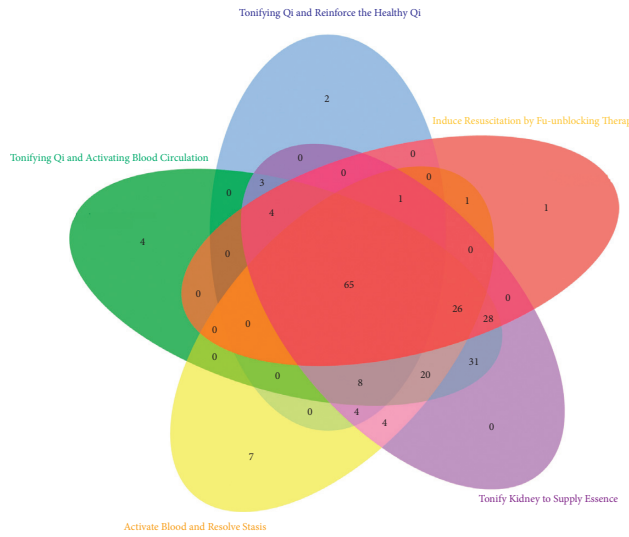

Figure 2: Peculiarities of 5 kinds of treatment methods of TCM enrichment pathways. Different colors represent the drug enriched pathway of different treatment methods, and the intersection part is two or more common pathways.

Table 2: Peculiar pathway of governance.

Governance	Special pathway
Tonifying Qi and reinforce the healthy Qi	Thermogenesis
Tonifying Qi and activating blood circulation	Fructose and mannose metabolism Glucagon signaling pathway Starch and sucrose metabolism
Activating blood and resolving stasis	Aldosterone synthesis and secretion Glycine, serine and threonine metabolism Histidine metabolism Phenylalanine metabolism
Induce resuscitation by fu-unblocking therapy	Tight junction

apoptosis [168]. The main characteristics of patients with depression and Qi deficiency syndrome are low immunity, malnutrition, or a long course of the disease, which leads to insufficient vitality. This phenomenon is related to insufficient body energy supply and insufficient cell replenishment, which is compatible with the special pathway of TQRHQ. In TQABC, the pathways of fructose and mannose metabolism, glucagon signaling pathway, starch and sucrose metabolism, and pathways related to sugar metabolism have also been shown to have a negative impact on the nerves. The progress of degenerative diseases has also contributed [169]. In particular, the glucagon pathway affects blood glucose levels. The level of blood glucose directly affects the processing of Aβ precursor protein toxicity and the clearance of Aβ [170]. Therefore, this type of TCM can effectively intervene in the progression of NDs caused by metabolic syndrome. Regarding patients with secondary depression of Qi stagnation and blood stasis type, the disorder of nutrient metabolism is similar to the deficiency of Qi in TCM. The accumulation of Aβ is the manifestation of blood stasis. Therefore, the special pathway of TQABC is extremely important for the imbalance of the body because this type of herb can interfere with diseases by affecting blood sugar. In the analysis of ABRS, the pathway of aldosterone synthesis and secretion is only present in the treatment. Single nucleotide polymorphisms and altered gene expression of components of the renin-angiotensin system are associated with secondary depression in NDs [171]. Renin-angiotensin-aldosterone system (RAAS)-activated angiotensin-type (AT4) receptor is also beneficial in NDs [172]. Its intervention in histidine metabolism may also be a promising therapeutic target for brain diseases to restore patients' circadian rhythms and solve sleep-wake disturbances [173]. From the perspective of TCM, the congestion degree of blood stasis syndrome is stronger than that of Qi stagnation and blood stasis and is accompanied by blood deficiency. Hypokalemia caused by high aldosterone levels has similar symptoms to blood deficiency, with clinical phenomena such as fatigue and numbness of the limbs. Aldosterone is related to local tissue remodeling and fibrosis and participates in inflammation, as is that abnormal aldosterone represents blood stasis in patients, so ABRS is more effective for patients with secondary depression with blood stasis syndrome. The most unique of IRFT is the tight junction. Tight junctions affect the stability of the blood-brain barrier, and the abnormality of tight junctions may trigger and/or contribute to a "vicious circle" such as neurodegenerative disease processes [174]. This pathway is also closely related to inflammation [175]. It

is possible to build a therapeutic system together with the stem cell therapy by maintaining the stability of the blood-brain barrier and reducing inflammation. As a targeted method, these pathways are particular to secondary depression with different syndromes in TCM, ensuring a good curative effect.

Combining TCM theories of medicinal properties, pharmacodynamics, and holism with western scientific approaches would help to advance the efficacy of TCM. Combining the concepts of TCM with modern medicine and technology will promote novel treatment ideas.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Jiahao Feng and Li Liao drafted the manuscript; Lin Zhang and Fangfang Xu collected and analyzed the herb data; Jiahao Feng and Li Liao classified the herbal medicines and compiled knowledge about the diseases; Jun Zhang reviewed this manuscript. All authors approved the final version of this paper. Li Liao and Jiahao Feng contributed equally to this paper.

Acknowledgments

This manuscript was funded by the National Natural Science Foundation (Grant no. 81873363).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 7265769, 13 pages
https://doi.org/10.1155/2022/7265769

Research Article

A Network Meta-Analysis of the Clinical Efficacy and Safety of Commonly Used Chinese Patent Medicines in the Auxiliary Treatment of Poststroke Depression

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Received 22 July 2021; Revised 21 September 2021; Accepted 20 December 2021; Published 6 January 2022

Academic Editor: Talha Bin Emran

Background. Poststroke depression (PSD) is a serious complication of clinical cerebrovascular disease. Patients not only have depression-related emotional symptoms but also have physical symptoms, such as autonomic dysfunction. At the same time, patients with varying degrees of depression will delay the neurological function of stroke patients. The recovery time of cognitive function and limb function will increase the risk of accidental death and even aggravate the mortality of cerebrovascular disease. Through combining data analysis and related literature, seven types of Chinese patent medicines (CPMs) widely used in the clinical treatment of PSD have been screened out. These herbs exhibit some clinical comparability under the conditions that the syndrome type and dosage form are relatively uniform. Therefore, in this study, the network meta-analysis method was used to evaluate the safety and efficacy of the seven CPMs screened out, and the probability ranking was performed to screen the best clinical auxiliary treatment plan of CPM. *Methods*. We searched the Chinese databases, including CNKI, WANFANG, and VIP, as well as the English databases, including the Cochrane Library, EMBASE, and PubMed, from inception to May 31, 2020, to identify randomized controlled trials (RCTs) on seven kinds of CPMs that were the subjects of the clinical research. The bias risk and quality of the included studies were analyzed with the Cochrane Handbook (version 5.1), ADDIS, and R software, and the results were compared in a network meta-analysis (NMA). *Results*. In terms of clinical effectiveness, the seven kinds of CPMs all improved clinical curative effects, with Jieyu Anshen capsule adjuvant treatment having the most significant effect [odds ratio (OR) = 5.00, 95% CI (1.72–9.48)]. Wuling capsule AT can effectively reduce the score index of scale factors for the HAMD score, NIHSS score, and TESS score [mean difference (MD) = −3.95, 95% CI (−4.88–3.00); OR = −3.25, 95% CI (−5.46)–1.05); OR = 0.22, 95% CI (0.05–0.79), resp.]. *Conclusion*. The mechanisms of seven CPMs in the adjuvant treatment of PSD have advantages. In terms of safety and efficacy, the CPMs had better clinical adjuvant treatment performance. Although this study concluded that the Jieyu Anshen capsule is the preferred drug for clinical treatment, a clear conclusion still needs to be verified in a high-quality randomized controlled study. In clinical practice, accurate selection and application can be carried out according to the specific characteristics of patients.

1. Introduction

Poststroke depression (PSD) is a serious complication of cerebrovascular disease, which is frequently observed within three to six months following stroke onset, with an incidence of approximately 22–75% [1]. Patients not only have symptoms related to autonomic nervous system and other physiological distress, but also have depression-associated emotional symptoms. Different degrees of depression can also attenuate the recovery of neurological functions, cognitive functions, and limb functions in these patients. Depression can further increase the risk of accidental death and even aggravate the mortality of cerebrovascular disease [2, 3]. Thus, it is of great importance to improve clinical efficacy, enhance neurological functions, and enhance the quality of life of patients. To date, conventional antidepressant medications are widely used along with western drugs. Although they are effective for increasing monoamine transmitter levels in the synaptic space of neurons, relieving depressive symptoms, and prolonging the treatment duration, patients can experience varying degrees of side effects and may easily relapse after treatment discontinuation. This severely limit patient's adherence to medication and the curative effects following stroke [4, 5].

Traditional Chinese medicine (TCM) has been shown to offer advantages for the treatment of strokes. Recently, Chinese patent medicine (CPM), in combination with western drugs, is commonly applied for treating this disease. CPM not only effectively avoids drug resistance, toxicity, addiction, and other defects that are seen with long-term application of western drugs but also mediates the visceral functions. CPM can rapidly relieve depression, reduce rehabilitation times, and enhance the quality of life. According to previous literature, seven types of oral CPMs widely used for the treatment of PSD were screened out. These are considered to be most representative, and they have some clinical comparability under the conditions such that the syndrome types and dosage forms are relatively uniform. However, the majority of meta-analyses have only described the efficacy of oral CPM in the treatment of PSD, and no evidence-based assessment has been conducted on the safety and efficacy of the seven representative CPMs in treating PSD. Hence, this network meta-analysis (NMA) aimed to provide the clinically relevant evidence of direct and indirect comparisons and comprehensively analyze the efficacy and safety of CPMs in the treatment of PSD. Additionally, the most ideal therapeutic approach was chosen to facilitate evidence-based clinical decisions for the optimization of combinatorial drug therapies.

2. Materials and Methods

2.1. Information Sources. Using computer retrieval technology, we searched for clinical randomized controlled trials (RCTs) of seven types of oral CPMs for the adjuvant treatment (AT) of PSD [6]. Primary searching was conducted from the establishment of the database to 31 May 2020. We searched Chinese databases, including CNKI, WANFANG, CBM, and VIP, as well as English databases, including the Cochrane Library, EMBASE, Web of Science and PubMed. The key terms included CPM, Wuling capsule, Shugan Jieyu capsule, Yangxue Qingnao granule, Jieyu Anshen capsule, Chaihu Shugan powder, Danzhi Xiaoyao pills, Xiaoyao pills, depression syndrome after stroke, PSD, and RCT. Different combinations of keywords, free words, and subject words were chosen for different databases.

During literature searching, the free words and subject words were independently searched, and the relevant keywords were employed for comprehensive searches. In addition, potential trial registrations were searched through the ClinicalTrials.gov and WHO international clinical trials registration platform. The references in the relevant journals were searched and tracked. Different search engines, such as Baidu academic, Google scholar and others, were used for manual searches. Data from major researchers and relevant authors were included to supplement incomplete reports or unpublished data from the original articles, in order to ensure comprehensive primary searches. In accordance with the Participant-Intervention-Comparator-Outcomes-Study (PICOS) principles, the studies that met the standards were included.

2.2. Eligibility Criteria. The selection criteria of this NMA were in accordance with the five main principles of PICOS.

2.2.1. Characteristics of Participants. The participants were patients with PSD and their inclusion was not limited by age, gender, or race. The diagnostic standard for PSD was based on the "Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke in China" (2014 Edition) revised by the Cerebrovascular Diseases Group of the Neurological Branch of the Chinese Medical Association in 2014, and stroke was in the sequelae period or recovery period [7, 8]. The diagnostic standard for depression was based on the "Classification and Diagnosis Criteria of Chinese Mental Disorders" (3rd Edition) [9]. The efficacy of TCM was assessed based on the diagnostic standards for stroke and depression [10].

2.2.2. Intervention and Comparator Types. When the judgment criteria of diagnosis and curative effects are clear and consistent, the experimental group is subjected to CPM treatment (Wuling capsule, Shugan Jieyu capsule, Yangxue Qingnao granule, Jieyu Anshen capsule, Chaihu Shugan powder, Danzhi Xiaoyao pill, or Xiaoyao pill) combined with western drugs, while the control group is subjected to western drug administration.

2.2.3. Types of Outcomes. Outcomes were described as (1) HAMD score, (2) TESS score, (3) NIHSS score, and (4) clinical efficacy.

2.2.4. Type of Study. All of the included studies were RCTs with blind or assignment concealment and no language limits.

2.3. Exclusion Criteria. We excluded nonrandomized controlled trials, case reports, experience summaries, self-controlled studies, review articles, animal studies, and repeated publications. The diagnosis of PSD is not always clear and studies sometimes combine stroke with other diseases. The efficacy judgment standard for the control and experimental groups was not always clear, and the treatment measures involved other treatments that could affect the final outcome of the causality judgment literature. Studies with incomplete data and unclear research findings or no connection with the full-text authors were also excluded.

2.4. Literature Screening. The electronic databases were searched by three researchers, and EndnoteX9 software was used to search the redundant information. The studies were combined when retrieval results were found in different databases. All data in the databases were retrieved and the full texts were downloaded. Two independent researchers conducted data extraction according to the preformulated method and the results were cross-checked and reviewed. Figure 1 shows the PRISMA flowchart describing the process of study selection [11, 12].

2.5. Data Extraction. The following data were extracted: (i) baseline information of the included RCTs, such as the first author, year, research topic, published journal, and so on; (ii) relevant information on the control and treatment groups in this study, such as the number of cases, age, treatment course, intervention measures, and outcome indicators; (iii) design types and quality evaluations of the included RCTs; (iv) outcome measures such as HAMD, TESS, NIHSS scores, and clinical efficacy.

2.6. Quality Evaluation. The quality of each RCT was evaluated by RevMan based on the Cochrane manual, including assignment concealment, random method, outcome data integrity, blind method, number of dropped cases, selective report, follow-up, and other biases, which was categorized into three groups: uncertainty risk, low risk, and high risk. The two first authors independently completed the quality evaluations of the included RCTs. If the results showed significant differences, a third researcher was invited to discuss and interpret the results and performed a quality evaluation. Literature quality and risk of bias assessments were also conducted based on the Cochrane manual. R v3.3.1 and ADDIS V1.16.5 were employed for statistical analysis, data integration, and NMA [13–15].

2.7. Publication Bias. The publication bias was assessed by R software when >5 RCTs were included. A symmetric inverted funnel shape indicates low or no publication bias. On the contrary, an asymmetrical shape indicates a potential publication bias.

2.8. Statistical Analysis. RevMan software was used for the assessments of literature quality and risk of bias. ADDIS and R software were employed for direct and indirect result comparisons and 95% CI calculations in the NMA. Meanwhile, anecdotal sequence and network relationship diagrams of the seven types of oral CPMs were constructed, in order to reveal the indirect comparative relationships among them. The node indicates a CPM type, the line denotes a direct or indirect comparative relationship between 2 CPMs, and the line thickness reflects the number of included RCTs. Subsequently, all direct and indirect comparisons were evaluated to determine the most effective CPM for PSD among these seven types of CPMs and estimate the rank probability of CPMs using the Markov chain Monte Carlo (MCMC) method. The 'NETMETA' program in R package was used, and the Bayes MCMC algorithm was called to analyze the random effects model results [16].

The odds ratio (OR) and 95% confidence interval (CI) were utilized for safety and efficacy analysis, such as the occurrence of side effects and recovery time of PSD symptoms. All measurement data were presented as standardized and weighted mean differences. According to the NMA probability ranking, the higher the total effective rate (Rank 1), the greater the effects, while the smaller the HAMD, NIHSS, and TESS scores (Rank 1), the greater the effects. The data of random effects model were called by ADDIS software according to the Bayesian MCMC algorithm for prior evaluation and processing (four chains were subjected to simulation modelling, and the initial value, iteration step, number of iterations and number of simulation iterations were adjusted to 2.5, 10, 20000, and 50000, resp.) [17].

We evaluated the methodological and clinical heterogeneity of the included studies and compared the fitting degrees of the random and fixed effects models. If there was statistical homogeneity ($I^2 \leq 50\%$, $P \geq 0.1$) in the subgroup, the fixed effects model was adopted for NMA. If no statistical heterogeneity ($I^2 > 50\%$, $P < 0.1$) was found, the random effects model was employed for NMA, and the potential causes of heterogeneity were determined based on methodological and clinical aspects. Descriptive analyses were performed when the RCT data could not be meta-analyzed.

The point split model was utilized to examine for inconsistency. If no statistical difference ($P > 0.05$) was observed among the studies in the subgroup, the consistency model was adopted for NMA; otherwise, the inconsistent model was applied. Convergence efficiency was tested by the potential scale reduced factor (PSRF). The results of consistency model analysis were considered reliable when a good convergence efficiency was achieved (PSRF = ~1).

3. Results

3.1. Results for Literature Searching. There were 547 studies during initial searching, and 52 clinical control studies were ultimately included after step-by-step screening. Figure 1 shows the procedure and data for literature screening.

3.2. Baseline Information and Quality Evaluation of Inclusion Studies. Fifty-two RCTs with 4711 patients with PSD were included. The experimental group included 18 Wuling capsules, 16 Shugan Jieyu capsules, 6 Yangxue Qingnao

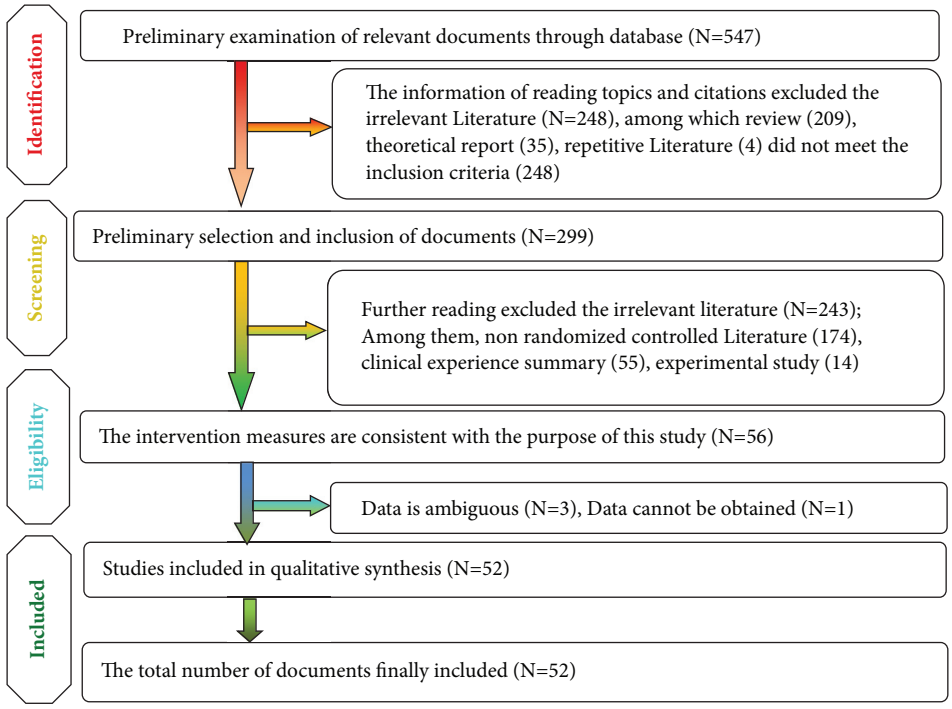

Figure 1: A flowchart of literature screening.

granules, 5 Danzhi Xiaoyao pills, 4 Xiaoyao pills, 2 Jieyu Anshen capsules, and 1 Chaihu Shugan powder combined with western medicines. Studies in the control groups all involved western medicines. The dose and method of administration of western medicines in both groups were the same. Table 1 shows the baseline information of the included RCTs [18–69]. Fifty-two RCTs were double arm trials and all of them mentioned randomized grouping, and nine mentioned the instructions for a blind method and did not mention the concealment of random allocation, selective reporting, and other biases (see Figure 2 for quality evaluation).

3.3. Consistency Analysis and Network Diagram. Figure 3 shows the included two-arm studies. The consistency analysis of four outcome indicators, that is, the clinical total effective rate, HAMD score, NIHSS score, and TESS score, was conducted. The PSFR value of the parameter was 1.00, indicating that the data results had good convergence, so the NMA was conducted under the consistency model. The network relationship was between interventions in the treatment of PSD. The numbers in the figure indicate the number of randomized controlled studies that were directly compared. The solid line in the figure indicates that there was a direct comparison between the two interventions, and the unconnected line indicates that the original study has not been directly compared. RCT can compare indirect relationships through network meta-analysis.

3.4. NMA Results

3.4.1. Effective Rate. The total effective rate is the OR, as the effect size. Table 2 shows that the intervention measures were compared with blank controls. Wuling capsule [OR = 4.35, 95% CI (3.03–6.26)], Xiaoyao pill [OR = 4.05, 95% CI (1.98–9.03)], Danzhi Xiaoyao pill [OR = 3.81, 95% CI (2.09–7.03)], Chaihu Shugan powder [OR = 3.12, 95% CI (0.57–8.97)], Shugan Jieyu capsule [OR = 3.87, 95% CI (2.77–6.05)], Jieyu Anshen capsule [OR = 5.00, 95% CI (1.72–9.48)], and Yangxue Qingnao granule [OR = 2.47, 95% CI (1.50–4.27)] showed clinical efficacy, and the differences were statistically significant. A pairwise comparison of seven interventions found that the Wuling capsule was better than Danzhi Xiaoyao pills [OR = 1.14, 95% CI (0.56–2.32)], Shugan Jieyu capsules [OR = 1.11, 95% CI (0.68–1.86)], or Yangxue Qingnao granules [OR = 1.76, 95% CI (0.93–3.26)] adjuvant therapy. Xiaoyao pills were better than Danzhi Xiaoyao pills [OR = 1.08, 95% CI (0.38–2.91)], Shugan Jieyu capsules [OR = 1.05, 95% CI (0.47–2.45)], or Yangxue Qingnao granules [OR = 1.63, 95% CI (0.69–4.15)] adjuvant therapy. The Danzhi Xiaoyao pill was better than the Shugan Jieyu capsule [OR = 0.98, 95% CI (0.49–2.02)] or Yangxue Qingnao granules [OR = 1.55, 95% CI (0.70–3.36)] adjuvant therapy. Chaihu Shugan powder was better than Shugan Jieyu capsules [OR = 0.81, 95% CI (0.14–8.57)] or Yangxue Qingnao granules [OR = 1.29, 95% CI (0.21–13.68)] adjuvant therapy. The Shugan Jieyu capsule was better than Yangxue Qingnao granules [OR = 1.56, 95% CI (0.84–3.18)]. The

Table 1: Basic information included in the study.

Included study	Cases (T/C)	Age	Intervention measures		Course of treatment (d)	Outcome indicators
			Test group (T)	Control group (C)		
Han xuqing 2014 [18]	28/30	59.29 ± 10.4	Wuling capsules 2 capsules, bid + Citalopram 10 mg/d, qd	Citalopram 10 mg/d, qd	24	①②
Wu yuhong 2013[19]	35/35	55~77	Wuling capsules 3 capsules, tid + Dealixin 1 tablet, bid	Dealixin 1 tablet, bid	56	①②
Guo ying 2013 [20]	95/95	68.14 ± 7.62	Wuling capsules 3 capsules, tid + Dealixin 1 tablet, bid	Dealixin 1 tablet, bid	42	①②③④
Mo shaozhen 2015[21]	69/69	45~75	Wuling capsules 3 capsules, tid + Dealixin 1 tablet, tid	Dealixin 1 tablet, tid	42	①②
Ma yunzhi 2012 [22]	30/30	64.8 ± 9.3	Wuling capsules 3 capsules, tid + Dealixin 1 tablet, tid	Dealixin 1 tablet, tid	42	①②③④
Zhou hongye 2015 [23]	34/32	47~74	Wuling capsules 3 capsules, tid + Dealixin 1 tablet, tid	Dealixin 1 tablet, tid	84	①⑤
Chen liang 2015 [24]	34/34	46~75	Wuling capsules 3 capsules, tid + Dealixin 1 tablet, tid	Dealixin 1 tablet, tid	42	①②
Zhang lili 2010 [25]	45/45	45~74	Wuling capsules 3 capsules, tid + Dealixin 1 tablet, tid	Dealixin 1 tablet, tid	42	①②
Liang yuan 2014 [26]	30/30	55.12 ± 5.2	Wuling capsules 3 capsules, tid + Dealixin 1 tablet, tid	Dealixin 1 tablet, tid	42	①②
Zhang danni 2016 [27]	50/50	52~77	Wuling capsules 3 capsules, tid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	42	①②
Ma binfeng 2017 [28]	63/63	58~86	Wuling capsules 3 capsules, tid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	56	①②
Shi qi 2008 [29]	30/26	46~82	Wuling capsules 3 capsules, tid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	42	①②
Kou jianhua 2019 [30]	40/40	42~75	Wuling capsules 3 capsules, tid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	84	①②④
Kong xiangfang 2014 [31]	38/38	43~76	Wuling capsules 3 capsules, tid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	56	①②④
Yuan jun 2014 [32]	85/85	45~75	Wuling capsules 3 capsules, tid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	28	①②④
Wan ailan 2006 [33]	35/35	59.23 ± 8.3	Wuling capsules 3 capsules, tid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	42	①②
Liu junqiong 2014 [34]	32/32	52~78	Wuling capsules 3 capsules, tid + Sertraline 50 mg/d, qd	Sertraline 50 mg/d, qd	56	①②
Xie yan 2018 [35]	49/49	52~78	Wuling capsules 3 capsules, tid + Sertraline 50 mg/d, qd	Sertraline 50 mg/d, qd	56	①②③④
Zhou peng 2017 [36]	34/34	18~68	Xiaoyao pill 8 pills, tid + Venlafaxine 75 mg/d, qd	Venlafaxine 75 mg/d, qd	28	①②
Wang jianqiang 2014 [37]	60/52	45~75	Xiaoyao pill 8 pills, tid + Deanxit 1 tablet, bid	Deanxit 1 tablet, bid	42	①②③
Zou lihua 2009 [38]	30/30	67.9 ± 6.1	Xiaoyao pill 8 pills, tid + Deanxit 1 tablet, bid	Deanxit 1 tablet, bid	42	①②
Ceng miaolin 2018 [39]	43/43	33~79	Xiaoyao pill 8 pills, tid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	28	①②
Jiang limin 2019 [40]	74/74	36~76	Danzhi Xiaoyao powder 3 g/d, bid + Citalopram 10 mg/d, qd	Citalopram 10 mg/d, qd	28	①②
Xu erping 2006 [41]	35/35	55.2 ± 1.9	Danzhi Xiaoyao powder 6 g/d, bid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	56	①②
Wang zongyuan 2008 [42]	36/36	55~79	Danzhi Xiaoyao powder 6 g/d, bid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	84	①②
Zhang yumao 2014 [43]	40/40	47~73	Danzhi Xiaoyao powder 6 g/d, bid + Sertraline 50 mg/d, qd	Sertraline 50 mg/d, qd	56	①②③
Peng xianwen 2014 [44]	49/49	45~78	Danzhi Xiaoyao powder 6 g/d, bid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	28	①②
Cui yi 2016 [45]	30/30	45~80	Chaihu Shugan powder granules + citalopram 10 mg/d, qd	Citalopram 10 mg/d, qd	42	①②④
Wen jun 2015 [46]	60/60	50~83	Shugan Jieyu capsule 2 capsules, bid + Mirtazapine 30 mg/d, qd	Mirtazapine 30 mg/d, qd	42	①②③④
Chen aijun 2013 [47]	39/39	49~78	Shugan Jieyu capsule 2 capsules, bid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	42	①③

Table 1: Continued.

Included study	Cases (T/C)	Age	Intervention measures		Course of treatment (d)	Outcome indicators
			Test group (T)	Control group (C)		
Hou jihong 2015 [48]	36/36	69.3 ± 7.5	Shugan Jieyu capsule 2 capsules, bid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	56	①②
Wu hongyi 2015 [49]	40/40	50~65	Shugan Jieyu capsule 2 capsules, bid + Deanxit 1 tablet, qd	Deanxit 1 tablet, qd	28	①②
Zhao zheng 2013 [50]	40/40	43~75	Shugan Jieyu capsule 2 capsules, bid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	42	①②
Ding na 2014 [51]	40/40	56.42 ± 5.18	Shugan Jieyu capsule 2 capsules, bid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	56	①②
Na wanqiu 2012 [52]	41/39	71.12 ± 5.51	Shugan Jieyu capsule 2 capsules, bid + Sertraline 50 mg/d, qd	Sertraline 50 mg/d, qd	56	①②③
Hu jun 2013 [53]	45/44	56.42 ± 5.18	Shugan Jieyu capsule 2 capsules, bid + Sertraline 50 mg/d, qd	Sertraline 50 mg/d, qd	42	①②
Tan hongyang 2018 [54]	62/62	52~73	Shugan Jieyu capsule 2 capsules, bid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	21	①②③④
Lu yi 2015 [55]	65/65	45~72	Shugan Jieyu capsule 2 capsules, bid + Olanzapine 2.5 mg/d, qd	Olanzapine 2.5 mg/d, qd	56	①②
Xu ming 2012 [56]	65/65	55~74	Shugan Jieyu capsule 2 capsules, bid + Venlafaxine 75 mg/d, bid	Venlafaxine 75 mg/d, bid	42	①②
Liu wei 2016 [57]	38/38	50~75	Shugan Jieyu capsule 2 capsule, bid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	56	①②③
Chen wei 2014 [58]	58/57	45~73	Shugan Jieyu capsule 2 capsules, bid + Venlafaxine 75 mg/d, bid	Venlafaxine 75 mg/d, bid	42	①③
Yi kunchang 2018 [59]	48/48	48~77	Shugan Jieyu capsule 2 capsules, bid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	56	①②
Pan zhenshan 2014 [60]	42/42	65.12 ± 8.35	Shugan Jieyu capsule 2 capsules, bid + Mirtazapine 30 mg/d, qd	Mirtazapine 30 mg/d, qd	56	①②③
Li junling 2013 [61]	27/27	49~75	Shugan Jieyu capsule 2 capsules, bid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	56	①②
Xie yan 2017 [62]	45/45	55~70	Jieyu Anshen granules 5 g, bid + Deanxit 1 pill, qd	Deanxit 1 pill, qd	42	①②
Xia junbo 2013 [63]	40/40	34~72	Jieyu Anshen granules 5 g, bid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	56	①②
Mu ying 2014 [64]	48/48	40~78	Yangxue Qingnao granules 4 g/d, tid + Paroxetine 20 mg/d, qd	Paroxetine 20 mg/d, qd	28	①④
Jiang guohua 2018 [65]	60/60	60~77	Yangxue Qingnao granules 4 g/d, tid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	56	①②③④
Huang xiaohong 2012 [66]	50/50	61.5 ± 7.8	Yangxue Qingnao granules 4 g/d, tid + Sertraline 50 mg/d, qd	Sertraline 50 mg/d, qd	56	①②
Pan dong 2014 [67]	38/41	60~75	Yangxue Qingnao granules 4 g/d, tid + Sertraline 50 mg/d, qd	Sertraline 50 mg/d, qd	56	①②
Ceng zhaofu 2013 [68]	34/34	41~75	Yangxue Qingnao granules 4 g/d, tid + Fluoxetine 20 mg/d, qd	Fluoxetine 20 mg/d, qd	56	①②
Wang xuejun 2017 [69]	32/32	61.37 ± 6.26	Yangxue Qingnao granules 4 g/d, tid + Citalopram 10 mg/d, qd	Citalopram 10 mg/d, qd	42	①②③

① Total effective rate; ② HAMD score (Hamilton Depression Scale); ③ TESS total score (adverse reactions); ④ NIHSS score (neurological deficit score). The intervention measures were based on the routine treatment of stroke..

Jieyu Anshen capsule was better than Yangxue Qingnao granules [OR = 2.03, 95% CI (0.60–8.83)] as adjuvant therapy, and there was no obvious difference in other pairwise comparisons, as shown in Table 2.

Probability ranking was as follows: Jieyu Anshen capsule (0.39) > Chaihu Shugan powder (0.25) > Xiaoyao pill (0.14) > Wuling capsule (0.10) > Danzhi Xiaoyao Pill (0.07) > Shugan Jieyu capsule (0.06) > Yangxue Qingnao granule (0.00). See Table 3 and Figure 4(a) for details.

3.4.2. HAMD Score. Forty-eight studies reported a comparison of the relevant Hamilton Depression Scale scores, and the network relationship between the comparisons of various interventions is shown in Figure 4(b). Taking MD as the effect quantity, the 95% CI confidence interval was used for analysis and statistics. Table 2 shows that each of the following was compared with the blank control and Wuling capsule [MD = −3.95, 95% CI(−4.88–−3.00)], Xiaoyao pills [MD = −5.19, 95% CI (−7.07–3.27)], Danzhi Xiaoyao pills

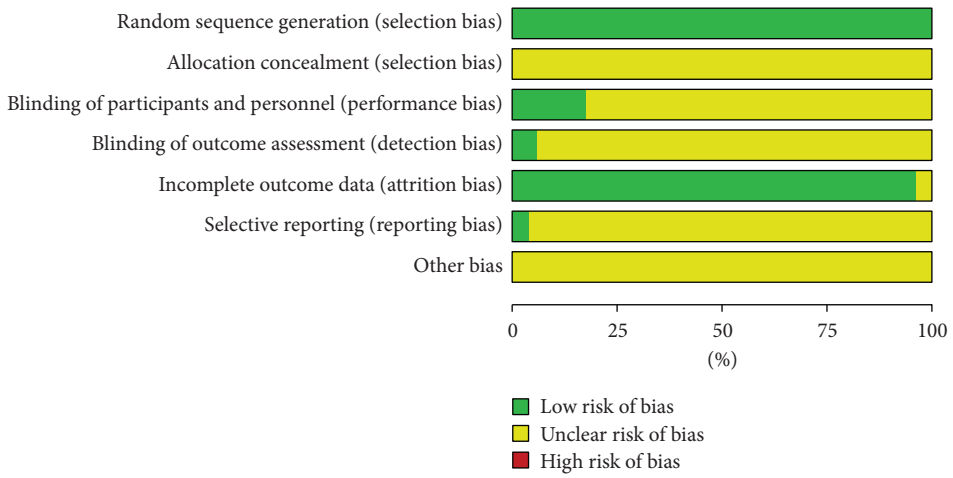

Figure 2: Bias risk assessment for inclusion in the study.

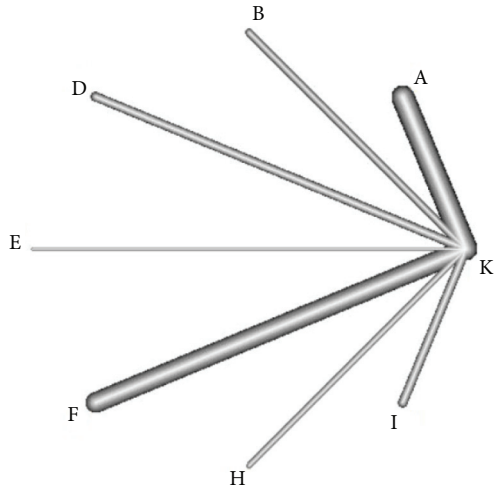

Figure 3: Evidence network diagram of CPMs in the auxiliary treatment of PSD. All abbreviations of CPM represent the combination group of CPM combined with western medicine, rather than CPM alone (code A: Wuling capsule combined with western medicine, B: Xiaoyao pill combined with western drugs, D: Danzhi Xiaoyao pill combined with western drugs, E: Chaihu Shugan powder combined with western drugs, F: Shugan Jieyu capsule combined with western medicine, H: Jieyu Anshen capsule combined with western medicine, I: Yangxue Qingnao granule combined with western medicine. K: blank control; this blank control refers to the type of CPM combined with western drug/positive control drug applied in the study, that is, the dosage and method of administration of the two groups of western medicine/positive control drug are consistent, so it belongs to the blank control trial design).

Table 2: Results of network meta-analysis.

Therapeutic method		Clinical effective rate	HAMD score	NIHSS score	TESS score
Method 1	Method 2				
Wuling capsule	Xiaoyao pill	1.08[0.44,2.38]	1.24[−0.88,3.38]	—	1.05[0.04,6.21]
	Danzhi Xiaoyao pill	1.14[0.56,2.32]*	−0.17[−2.30,1.80]	—	1.31[0.08,2.00]
	Chaihu Shugan powder	1.40[0.13,8.00]	−0.28[−5.07,4.61]	−2.62[−8.57,3.19]	—
	Shugan Jieyu capsule	1.11[0.68,1.86]*	0.26[−1.09,1.64]	1.10[−3.31,5.56]	0.84[0.16,4.36]
	Jieyu Anshen capsule	0.87[0.22,2.88]	−1.94[−4.66,0.90]	—	—
	Yangxue Qingnao granule	1.76[0.93,3.26]*	−1.29[−3.24,0.66]	0.50[−3.86,4.86]	0.78[0.08,6.23]
	Blank control	4.35[3.03,6.26]*	−3.95 [−4.88,−3.00]*	−3.25 [−5.46,−1.05]*	0.22 [0.05,0.79]*

Table 2: Continued.

Therapeutic method		Clinical effective rate	HAMD score	NIHSS score	TESS score
Method 1	Method 2				
Xiaoyao pill	Danzhi Xiaoyao pill	1.08[0.38,2.91]*	−1.40[−4.18,1.15]	—	1.23[0.01,6.62]
	Chaihu Shugan powder	1.27[0.11,8.52]	−1.50[−6.64,3.65]	—	—
	Shugan Jieyu capsule	1.05[0.47,2.45]*	−0.97[−3.14,1.20]	—	0.80[0.02,9.13]
	Jieyu Anshen capsule	0.82[0.16,3.07]	−3.18[−6.42,0.07]	—	—
	Yangxue Qingnao granule	1.63[0.69,4.15]*	−2.54[−5.08,0.04]	—	0.73[0.01,2.65]
	Blank control	4.05[1.98,9.03]*	−5.19 [−7.07,−3.27]*	—	0.21[0.01,4.14]*
Danzhi Xiaoyao pill	Chaihu Shugan powder	1.21[0.12,7.34]	−0.10[−5.11,5.03]	—	—
	Shugan Jieyu capsule	0.98[0.49,2.02]*	0.43[−1.59,2.61]	—	0.65[0.05,8.75]
	Jieyu Anshen capsule	0.74[0.16,2.61]	−1.77[−4.82,1.60]	—	—
	Yangxue Qingnao granule	1.55[0.70,3.36]*	−1.13[−3.57,1.48]	—	0.59 [0.03,11.33]
	Blank control	3.81[2.09,7.03]*	−3.78 [−5.55,−1.84]*	—	0.17[0.01,1.78]*
Chaihu Shugan powder	Shugan Jieyu capsule	0.81[0.14,8.57]*	0.55[−4.31,5.35]	3.72[−2.84,10.49]	—
	Jieyu Anshen capsule	0.62[0.07,9.94]	−1.62[−7.06,3.70]	—	—
	Yangxue Qingnao granule	1.29[0.21,13.68]*	−1.03[−6.04,4.03]	3.14[−3.39,9.76]	—
	Blank control	3.12[0.57,8.97]*	−3.65[−8.45,1.05]	−0.63[−5.99,4.87]	—
Shugan Jieyu capsule	Jieyu Anshen capsule	0.77[0.18,2.88]	−2.20[−5.00,0.66]	—	—
	Yangxue Qingnao granule	1.56[0.84,3.18]*	−1.57[−3.55,0.46]	−0.60[−6.06,4.86]	0.93 [0.14,5.67]*
	Blank control	3.87[2.77,6.05]*	−4.22 [−5.23,−3.17]*	−4.37 [−8.19,−0.57]*	0.26 [0.10,0.58]*
Jieyu Anshen capsule	Yangxue Qingnao granule	2.03[0.60,8.83]*	0.65[−2.44,3.76]	—	—
	Blank control	5.00[1.72,9.48]*	−2.00[−4.67,0.56]	—	—
Yangxue Qingnao granule	Blank control	2.47[1.50,4.27]*	−2.65 [−4.37,−0.97]*	−3.76[−7.56,0.03]	0.28 [0.05,1.42]*

Table 3: Ranking list of different interventions.

Intervention measures	Clinical effective rate	HAMD score	NIHSS score	TESS score
Wuling capsule	0.10	0.00	0.00	0.00
Xiaoyao pill	0.14	0.00	0.14	—
Danzhi Xiaoyao pill	0.07	0.00	0.05	—
Chaihu Shugan powder	0.25	0.05	—	0.39
Shugan Jieyu capsule	0.06	0.00	0.01	0.01
Jieyu Anshen capsule	0.39	0.06	—	—
Yangxue Qingnao granule	0.00	0.00	0.04	0.02
Blank control	0.00	0.88	0.75	0.58

[MD = −3.78, 95% CI(−5.55–1.84)], Shugan Jieyu capsules [MD = −4.22, 95% CI(−5.23–3.17)], and Yangxue Qingnao granules [MD = −2.65, 95% CI(−4.37–0.97)], and all were statistically significant, and no obvious difference was found between other pairwise comparisons ($P > 0.05$).

Probability rankings were as follows: Wuling capsule (0.00) = Shugan Jieyu capsule (0.00) = Yangxue Qingnao granule (0.00) = Xiaoyao pill (0.00) = Danzhi Xiaoyao pill (0.00) > Chaihu Shugan powder (0.05) > Jieyu Anshen capsule (0.06), as shown in Table 3 and Figure 4(b).

3.4.3. NIHSS Score. Eleven studies reported NIHSS score analysis. OR was the effect measure. Table 2 shows that each intervention was compared with the blank control. Wuling capsule [OR = −3.25, 95% CI (−5.46–1.05)] and Shugan Jieyu capsule [OR = −4.37, 95% CI (−8.19–0.57)] ATs have been shown to be effective in reducing NIHSS scale scores, and the differences were statistically significant. No remarkable difference was found between the two intervention measures ($P > 0.05$), as shown in Table 2.

Probability rankings were as follows: Wuling capsule (0.00) > Shugan Jieyu capsule (0.01) > Yangxue Qingnao granule (0.02) > Chaihu Shugan powder (0.39). See Table 3 and Figure 4(c) for details.

3.4.4. TESS Score. Fourteen studies reported adverse reaction analysis. OR was the effect quantity. Table 2 shows that each intervention was compared with the blank control. Wuling capsule [OR = 0.22, 95% CI (0.05–0.79)], Xiaoyao pill [OR = 0.21, 95% CI (0.01–4.14)], Danzhi Xiaoyao Pill

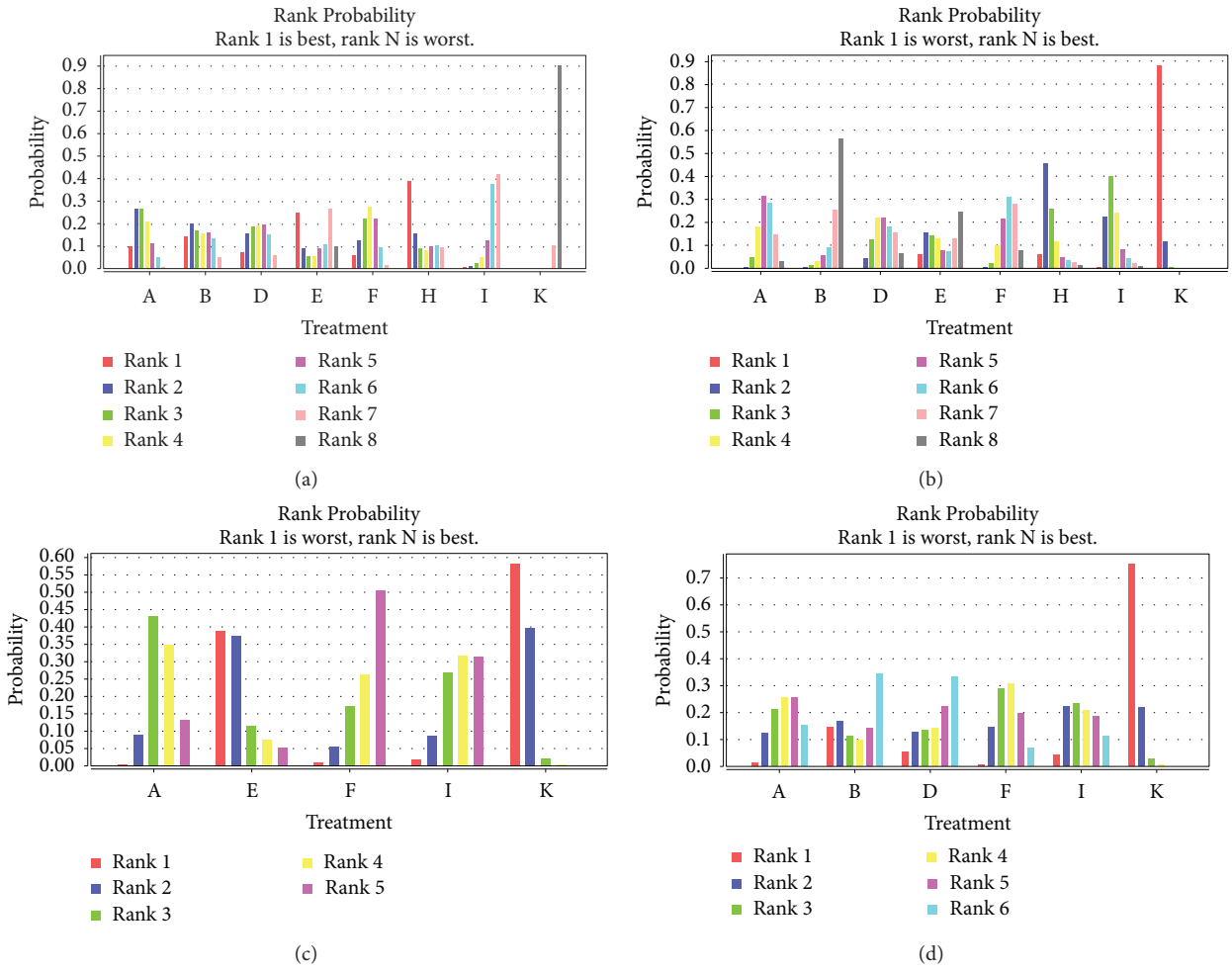

Figure 4: Ranking diagram of different outcome indicators for each intervention: (a) total effective rate; (b) HAMD score; (c) NIHSS score; (d) TESS total score. The higher the total effective rate (Rank 1), the greater the effects. The smaller the HAMD, NIHSS, and TESS (Rank 1), the greater the effects.

[OR = 0.17, 95% CI (0.01–1.78)], Shugan Jieyu capsule [OR = 0.26, 95% CI (0.10–0.58)], and Yangxue Qingnao granules [OR = 0.28, 95% CI (0.05–1.42)] ATs have shown to be effective in reducing the incidence of clinical adverse reactions, and the differences were statistically significant. There was no remarkable difference in other pairwise comparisons ($P > 0.05$). See Table 2.

Probability rankings were as follows: Wuling capsule (0.00) > Shugan Jieyu capsule (0.01) > Yangxue Qingnao granule (0.04) > Danzhi Xiaoyao Pill (0.05) > Xiaoyao pill (0.14). See Table 3 and Figure 4(d).

4. Discussion

TCM suggests that PSD belongs to the combined category of "stroke" and "depression syndrome." We should reasonably apply the integrated regulation and individual syndrome differentiation and treatment methods of TCM and use products that sooth the liver and relieve the depression, strengthening the spleen and stomach, alleviating depression to regulate qi, nourishing yin and promoting body fluids, and supplementing qi and strengthening the stomach. In this way, it can not only balance qi, blood, yin and yang, and dredging meridian and relax emotions but also effectively exert the clinical advantages of pure Chinese medicine preparation.

TCM is usually applied for promoting qi, soothing the liver, dredging collaterals, relieving depression, nourishing yin, activating blood, and removing blood stasis. It is not only a multitarget herb but also effectively induces the synergy of effective antidepressive components. In this NMA, seven types of oral CPMs were screened by data mining, including Wuling capsule, Shugan Jieyu capsule, Jieyu Anshen capsule, Yangxue Qingnao granule, Chaihu Shugan powder, Danzhi Xiaoyao pill, and Xiaoyao pill, which were prepared by natural Chinese herbal medicines. On the basis of definite curative effect, Chinese herbal medicine can decrease the occurrence of side effects and greatly improve medication adherence and tolerance. With respect to dosage form, CPMs can not only prevent aggravation of Chinese medicine decoction but also enhance the flavor of CPMs through sucrose-based auxiliary materials, which are preferred by patients clinically. Hence, CPMs have potential clinical applications in PSD [70, 71].

The network meta-analysis concluded that the first ranking for clinical effectiveness was the Jieyu Anshen capsule adjuvant therapy, the second was Chaihu Shugan powder, and the third was the Xiaoyao pill. Through these three proprietary Chinese medicine formulas, it can be found that all have good effects of relieving depression and soothing the liver, invigorating the spleen and regulating qi, nourishing blood, and calming the nerves. Among them, the Jieyu Anshen capsule was also added with Rhizoma *Acorus gramineus*, *Polygala tenuifolia*, Curcumae Radix, Pinellia, and other important products for calming the nerves, resolving phlegm. Modern studies have found that the water extract of *Acorus tatarinowii* has a certain antidepressant effect, which may be related to the improvement of 5-HT nerve function in the brain. At the same time, compatibility with saikosaponin can effectively enhance the ability to inhibit 5-HT reuptake to enhance the antidepressant effect. The combination of Polygala, Dan Nan Xing, and Pinellia ternata can enhance the effects of relieving depression, calming nerves, exempting phlegm, and resuscitation, which coincides with the syndrome of depression caused by stroke. Therefore, the combination of various medicines not only takes into account the pathogenic factors of qi and blood stagnation caused by apoplexy but also pays attention to the pathogenic characteristics of the depression syndrome, so it can accelerate the repair of nerve cells and nerve functions and has a high clinical efficacy [72, 73]. Chaihu Shugan powder and Xiaoyao pills are evolved from the classical famous prescription Sini powder, and both contain products for soothing the liver and relieving depression, nourishing the blood, nourishing the liver, and softening the liver. At the same time, they are combined with herbals for regulating qi and activating blood circulation, which can effectively disperse the stagnation of liver qi and stasis in the circulation of blood, so as to make liver qi smooth, blood deficiency supplement, and strengthen spleen. Pharmacological studies have confirmed that the two prescriptions not only regulate the cerebral cortex and improve cerebral microcirculation but also regulate immune and antioxidant functions, so they can promote the rehabilitation of patients with PSD.

Regarding the HAMD, NIHSS, and TESS scale scores, the AT of Wuling capsules can effectively reduce the relevant scale factor scores. This medicinal preparation is a dry powder of mycelium produced by fermentation of strains isolated from Wuling Shen. It contains a variety of adenosine, polysaccharides, amino acids, vitamins, and trace elements, which can have antianxiety and antidepressant effects and has two-way regulation of cerebral cortex function. It can inhibit the synthesis of the neurotransmitter γ-aminobutyric acid and improve the binding of related receptors in cerebral cortex. Moreover, it can enhance the brain energy reserves to protect the damaged brain nerve cells, so as to promote the healing force of nerve function defects, nerve cell repair force, calming, and tranquilizing force and has a better force in reducing the toxic and side effects of drugs. Therefore, it is considered that the auxiliary treatment of CPM has certain clinical value, safety, and reliability [74].

Through the comprehensive data analysis, the most effective CPM was selected to provide certain reference values for clinicians. There are some limitations to this study, including selection bias, clinical heterogeneity, and publication bias, which may influence the study outcomes. However, we suggest that this NMA can provide reliable reference value for clinical practice and evidence-based medicine as well as selecting the most appropriate treatment option for PSD to a certain extent. The protocol for this NMA has been registered on the international system review expectation register (CRD42020164543), which follows the guidelines of "Cochrane Intervention System Review Manual" and "PRISMA-P statement." There will be a description of the amendment with date and reason if the protocol needs to be amended.

5. Conclusion

In summary, the Jieyu Anshen capsule is the first choice to improve clinical efficacy in the treatment of PSD. To reduce the HAMD and NIHSS scale scores and improve security, Wuling capsules should be the first choice for adjuvant treatment for the best effect. However, the specific disease should also be combined with the actual situation of patients and syndrome differentiation to make a reasonable choice for medication. Through extensive collection of the literature, related combination, and statistic analysis, this study provided reference value for clinicians when optimizing the choice of proprietary CPM for adjuvant therapy. However, the study is included in the single center clinical randomized control trial, and the number of samples included was relatively small, which made the statistical efficiency low and affected the stability of long-term efficacy evaluation results. Therefore, the design of future trials should be verified by large-scale, multicenter, prospective, double-blind randomized controlled trials, and the objective criteria should be used to evaluate the indicators, so as to reduce the risk of personal bias to the greatest extent, and provide a reliable basis for evaluation of results that can effectively guide the clinical selection of prescriptions and drugs.

Abbreviations

HAMD:	Hamilton Depression Scale
TESS:	Treatment Emergent Symptom Scale
NIHSS:	National Institute of Health Stroke Scale
PRISMA-P:	Preferred reporting items for systematic reviews and meta-analyses protocols
NMA:	Network meta-analysis
RCTS:	Randomized controlled trials
ADDIS:	The Aggregate Data Drug Information System
MCMC:	Markov China Monte Carlo
CI:	Confidence interval
OR:	Odds ratio
RR:	Risk ratio
MD:	Mean difference
PSRF:	Potential scale reduced factor

PICOS: Participants, interventions, comparators, outcomes, study design.
TCM: Traditional Chinese medicine.

Data Availability

The data that support the findings of this study are publicly available and can be obtained from the corresponding author upon reasonable request.

Ethical Approval

The study protocols were approved by the institutional review board and ethics committee. Further ethical approval and informed consent are not required. The findings will be disseminated through academic conference reports or peer-reviewed journals. This NMA has been registered on the international system review expectation register (CRD42020164543), which follows the guidelines of "Cochrane Intervention System Review Manual" and "PRISMA-P statement." There will be a description of the amendment with date and reason if the protocol needs to be amended.

Disclosure

PROSPERO registration number is CRD42020164543. The protocol for this systematic review was registered on PROSPERO and has been published in the following medicine journals: https://pubmed.ncbi.nlm.nih.gov/32756126/.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Ying Yu and Gong Zhang contributed equally to this work. Ying Yu and Gong Zhang conceptualized the study. Tao Han, Hongjie Liu, and Hailiang Huang were responsible for project administration. Ying Yu and Gong Zhang were responsible for data curation. Ying Yu and Gong Zhang were responsible for formal analysis. Ying Yu and Gong Zhang developed the methodology. Ying Yu and Gong Zhang provided software. Tao Han and Hailiang Huang supervised the study. Ying Yu and Gong Zhang wrote the original draft. Ying Yu and Gong Zhang reviewed and edited the manuscript.

Acknowledgments

This research was funded by the Preliminary Mechanism and Efficacy Evaluation by the Excellent Scientific Research and Innovation Teams at Shandong University of Traditional Chinese Medicine in the Treatment of Major Diseases (no. 220316). The financial support during study design, data collection, data analysis, data interpretation, and manuscript writing was provided by the funder (Tao Han).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2021, Article ID 2165632, 14 pages
https://doi.org/10.1155/2021/2165632

Research Article

Identification of Potential Biomarkers of Depression and Network Pharmacology Approach to Investigate the Mechanism of Key Genes and Therapeutic Traditional Chinese Medicine in the Treatment of Depression

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Received 22 July 2021; Revised 27 October 2021; Accepted 26 November 2021; Published 31 December 2021

Academic Editor: Md. Areeful Haque

Background. To explore the potential target of depression and the mechanism of related traditional Chinese medicine in the treatment of depression. *Method*. Differential gene expression in depression patients and controls was analyzed in the GEO database. Key genes for depression were obtained by searching the disease databases. The COREMINE Medical database was used to search for Chinese medicines corresponding to the key genes in the treatment of depression, and the network pharmacological analysis was performed on these Chinese medicines. Then, protein-protein interaction analysis was conducted. Prediction of gene phenotypes was based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment scores. *Results*. The total number of differentially expressed genes in the GEO database was 147. Combined with the GEO dataset and disease database, a total of 3533 depression-related genes were analyzed. After screening in COREMINE Medical, it was found that the top 4 traditional Chinese medicines with the highest frequency for depression were *Paeonia lactiflora* Pall., Crocus sativus L., *Bupleurum chinense* DC., and *Cannabis sativa* L. The compound target network consisted of 24 compounds and 138 corresponding targets, and the key targets involved PRKACA, NCOA2, PPARA, and so on. GO and KEGG analysis revealed that the most commonly used Chinese medicine could regulate multiple aspects of depression through these targets, related to metabolism, neuroendocrine function, and neuroimmunity. Prediction and analysis of protein-protein interactions resulted in the selection of nine hub genes (ESR1, HSP90AA1, JUN, MAPK1, MAPK14, MAPK8, RB1, RELA, and TP53). In addition, a total of four ingredients (petunidin, isorhamnetin, quercetin, and luteolin) from this Chinese medicine could act on these hub genes. *Conclusions*. Our research revealed the complicated antidepressant mechanism of the most commonly used Chinese medicines and also provided a rational strategy for revealing the complex composition and function of Chinese herbal formulas.

1. Introduction

Depression is a prevalent mental disorder ranked as the leading nonfatal cause of disability by the World Health Organization [1]. Major depressive disorder (MDD) is one of the most common psychiatric disorders resulting in a lifetime disability. It is also an important illness associated with suicidal ideation and completed suicide [2–4]. Depression influences various diseases, such as cardiovascular disease and diabetes mellitus [5]. Research on the treatment of depression has been extensive and has shown that depression can be treated with three different forms of psychotherapies: (1) antidepressants and other medications that augment antidepressant action, (2) evidence-based psychotherapy such as cognitive-behavior therapy (CBT) and interpersonal psychotherapy (IPT), and (3) somatic nonpharmacological treatments including electroconvulsive therapy (ECT), repetitive transcranial magnetic stimulation (rTMS), and vagus nerve stimulation (VNS) [6]. However, the treatment effects were limited.

Traditional Chinese medicine (TCM) has been practiced over centuries in Asia and relies on tremendous empirical knowledge [7]. The use of TCM in the treatment of depression has a long history. TCM was found to be superior to antipsychotic drugs in its effects on antianxiety/depression and antipsychomotor inhibition. Traditional Chinese medicine has been proven to have mild antidepressant benefits with few side effects [8]. Xiaoyaosan (XYS) is a traditional Chinese medicine prescription and has been widely used for centuries to treat depressive conditions. XYS was divided into five different polar fractions to explore the antidepressant activity. The results obtained showed that the petroleum ether fraction of XYS is the most effective fraction, suggesting that lipophilic components probably contribute to the antidepressant effect of XYS [9]. Gao et al. [10] showed that the petroleum ether fraction of Bupleuri Radix (PBR) produces an antidepressant effect by regulating glycometabolism, amino acid metabolism, sphingolipid metabolism, glycerophospholipid metabolism, and fatty acid metabolism. Kaixin Jieyu Fang can alleviate cerebral white matter damage, and the underlying mechanism is associated with the regulation of Bcl-2/Bax protein and mRNA expression, which is one of the possible mechanisms behind the protective effect of Kaixin Jieyu Fang against vascular depression [11].

Paeonia lactiflora Pall. has analgesic, anti-inflammatory, antioxidant, antidepression, anticardiovascular diseases; antineurodegenerative diseases; and other biological activities [12]. Total glucosides of paeony can also reduce the extent of cerebral infarction by increasing the content of ATP in the hippocampus and enable ischemic site reperfusion to treat depression [13]. *Paeonia lactiflora* Pall. and *Bupleurum chinense* DC. are the basic combination of *Bupleurum* prescriptions such as Xiaoyao powder, Sini powder, and Xiaochaihu decoction. The two drugs together play the function of nourish blood, soften the liver, and have the characteristics of dispersing and collecting, harmonizing qi and blood, and combining dispersing and softening, which accord with the physiological characteristics of the liver of "body Yin and use Yang" [14]. The drug pair of *Paeonia lactiflora* Pall. and *Bupleurum chinense* DC. improved depressive symptoms in rats by reversing the decrease in monoamine neurotransmitters. At the same time, it can enhance the expression of neurotrophic factors, brain-derived neurons, and receptor tyrosine protein kinases in the hippocampus of rats and achieve an antidepression effect [15, 16]. *Paeonia lactiflora* Pall. and *Bupleurum chinense* DC. [17] participate in the Ca^{2+} signaling pathway to the target of action and thus can inhibit the damage caused by Ca^{2+} overload. Hosseinzadeh and Noraei [18] studied *Crocus sativus* L. water extract and its effective component (saffron element and saffron formaldehyde) in hypnosis, fight anxiety, autonomic activity, and the action of sport harmonious ability respect through experiment of sodium of pentobarbital, maze, opening experiment, and Rotarod experiment. Emp oil can partially improve the depressive behavior and cognitive ability of mice and reduce the expression of IL-1β. The CBD can improve the anxiety symptoms of mice, increase learning and cognitive functions, and inhibit the cortex and hippocampus of brain tissue [19]. Cannabidiol (CBD) is one of the phenols in *Cannabis sativa* L. oil [20]. CBD can quickly cross the blood-brain barrier and can be used as a treatment for nervous system diseases caused by stress [21] and [22]. The results of forced swimming experiments in mice suggest that CBD can reduce the immobilization time in mice and may have antidepressant effects.

The traditional Chinese medicine compound preparation is easy to obtain and rich in various active ingredients. It has the characteristics of acting on multiple targets, and a single component cannot completely reveal its pharmacological activity. In recent years, the application of high-throughput platforms in gene expression has been widely used in clinical studies such as disease prognosis and targeted drug development. Therefore, it is of great significance to explore the key molecules in depression and identify effective Chinese medicine for the treatment of depression. In this study, 15 datasets generated by depression microarray technology and 5 disease gene databases were analyzed to explore the key genes of depression, and the mechanism of related traditional Chinese medicine in the treatment of depression was approached by network pharmacology analysis.

2. Method

2.1. The Prediction of Known Therapeutic Targets Acting on Depression. The Gene Expression Omnibus (GEO) database [23] (http://www.ncbi.nlm.nih.gov/geo) is the largest, most comprehensive, and publicly available source of gene expression data. We searched all depressive gene expression datasets from the GEO database up to July 2021 using the keywords "depression" and "depressive disorder." The datasets of nonhuman, nonbrain, noncase-control experiments, and nonmajor depression were removed.

In addition, five disease databases of the DrugBank, GeneCards, OMIM, PharmGkb, and TTD were retrieved. The websites of these five databases are listed in Supplementary Table S1.

2.2. Data Processing. Genes collected from the GEO database were analyzed to determine the different genes between the brain tissues of patients with depression and normal people, and then the genes were combined. Finally, we combined the total of differentially expressed genes (DEGs) with the depression-related genes found in the disease database and excluded the duplicates.

2.3. Screening of Traditional Chinese Medicine for the Treatment of Depression. COREMINE Medical (https://www.pubgene.com/coremine-medical/) is the world's most advanced medical information retrieval platform jointly developed by Norway, the Chinese Academy of Sciences, the Chinese Academy of Medical Sciences, the US National Library of Medicine, and other institutions. By entering the name of depression disease into this database, potential

drugs associated with depression disease can be retrieved. According to the frequency statistics of the screened TCM, the top herbs with the highest frequency were obtained.

2.4. Chemical Ingredient Database and Putative Target Building of the Herbs. To determine the chemical ingredients of the four herbs, we performed a search by the Traditional Chinese Medicine Systems Pharmacology Database TCMSP. We type complete Pinyin of each drug in the database and then combine with the existing research results in the literature at the same time, through multiple preexperiments. Finally, we selected oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 as TCMSP property parameters to predict the effective components in the four herbs.

2.5. Construction of Drug-Target-Disease Interaction Network. After obtaining compound-related targets and depression disease targets in the four herbs, the intersection target genes of the two were obtained. The visualization of the drug-target-disease interaction network was constructed with Cytoscape 3.8.0.

2.6. Protein-Protein Interaction Analysis. After obtaining the relevant targets of the four herbs and depression disease targets, we took the intersection target genes of the two and constructed the protein interaction network (PPI) online by STRING (http://string-db.org/), which was made to explore the interaction between known and predicted compounds and proteins [24]. Only proteins that interacted directly with each compound in HPXL were selected as the presumed targets. To ensure the reliability of the data, we set the "minimum required interaction score" to communicate the protein-protein interaction information with a combined score ≥0.9.

Then, Cytoscape 3.8.0 was applied to visualize the structure of the protein network. In the process of protein network construction, a force-oriented algorithm was used to distribute nodes reasonably to produce a clear visual effect for protein interaction data (PPI). To use a short sentence for the overview process of how we generated a PPI network graph, we downloaded and saved TSV files from the previous analysis results and then imported them into Cytoscape 3.8.0 software. CytoNCA plug-in was used for topology analysis. We selected core targets whose degree centrality was greater than the average degree value for further analysis. The PPI network diagram of the core targets was constructed using Cytoscape 3.8.0.

2.7. Gene Ontology (GO) Analysis. Gene Ontology (GO) analysis [25] was conducted on the intersection of drug targets and disease targets through the clusterProfiler R package. The clusterProfiler R package was utilized to obtain significant enrichment results and figures of GO analysis. In this study, only the top ten most significant GO items were retained.

2.8. Molecular Docking. AutoDockTools 1.5.6 software was used to measure semiflexible molecular docking. The compounds' 3D structures were downloaded from the PubChem database as ligands. Core targets were used as target proteins, and proteins were obtained from the Research Collaboratory for Structural Bioinformatics Protein Databank (RCSB PDB) as receptors. Polar hydrogen was added to the treated receptor files using AutoDock computational software.

Next, the parameters of the docking box were set with the AutoGrid tool to find and record the optimal docking position between the receptor and the ligand. If analysis of the affinity ($kJ \cdot mol^{-1}$) of molecular docking indicated binding energy less than 0, we assumed that the ligand and receptor could spontaneously bind. In this study, affinity $\leq -5.0\ kJ \cdot mol^{-1}$ was selected as the screening basis. Finally, PYMOL software was used to analyze and observe the docking results of the compounds and proteins.

The flowchart of strategy based on network pharmacology is shown in Figure 1.

3. Results

3.1. Disease Target Genes. Thirteen case-control brain gene expression datasets of human major depressive disorder were obtained. GSE53987 in these 13 datasets contains data from the hippocampus (HPC), prefrontal cortex (PFC), and associative striatum (STR), so it is divided into three sub-datasets: GSE53987–HPC, GSE53987–STR, and GSE53987–PFC. Therefore, this study contains 15 datasets of severe depression, and the GEO addresses corresponding to these datasets are shown in Table 1.

3.2. Prediction of Compounds-Related Targets. After preliminary screening, it was found that, by comparing the gene expression between the depression group and the control group, the total number of differential genes in the GEO database was 147. There were 50 upregulated genes and 89 downregulated genes in 15 GSE datasets. The volcanic map analysis of differential genes in each dataset is shown in Figure 2. The heat map of dataset 14-GSE54575 is shown in Figure 3, where the red represents the upregulated gene expression and the blue represents the downregulated gene expression.

Based on the GeneCards database, 2993 known depression treatment targets were obtained, while 1 known depression treatment target was obtained based on the OMIM database. There were 614 targets in the PharmGkb database, 57 targets in the TTD database, and 14 targets in the DrugBank database. After eliminating duplicated targets from these five databases, a total of 3447 known depression therapeutic targets were collected. Details can be found in Figure 4. Combined with the GEO dataset and disease database, a total of 3533 depression-related genes were analyzed.

3.3. Composite Ingredients and Putative Targets for the Four Herbs. After screening in COREMINE Medical, it was found that the top 4 traditional Chinese medicines with the

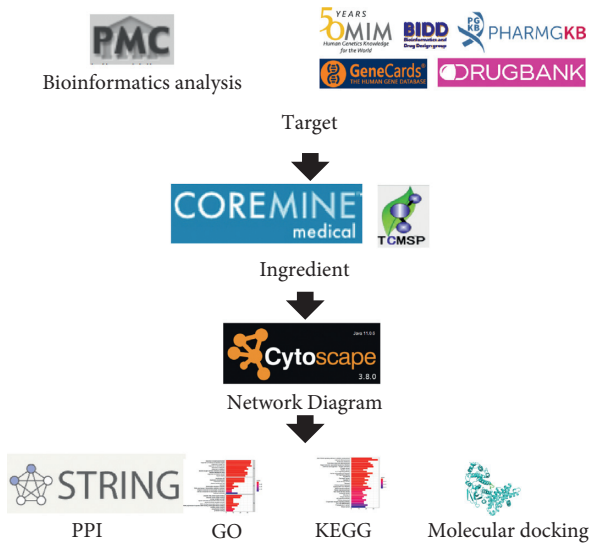

Figure 1: Flowchart of the network pharmacology-based strategy. TCMSP: Traditional Chinese Medicine Systems Pharmacology; GeneCards: retrieve human genetic information; OMIM: Online Mendelian Inheritance in Man; PPI: protein-protein interaction; TTD: Therapeutic Target Database.

Table 1: Information of GEO datasets.

Number	The dataset	Link of the GEO database
1	GSE53987-HPC	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53987
2	GSE53987-STR	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53987
3	GSE53987-PFC	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53987
4	GSE54562	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54562
5	GSE54563	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54563
6	GSE54564	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54564
7	GSE54565	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54565
8	GSE54566	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54566
9	GSE54567	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54567
10	GSE54568	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54568
11	GSE54570	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54570
12	GSE54571	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54571
13	GSE54572	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54572
14	GSE54575	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54575
15	GSE12654	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE12654

highest frequency for depression were *Paeonia lactiflora* Pall. (Paeoniaceae, *Paeoniae radix alba*), *Crocus sativus* L. (Iridaceae, croci stigma); *Bupleurum chinense* DC. (Apiaceae, bupleuri radix), and *Cannabis sativa* L. (Cannabaceae, cannabis fructus). *Paeonia lactiflora* Pall. (Paeoniaceae, paeoniae radix alba), sweet in flavor and flat in nature, belongs to the spleen, stomach, and large intestine meridians. *Crocus sativus* L. (Iridaceae, croci stigma), which belongs to the heart and liver meridians, has the effects of promoting blood circulation to remove blood stasis, cooling blood and detoxification, relieving depression, and calming nerves. *Bupleurum chinense* DC. (Apiaceae, bupleuri radix), belonging to the liver, gallbladder, and lung meridians, has the effects of soothing the liver, relieving depression, and raising yang. *Cannabis sativa* L. (Cannabaceae, cannabis fructus), belongs to the liver and spleen meridians, which help to nourish blood and regulate menstruation, suppress yin and stop sweating, soften the liver and relieve pain, and suppress liver yang. Modern research on depression focuses on the stagnation of liver and qi, and emotional disorders; therefore, the four Chinese medicines mentioned above are chosen to perform follow-up network pharmacological analysis.

3.4. Known Therapeutic Targets Acting on Depression. A total of 41 chemical ingredients (Supplementary Table S2) of the four herbal medicines were retrieved from TCMSP and related literature: 6 ingredients in *Cannabis sativa* L. (Cannabaceae, cannabis fructus), 5 in *Crocus sativus* L. (Iridaceae, croci stigma), 17 in *Bupleurum chinense* DC. (Apiaceae, bupleuri radix), and 13 in *Paeonia lactiflora* Pall. (Paeoniaceae, paeoniae radix alba). According to the target prediction system in the TCMSP database, we assumed the possible targets of 4 Chinese herbal medicines. As there may be interactions between these medicines and therapies, we

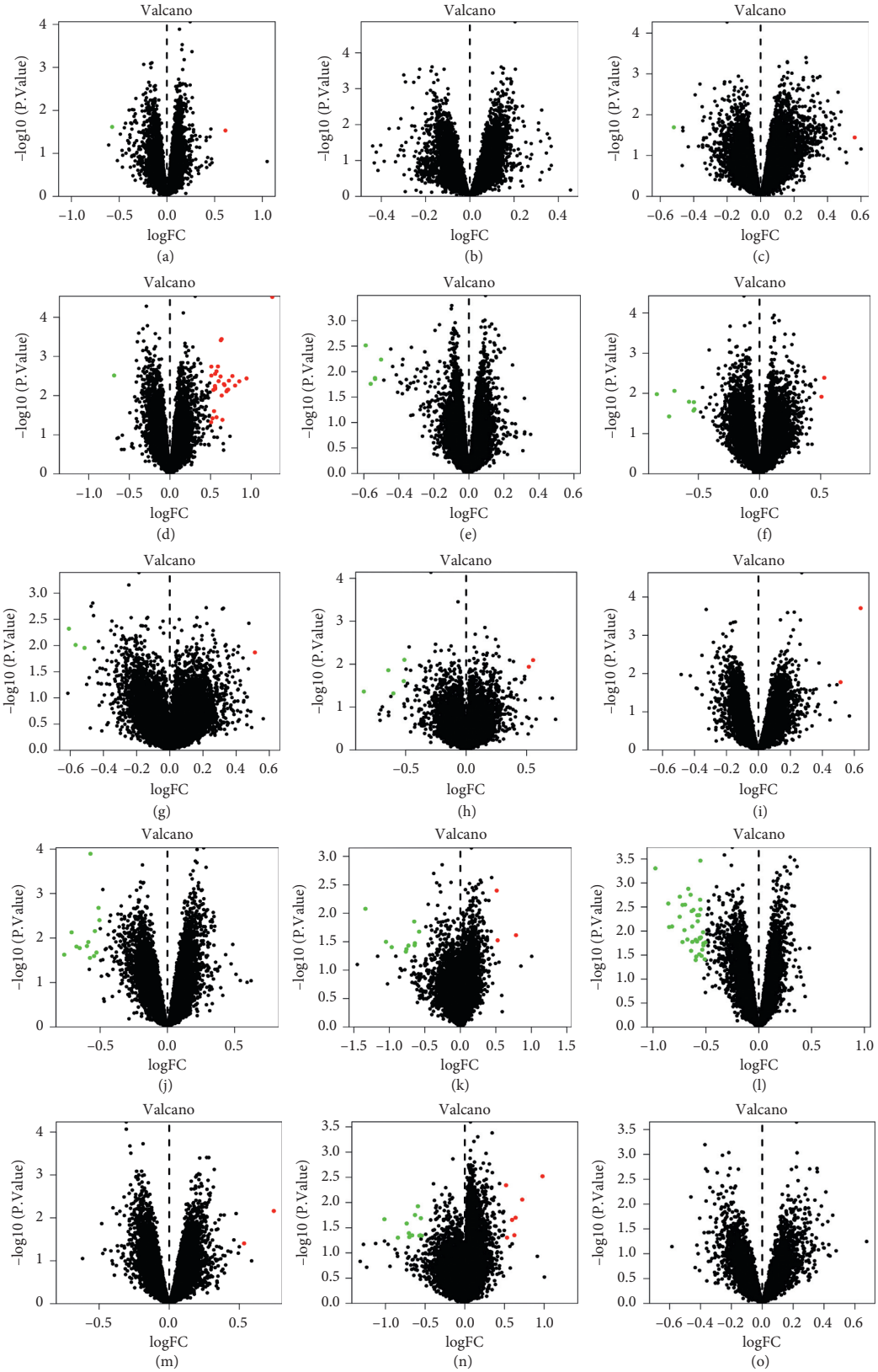

Figure 2: Volcano diagram of differential genes. A: GSE53987-HIP; B: GSE53987-PFC; C: GSE53987-STR; D: GSE54562; E: GSE54563; F: GSE54564; G: GSE54565; H: GSE54566; I: GSE54567; J: GSE54568; K: GSE54570; L: GSE54571; M: GSE54572; N: GSE54575; O: GSE12654. Red dots represent upregulated gene expression and the green dots represent downregulated gene expression.

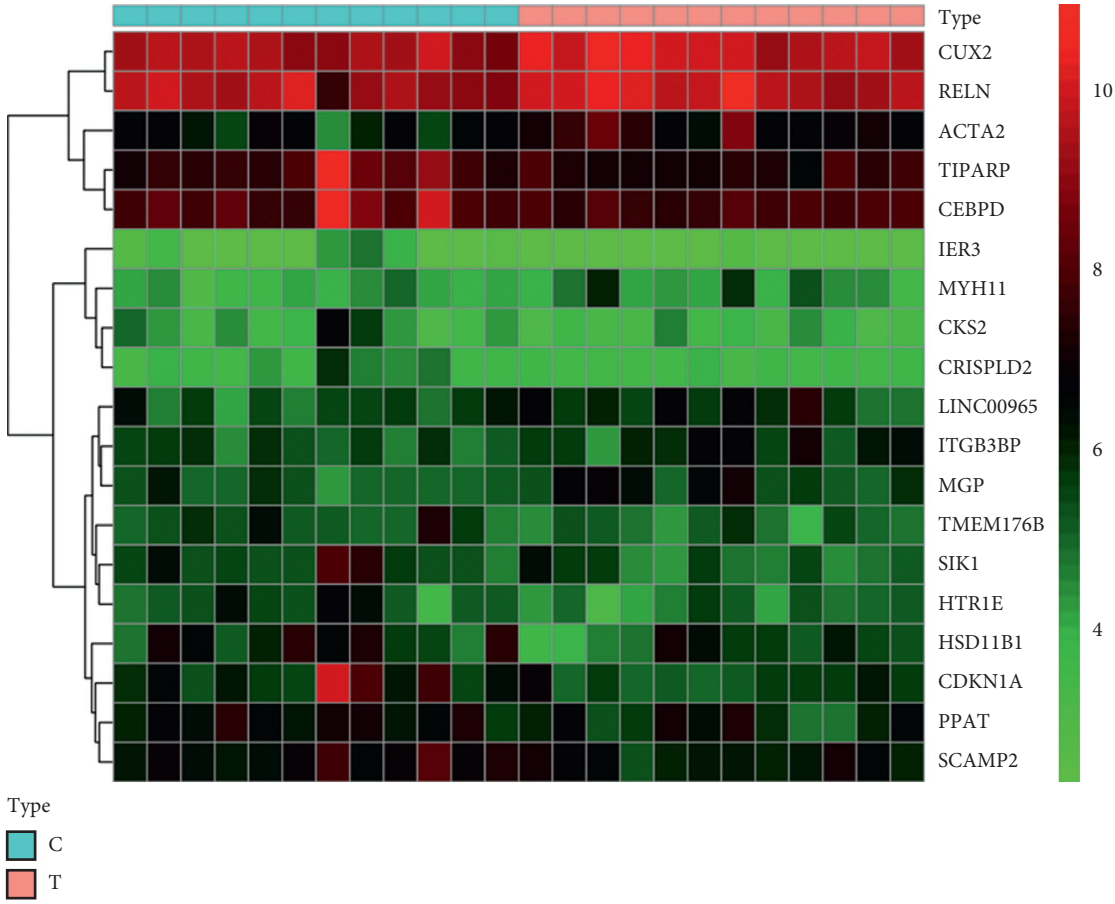

Figure 3: Heat map of GSE54575. C: gene analysis of brain tissue in the normal group. T: analysis of brain tissue genes in the depression group.

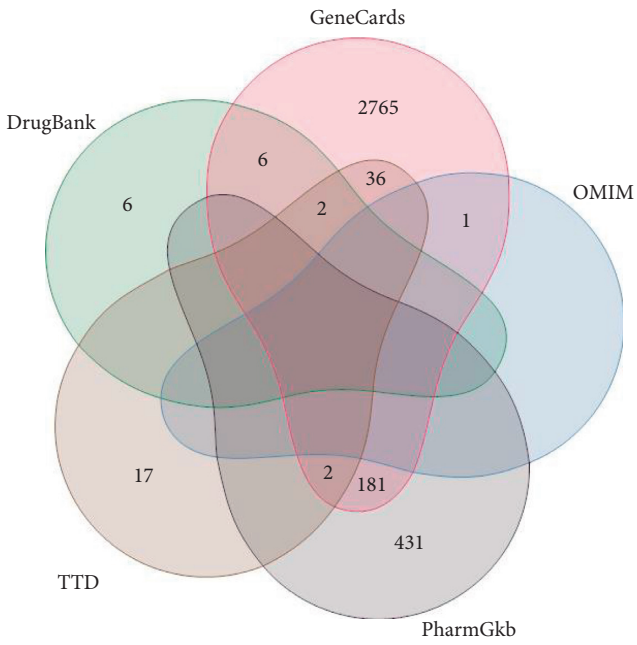

Figure 4: Targets of disease databases.

eliminated target overlap and obtained 867 target genes of active components; details about the assumed targets are contained in Supplementary Table S3.

3.5. Construction of the Drug-Target-Disease Interaction Network. The construction of a drug-target-disease interaction network led to a total of 138 target genes in the drug-disease intersection, as shown in Figure 5. The intersection of drug targets and depression disease targets in herbs and files of the properties of each node were imported into Cytoscape to construct the network diagram of "drug-target-disease interaction."

Different shapes and colors were applied in the network to clearly and intuitively present drug ingredients and the network relationship between drug action targets and disease targets. In the network diagram, different nodes represent drugs, components, and targets, and the wires represent the interaction between the nodes. In this network diagram, there are 24 active ingredients, 122 nodes, and 452 edges of molecules related to drugs, diseases, and compounds, as shown in Figure 6. In the network, the degree value of a node represents the number of routes connected to the nodes in the network. According to the topological properties of the network, the nodes with higher degree values are screened for analysis. These nodes with many connecting compounds or targets act as hubs in the whole network and may be the key compounds or targets.

There were 24 compounds and 138 gene targets in this network, and each compound interacted with an average

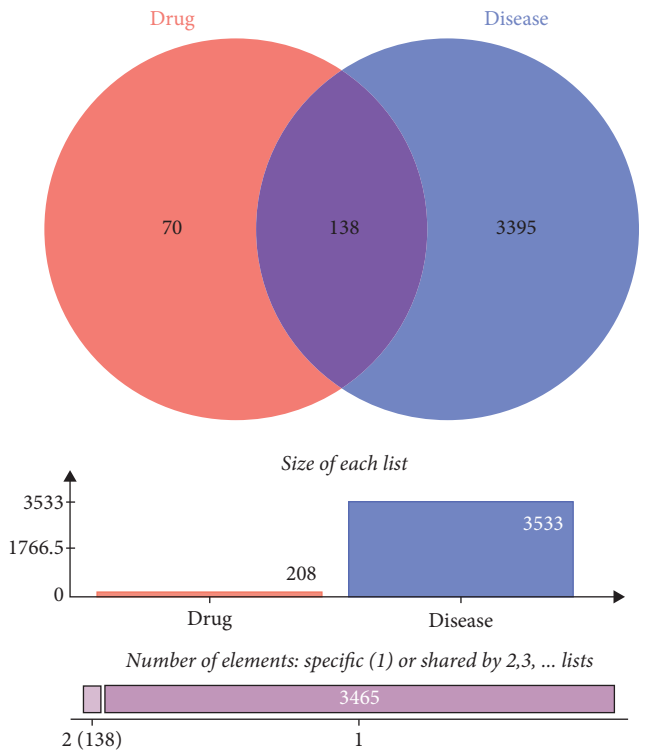

Figure 5: Venn diagram of ingredient-disease target

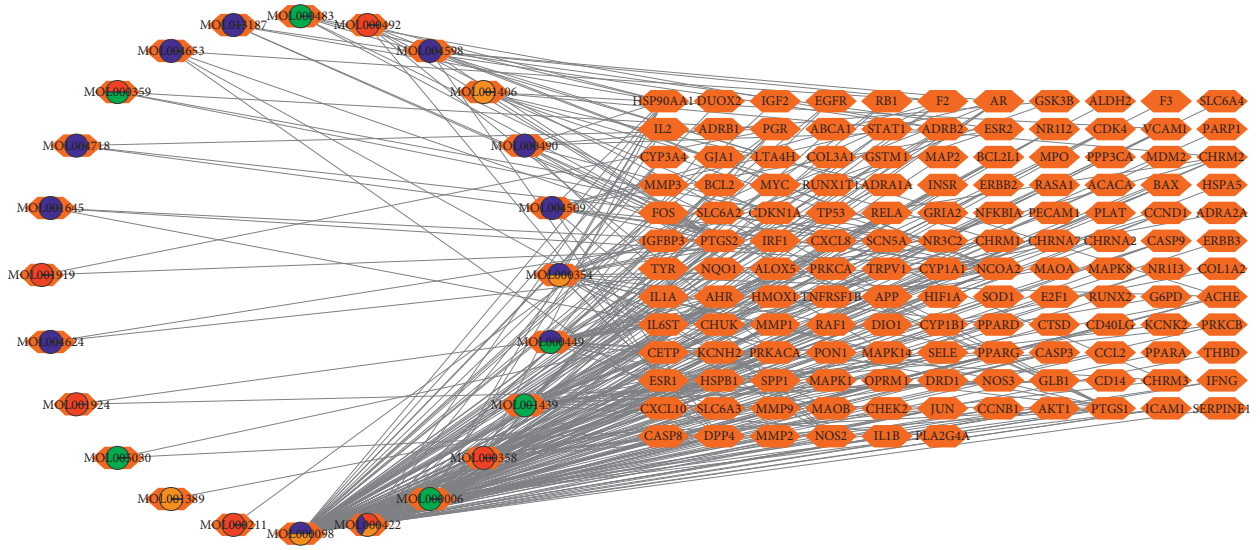

Figure 6: Drug-target-disease network diagram. The green dots represent the hemp kernel's compound molecule. The orange dots represent the molecular compounds in the saffron. The blue dots represent the compound molecules of *Bupleurum*. The red dots represent the compound molecules of *Paeonia paeoniae*. Each edge represents the interaction between the compound molecule and the target.

of 5.75 targets. Each target interacts with an average of 3.3 compounds. Thus, traditional Chinese medicine has various interactions with drugs. Different drugs can have the same molecular compounds. The same compound molecules can be applied to different targets. Different compound molecules can also be applied to the same target. A target can be associated with many diseases. A kind of disease can involve multiple targets and form a complex network.

3.6. PPI Network of Therapeutic Targets for Herbs against Depression. We screened 141 common drug-disease targets. These targets were put into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database to construct a protein-protein interaction (PPI) network (Figure 7). The minimum interaction threshold is set to "Medium Confidence = 0.9", and after removing free nodes, the PPI network is obtained. The results obtained from STRING database analysis are shown in Figure 7, where

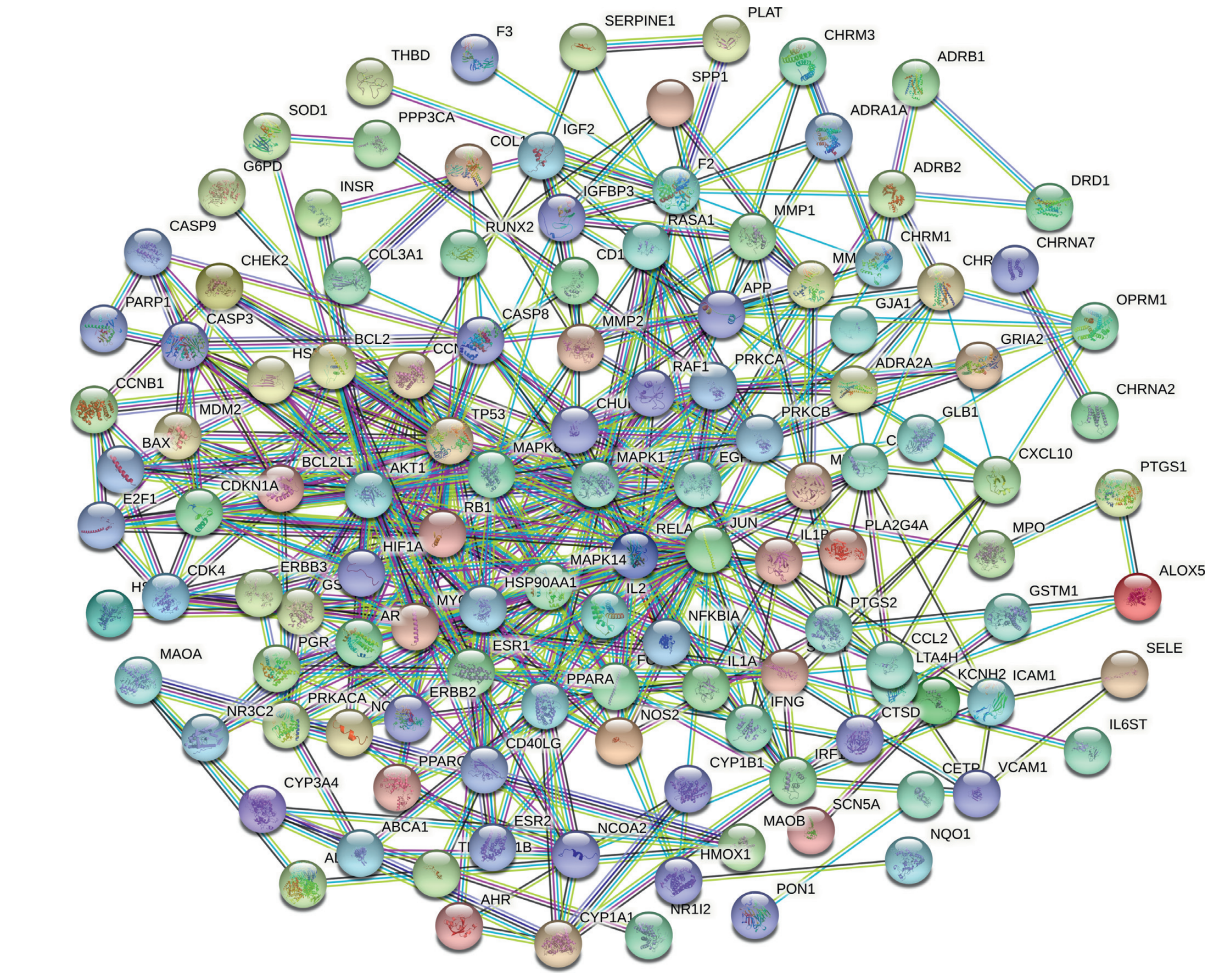

FIGURE 7: PPI network of constituents, common disease targets.

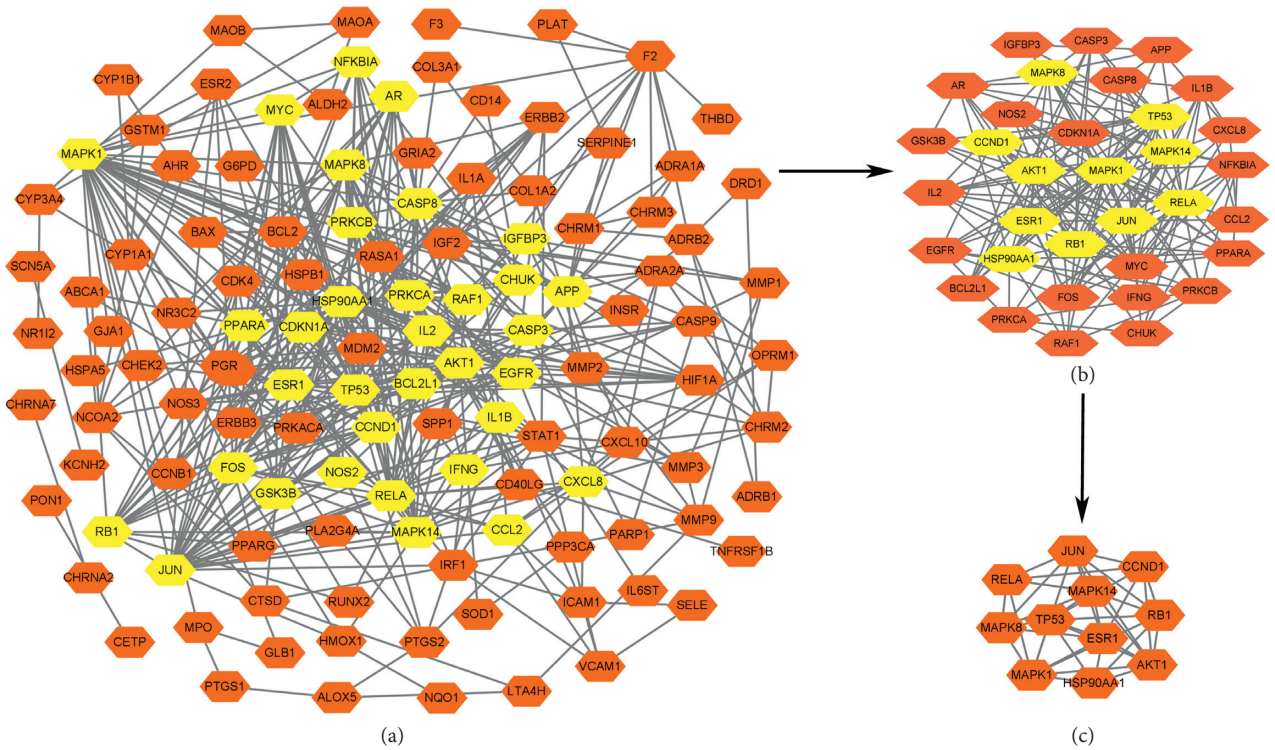

FIGURE 8: Core targets of HERBS in the treatment of depression.

nodes represent proteins, the lines between nodes represent the interactions between two proteins, and different colors correspond to different types of interactions. We applied Cytoscape 3.8.0 and CytoNCA to calculate the mean centrality value of network nodes. We screened the nodes with a centrality value greater than the mean and finally obtained 11 core targets (Figure 8). These core genes included HSP90AA1, AKT1, MAPK1, CCND1, MAPK14, RB1, TP53, JUN, ESR1, RELA, and MAPK8. Results indicated that these targets played an important role in the PPI network and could also be the key targets of herbs in treating depression.

3.7. Results of Drug-Disease Intersection Target Genes after GO Analysis. To clarify the potential mechanism of herbs acting on depression, the clusterProfiler R package was used to conduct GO analysis at the intersection of drug and disease targets. It included three aspects: biological process (BP), cellular component (CC), and molecular function (MF). A screening standard of $P < 0.05$ was set. Each category was sorted according to the number of enriched genes, and the top 10 items are displayed in the form of a bar graph (Figure 9). A total of 2,207 items were obtained by BP analysis, and the first 10 items were mainly reactions: response to lipopolysaccharide; response to molecule of bacterial origin; response to metal ion; response to nutrient levels; response to antibiotic; response to radiation; reactive oxygen species metabolic process; cellular response to drug; and response to oxidative stress and neuron death. A total of 109 items were obtained by CC analysis, and the first 10 items mainly involved membrane raft; membrane microdomain; membrane region; plasma membrane raft; caveolae; transcription regulator complex; RNA polymerase II transcription regulator complex; integral component of presynaptic membrane; intrinsic component of presynaptic membrane; and neuronal cell body. A total of 197 items were obtained by MF analysis, and the first 10 items were mainly ubiquitin-like protein ligase binding; ubiquitin protein ligase binding; nuclear receptor activity; ligand-activated transcription factor activity; steroid hormone receptor activity; DNA-binding transcription factor binding; RNA polymerase II-specific DNA-binding transcription factor binding; scaffold protein binding; phosphatase binding; and protease binding.

3.8. The Results after KEGG Analysis. We analyzed the data as well as related biological processes. The clusterProfiler R package was applied to conduct KEGG pathway enrichment analysis on the HERS gene set, and the results are shown (Figures 10 and 11). A total of 180 signaling pathways ($P < 0.05$) were obtained by KEGG pathway enrichment screening, including the IL-17 signaling pathway, apoptosis, pathways of neurodegeneration-multiple diseases, dopaminergic synapse, cell cycle, long-term depression, and neuroactive ligand-receptor interaction. Figure 10 shows the top 30 paths sorted from smallest to largest by P value. Figure 11 shows the signaling pathway of long-term depression.

3.9. Molecular Docking. To ensure that the accuracy of this study is within the acceptable level, we applied iGEM-DOCK molecular docking analysis to conduct molecular docking between 9 core genes of herbs acting on depression and the key effective compounds obtained by topological analysis. The more stable the binding structure of the small molecule receptor and ligand, the lower the energy level, and the larger the interaction type should be. According to the docking score (Table 2), MAPK14 and petunidin, MAPK14 and isorhamnetin, and MAPK1 and quercetin had the strongest combination and a low energy score. The specific docking structure is shown in Figure 12.

4. Discussion

Depression belongs to the category of "melancholia" and "zang manic" in Chinese medicine. Through the analysis of COREMINE Medical, we can know that hemp seed, saffron, *Bupleurum*, *Cannabis sativa* L. (Cannabaceae, cannabis fructus), and *Crocus sativus* L. (Iridaceae, croci stigma), four traditional Chinese medicines, can target the key genes of depression. It has a certain antidepressive effect and multitarget and multilevel effects. Hemp oil can partially improve the depressive behavior and cognitive ability of mice and reduce the expression of IL-1β. The inflammatory response of hemp seed oil has a certain effect on depression mice [19]. *Bupleurum chinense* DC. (Apiaceae, bupleuri radix) and radix paeoniae alba in antidepressant research are more, to explore the medication rules of traditional Chinese medicine in treating poststroke depression by "traditional Chinese medicine inheritance support system (V2.5)." The results showed that *Bupleurum chinense* DC. (Apiaceae, bupleuri radix) and Radix Paeoniae Alba were the top five Chinese medicines [26]. Vahdati Hassani et al. [27] suggested that crocin has antidepressant-like action by increasing CREB, BDNF, and VGF levels in the hippocampus. Zhang et al. [28] showed that crocin attenuated LPS-induced production of reactive oxygen species and microglial M1-polarization. Moreover, crocin inhibited LPS-induced anxiety and depressive-like behaviors in mice. Crocin blocked the effect of increased cytokine expression including IL-1β, IL-18, and TNF-α in the hippocampus of LPS-injected mice. Additionally, LPS-injected mice exhibited elevated expression of NF-κB, p65, NLRP3, caspase-1, and the adaptor protein ASC, which were inhibited by crocin treatment. In summary, crocin attenuates LPS-induced anxiety and depressive-like behaviors by suppressing the NF-kB and NLRP3 signaling pathways.

In this study, network pharmacological methods were used to explore the possible targets and pathways of *Cannabis sativa* L. (Cannabaceae, cannabis fructus), *Crocus sativus* L. (Iridaceae, croci stigma), *Bupleurum chinense* DC. (Apiaceae, bupleuri radix), and *Paeonia lactiflora* Pall. (Paeoniaceae, paeoniae radix alba). Among the selected 24 compounds corresponding to 138 action targets, the top 5 compounds with the strongest effect were finally analyzed, including petunidin, isorhamnetin, luteolin, quercetin, and kaempferol. At present, there are few studies on morning-glory as an antidepressant, and morning-glory is likely to be a

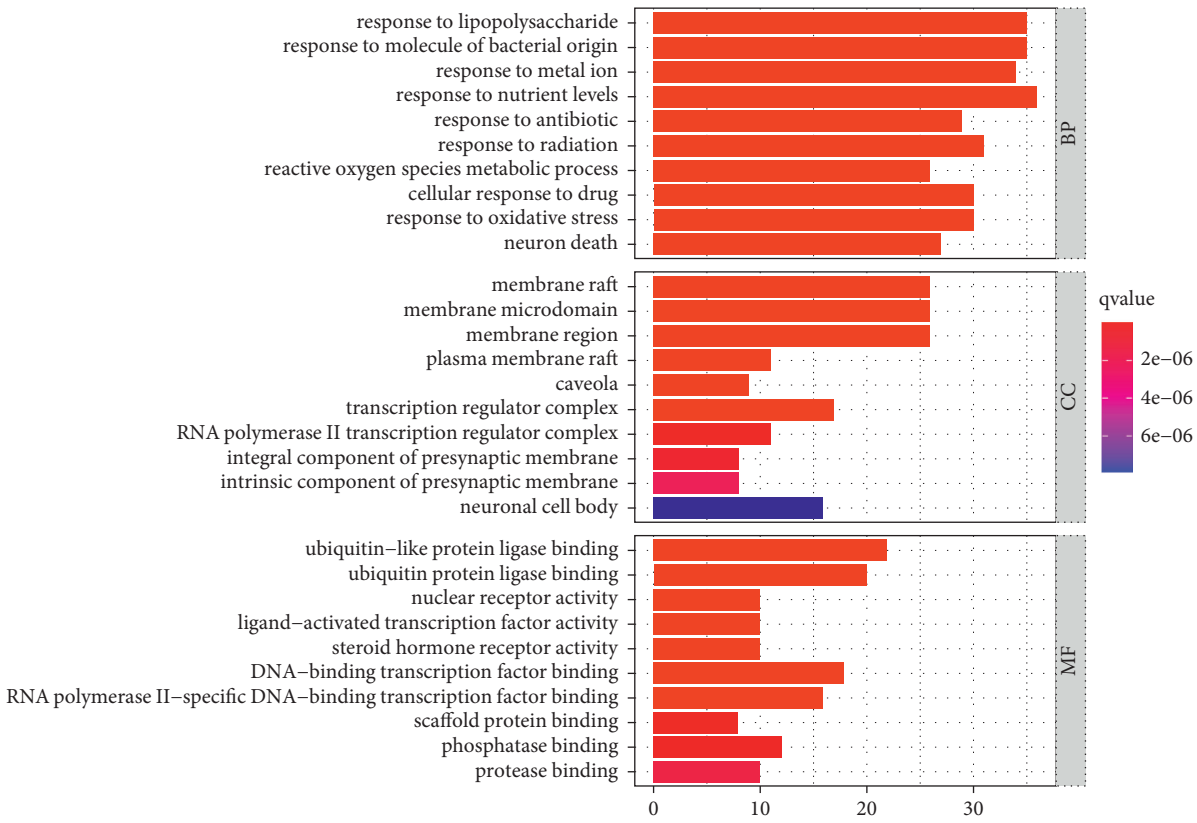

Figure 9: Bar plot of GO analysis results.

targeted drug for the treatment of depression. Petunidin belongs to *Bupleurum chinense* DC. (Apiaceae, bupleuri radix). Isorhamnetin belongs to the *Bupleurum chinense* DC. (Apiaceae, bupleuri radix) and *Crocus sativus* L. (Iridaceae, croci stigma). Luteolin belongs to *Cannabis sativa* L. (Cannabaceae, cannabis fructus). Quercetin belongs to *Bupleurum chinense* DC. (Apiaceae, bupleuri radix), and *Crocus sativus* L. (Iridaceae, croci stigma). Kaempferol belongs to *Bupleurum chinense* DC. (Apiaceae, bupleuri radix), *Crocus sativus* L. (Iridaceae, croci stigma), and *Paeonia lactiflora* Pall. (Paeoniaceae, paeoniae radix alba). It has been reported that isorhamnetin, kaempferol, and quercetin have antidepressant effects [29, 30]. Luteolin can improve depression-like behavior in CUMS mice, the mechanism of which may be related to the enhancement of antioxidant activity and the improvement of oxidative/antioxidant balance in brain tissue of mice [31]. Quercetin can significantly reverse corticosterone-releasing factor- (CRF-) induced anxiety and depression-like behavior in rats [32]. Kaempferol can enhance the antioxidant capacity of the cerebral cortex and upregulate the activity of the Akt/β-catenin cascade to induce anti-inflammatory effect and then play an antidepressant role in chronic social defeat stress (CSDS) mice [33]. This also provides a theoretical basis for the rationality of these four herbs in the treatment of depression.

According to the target of ingredient-disease intersection, these four traditional Chinese medicines can act on multiple targets of HSP90AA1, Akt1, MAPK1, CCND1, MAPK14, RB1, TP53, Jun, ESR1, Rela, and MAPK8 to produce synergistic antidepressant effects. GO and KEGG analysis revealed that the mechanism of depression treatment may be through response to oxidative stress and neuron death, steroid hormone receptor activity; IL-17 signaling pathway, apoptosis, pathways of neurodegeneration-multiple diseases, dopaminergic synapse, cell cycle, long-term depression, neuroactive ligand-receptor interaction play a role in improving the effect of depression. MAPKs are the core targets involved in the IL-17 signaling pathway. The possible effects are petunidin on MAPK14, isorhamnetin on MAPK14, quercetin on MAPK1, and luteolin on MAPK1.

Astrocytic p38α MAPK drives NMDA receptor-dependent long-term depression and modulates long-term memory [34]. Serotonin facilitates long-term depression induction in the prefrontal cortex via p38 MAPK/Rab5-mediated enhancement of AMPA receptor internalization [35]. MAPK signaling determines anxiety in the Juvenile Mouse brain but depression-like behavior in adults [36]. Enhanced MAPK1 function causes a neurodevelopmental disorder within the RASopathy clinical spectrum [37]. The P2RX7-MAPK1/2-SP1 axis inhibits MTOR independent HSPB1-mediated astroglial autophagy [38]. Enhanced MAPK1 function causes a neurodevelopmental disorder within the RASopathy clinical spectrum [37]. The P2RX7-MAPK1/2-SP1 axis inhibits MTOR independent HSPB1-mediated astroglial autophagy [38]. The mechanism of Alzheimer's disease is that MAPK14/P38A activates the targeting neurons to regulate autophagy. Mitophagy is primarily due to alternative autophagy and requires the

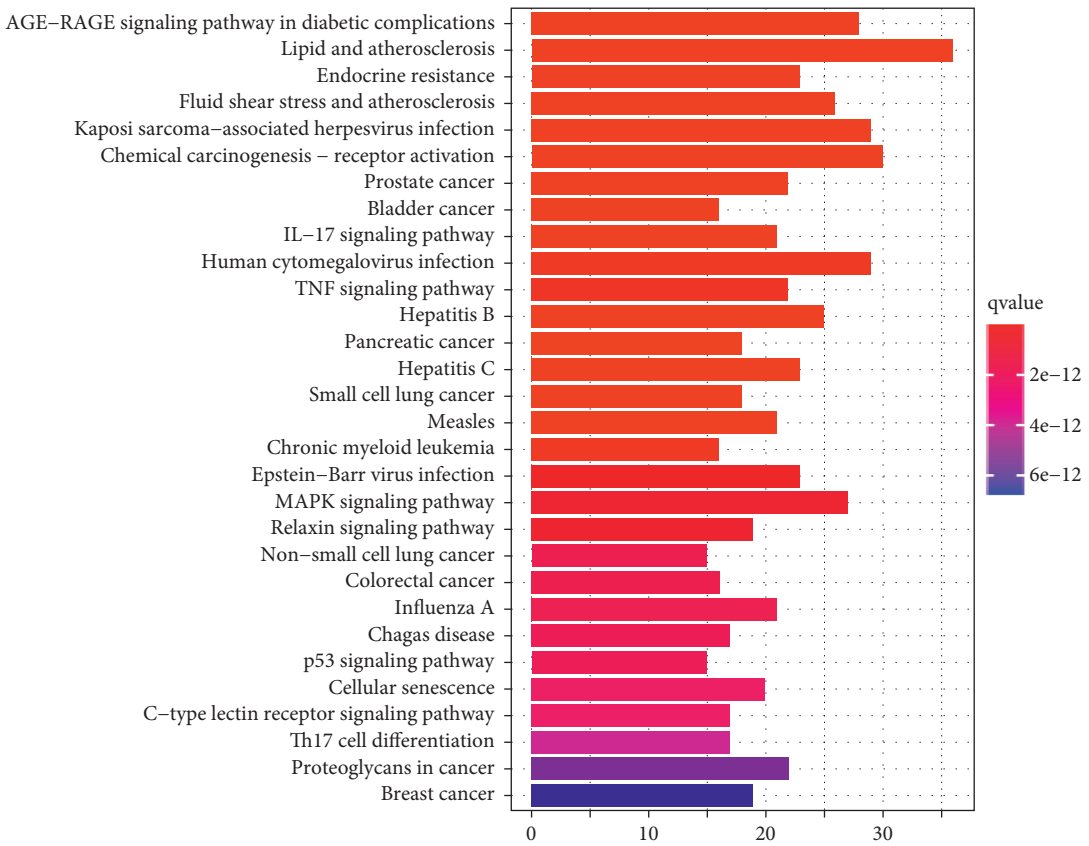

Figure 10: Bar plot of KEGG analysis results.

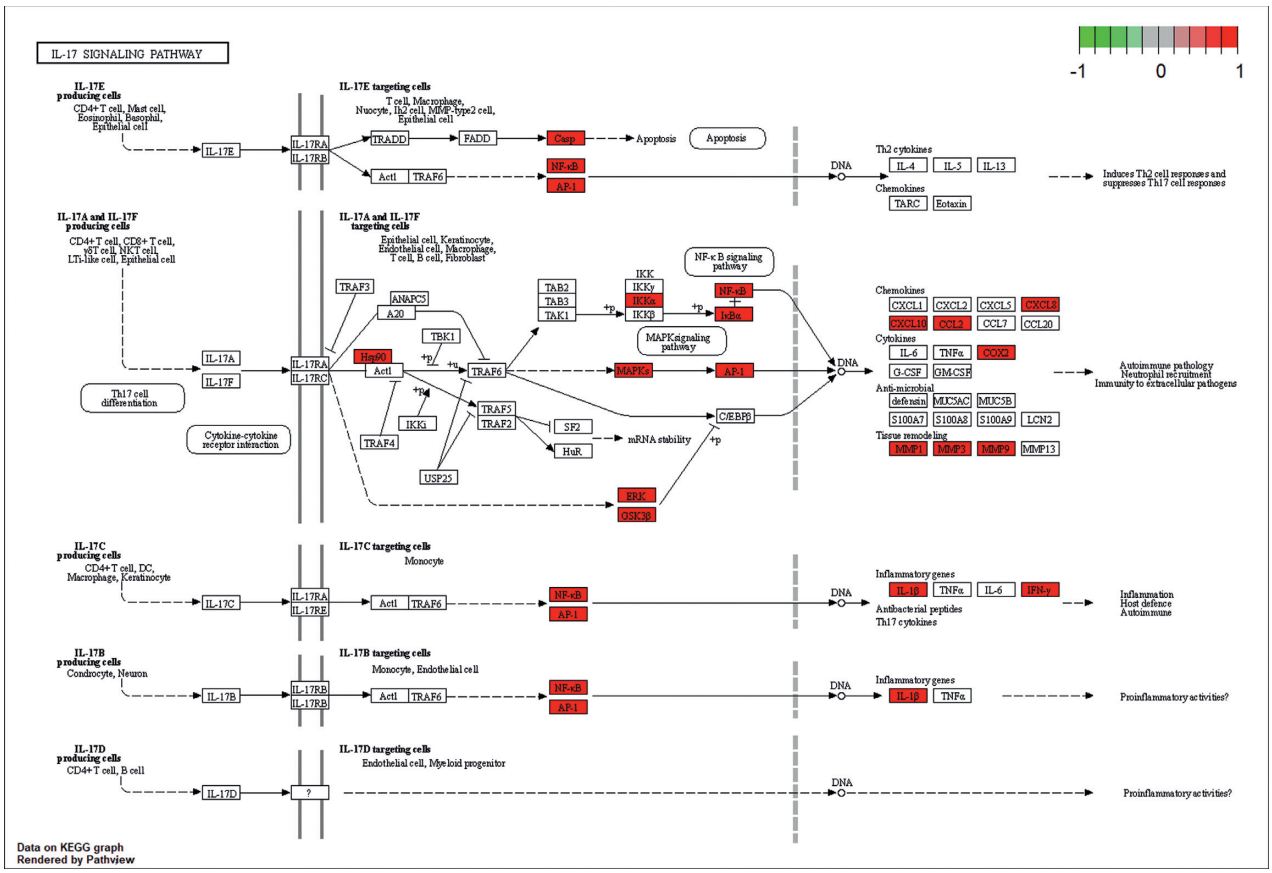

Figure 11: Signaling pathway of IL-17 signaling pathway.

Table 2: Molecular docking score.

Target	PDB ID	Compound	Affinity (kJ·mol^{-1})
JUN	P05412	Luteolin	−8.8
		Quercetin	−8.6
		Kaempferol	−8.4
		Beta-sitosterol	−6.8
MAPK8	P45983	Kaempferol	−5.5
RELA	P9WHG9	Isorhamnetin	−7.6
		Quercetin	−7.5
		Arachidonic acid	−5
TP53	Q96S44	Luteolin	−8.2
		Quercetin	−8
RB1	P13405	Quercetin	−8.4
		Luteolin	−8.9
MAPK14	Q16539	Petunidin	−9
		Isorhamnetin	−9
CCND1	*P24385*	Quercetin	−7.1
		Arachidonic acid	−4.9
		Luteolin	−7.3
MAPK1	P28482	Quercetin	-8.4
		Arachidonic acid	−5.8
		Luteolin	−8.5
AKT1	P31750	Luteolin	−6.3
		Quercetin	−6.3
		Kaempferol	−6.4
HSP90AA1	P07900	Kaempferol	−6.7
		Beta-sitosterol	−7.4
		(+)-Catechin	−6.8
		Isorhamnetin	−6.9
		Cubebin	−6.7
		Quercetin	−6.9
ESR1	P03372	(+)-Catechin	−7.1
		Isorhamnetin	−8.5

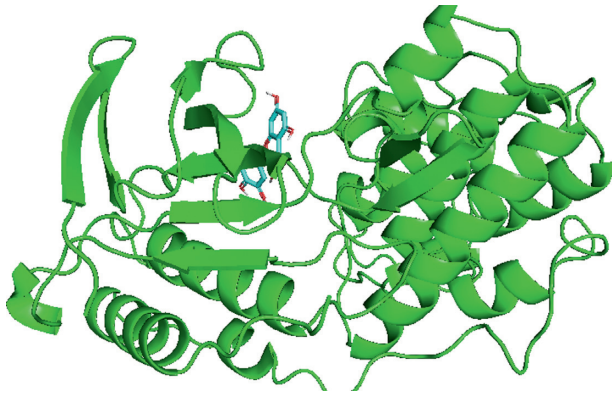

Figure 12: Docked conformation of MAPK14 and quercetin.

MAPK1 and MAPK14 signaling pathways [39, 40]. Depression is accompanied by increased IL-17 mRNA and protein levels in serum, thereby confirming the important role of IL-17 production in depression and its possible use as a biomarker of depression [41]. However, adipose tissue also expresses high levels of circulating interleukin-17 (IL-17A), mainly secreted by T helper 17 (Th17, CD4+) cells [42–44]. In addition to their local action, systemic cytokines derived from adipose tissue may also enter the central nervous system which leads to neuroinflammation [45]. Inflammatory factors cause excessive activation of IDO (indoleamine-2,3-dioxygenase), an enzyme present in microglia, astrocytes and neurons, and catabolizing the conversion of tryptophan into the neurotoxic kynurenine (KYN), thus reducing the availability of tryptophan for the production of serotonin. Kynurenine, in turn, influences the intensification of neurodegenerative processes [46, 47]. Altered neurogenesis and neuroplasticity and serotonin deficits contribute to depression. MAPK14, as an Alzheimer's therapeutic target, has otherwise primarily been considered as an anti-inflammatory mechanism to target innate immune responses in the brain [48, 49], particularly with regard to microglial activation, as well as reducing inflammation-induced synaptic toxicity. Quercetin alleviates anxiety and depression in 3-nitropropionic acid-induced Huntington's disease in rats [50]. Quercetin alleviates LPS-induced depression-like behavior in rats by regulating BDNF-related imbalance of copine 6 and TREM1/2 in the hippocampus and PFC [51].

5. Conclusion

Overall, this study systematically summarized key active compounds in *Paeonia lactiflora* Pall. (Paeoniaceae, paeoniae radix alba), *Crocus sativus* L. (Iridaceae, croci stigma), *Bupleurum chinense* DC. (Apiaceae, bupleuri radix), and *Cannabis sativa* L. (Cannabaceae, cannabis fructus) and comprehensively analyzed potential mechanisms of its action and pathways. The current findings revealed the complicated antidepressant mechanism of most commonly used Chinese medicines and provided a rational strategy for revealing the complex composition and function of Chinese herbal formulas.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Yucong Shi and Dan Chen contributed equally to this work. Y.S. and D.C. conducted the experiments and participated in the conception and the design of the study. Y.S. and S.M. conducted the experiments and performed the analysis. Y.S. and D.C. contributed to analyzing the data and drafting the manuscript. L.D and H.X. contributed to the conception, design, and analysis of the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (nos. 81830114, 82104707, and 82004232), Natural Science Foundation of Guangdong, China (nos. 2020A1515010756 and 2021A1515011215), Project of Administration of Traditional Chinese Medicine

of Guangdong Province (no. 20201074), and China Postdoctoral Science Foundation (no. 2020M683206).

Supplementary Materials

Table S1: database and website. Table S2: ingredients. Table S3: targets. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2021, Article ID 8614963, 13 pages
https://doi.org/10.1155/2021/8614963

Research Article

Bioinformatics and Network Pharmacology-Based Approaches to Explore the Potential Mechanism of the Antidepressant Effect of Cyperi Rhizoma through Soothing the Liver

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Received 21 July 2021; Accepted 13 December 2021; Published 28 December 2021

Academic Editor: Junqing Huang

Major depressive disorder (MDD) has become the second most common disease worldwide, making it a threat to human health. Cyperi Rhizoma (CR) is a traditional herbal medicine with antidepressant properties. Traditional Chinese medicine theory states that CR relieves MDD by dispersing stagnated liver qi to soothe the liver, but the material basis and underlying mechanism have not been elucidated. In this study, we identified the active compounds and potential anti-MDD targets of CR by network pharmacology-based approaches. Through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, we hypothesized that the anti-MDD effect of CR may be mediated by an altered response of the liver to lipopolysaccharide (LPS) and glucose metabolism. Through bioinformatics analysis, comparing normal and MDD liver tissue in rats with spontaneous diabetes, we identified differentially expressed genes (DEGs) and selected PAI-1 (SERPINE1) as a target of CR in combating MDD. Molecular docking and molecular dynamics analysis also verified the binding of the active compound quercetin to PAI-1. It can be concluded that quercetin is the active compound of CR that acts against MDD by targeting PAI-1 to enhance the liver response to LPS and glucose metabolism. This study not only reveals the material basis and underlying mechanism of CR against MDD through soothing the liver but also provides evidence for PAI-1 as a potential target and quercetin as a potential agent for MDD treatment.

1. Introduction

Major depressive disorder (MDD), a complicated mental disease, affects approximately 340 million people, and 1 million people die each year due to suicide [1]. MDD is characterized by multiple emotional and physical disorders, such as depressive mood, excessive guilt, insomnia, poor concentration, and suicidal ideation [2]. Various factors contribute to the initiation of MDD, including neuronal apoptosis, decreased neurotransmitter secretion, psychosocial factors, and abnormal immune and endocrine systems [3]. Recent research demonstrated that diabetes and inflammation are both key risk factors for MDD [4, 5]. The levels of numerous proinflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1B (IL-1B), and tumour necrosis factor-α (TNF-α), are elevated in MDD patients [6, 7]. It has been reported that lipopolysaccharide (LPS) induces systemic inflammation by elevating proinflammatory cytokines in both the periphery and brain, thus resulting in depression-like behaviour [8]. Additionally,

epidemiological studies have shown that depression is twice as frequent in people with diabetes compared with those without diabetes [9]. Although the mechanism of diabetes-induced MDD is not completely understood, metabolic disorders, such as persistent hyperglycaemia in diabetes, can change the function, neurochemistry, or structure of the brain to mediate MDD [10]. LPS and glucose metabolism are closely related to the liver, a central organ for systemic metabolism [11]. The liver is responsible for the clearance of LPS in the blood [12]. As an organ of action for insulin, the liver regulates blood glucose by producing glucose during fasting and storing glucose postprandially [13]. In type 1 and type 2 diabetes mellitus, hepatic processes are dysregulated and contribute to hyperglycaemia [14]. In brief, the liver may play a vital role in LPS and glucose metabolism to mediate MDD.

Cyperi Rhizoma (CR), a traditional herbal medicine, has been clinically used for menstrual or emotional disturbances in women and stomach disorders in Asia for centuries [15]. Modern pharmacological research has revealed the anti-inflammatory, antiapoptotic, and antibacterial properties of CR [16]. Recent studies have demonstrated that CR possesses therapeutic effects on nervous system diseases, such as 6-hydroxydopamine-induced neuronal damage [17]. In addition, CR extract exerts an antidepressant effect on mice characterized by shorter immobility time in the swimming test and the tail suspension test [18, 19]. Traditional Chinese medicine (TCM) theory states that CR relieves depression and anxiety by dispersing stagnated liver qi to soothe the liver. However, the material basis and antidepressant mechanism of CR have not been well established.

Plasminogen activator inhibitor-1 (PAI-1, SERPINE1) is a prothrombotic plasma protein secreted by endothelial tissue [20]. As a stress-related protein, PAI-1 has been implicated in numerous disease states, including MDD. Elevated plasma levels of PAI-1 have been observed in depressed patients and C57Bl/6J mice [21]. Another meta-analysis also demonstrated that the PAI-1 serum level was 0.27 SDs higher in MDD patients than it was in healthy controls [22]. Abnormal PAI-1 causes MDD through different mechanisms. First, PAI-1 may contribute to the cleavage of pro-brain-derived neurotrophic factor (BDNF) into its mature form, and BDNF is strongly implicated in depression [23]. In addition, PAI-1 is widely regarded as an inflammatory marker involved in the pathogenesis of depression [24]. PAI-1 participates in LPS-induced inflammation [25]. Inhibition of PAI-1 by histone deacetylase 2 (HDAC2) attenuates LPS-induced inflammation [26]. Furthermore, the PAI-1 levels were significantly higher among patients with metabolic syndrome, and 53.2% of these patients experienced depression [24]. For example, an elevated level of PAI-1 was detected in diabetes- and insulin-resistant states, which is associated with an imbalance in glucose and lipid homeostasis [27]. Given that the liver is a major site for PAI-1 synthesis and responds to a variety of hormonal changes and other pathological events [28], it can be deduced that liver disorders and MDD are closely associated.

In our present study, we selected active compounds and identified PAI-1 as a target of CR to combat MDD by regulating the liver response to LPS and glucose metabolism, which may shed light on the modern pharmacological connotation of the antidepressant effect of CR through soothing the liver.

2. Materials and Methods

2.1. Compounds and Targets Screening of CR. Chemical compounds and their related targets were collected from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (https://old.tcmsp-e.com/tcmsp), a unique pharmacology platform for Chinese herbal medicines. To further obtain the active compounds according to the ADME (adsorption, distribution, metabolism, excretion) parameters, we chose compounds meeting the requirements of both oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. OB is a vital indicator of the orally administered dosage of unchanged drug entering the human circulatory system [29]. DL was applied to filter out compounds with undesirable properties [30]. To obtain the gene symbol of each target, we used UniProt (https://www.uniprot.org) to convert protein names to gene symbols.

2.2. Screening of Potential Targets of CR in the Context of MDD. GeneCards (http://www.genecards.org/) was used to search for the therapeutic target genes of MDD. Key terms related to "major depressive disorder" were retrieved, and the requirement of relevance score ≥10 was set. We selected genes at the intersection of CR and MDD (C&M) as potential target genes through which CR exerts effects on MDD, and the corresponding compounds of CR were regarded as candidate components with antidepressant properties.

2.3. Network Construction. The drug-compound-target-disease network was established using the network visualization software Cytoscape (v3.8.2; Agilent Technologies Company, USA). Each node in the network represents a medicine, disease, target, or compound. If an interaction occurred between nodes, they were connected by a line. Topology analysis was achieved in CentiScaPe.

2.4. Protein-Protein Interaction (PPI) Network Construction. The PPI network of C&M targets was built using STRING version 11.0 (https://www.string-db.org/). The targets of C&M were entered into the STRING database. The species was limited to *Homo sapiens*, and an interaction score greater than 0.4 was required. The bitmap image and network information were downloaded from this website. After enrichment of all the nodes, we obtained the top 30 targets that were considered to play central roles in the PPI network.

2.5. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment. We conducted GO and KEGG enrichment analysis based on Bioconductor

software (http://bioconductor.org/). GO enrichment included the biological process (BP), molecular function (MF), and cellular component (CC) categories. We used clusterProfiler (version 4.1), an *R* package, to perform the GO and KEGG enrichment analysis of gene clusters. The results were considered significant when the P value < 0.05. The top 5 GO and KEGG enriched results are displayed in the bar plot.

2.6. Identification and Analysis of Differentially Expressed Genes (DEGs). The microarray data set GSE94988, which includes 3 control samples, 3 mild depression samples, and 3 major depression samples, was downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) using the GEOquery package (version 2.54.1) [31]. In the original research, the analysis of GSE94988 aims to explore the mechanism of pharmacokinetic perturbation in a CUMS-induced depression animal model with spontaneous diabetic GK rats [32]. The expression profile in GK rat livers was screened using Affymetrix Rat 230 2.0 Array. The original files from the GEO database were normalized by the *R* software (version 4.0.2) affy package. DEGs between 3 control samples and 3 major depression samples were screened using the limma package (version 3.42.2), which can fit a linear model for the expression of each gene. The screening criteria were $P < 0.05$, and $|\log2\text{-fold change (FC)}| > 1$ between the two groups. To better visualize these DEGs, volcano plots were made using the ggplot2 package (version 3.3.3), and heatmaps were generated using the ComplexHeatmap package (version 2.2.0) [33]. Venn diagrams were used to display the intersection between DEGs and C&M targets.

2.7. Molecular Docking between Targets and Compounds. The crystal structure of the PAI-1 protein (PDB id: 7AQF) was downloaded from RCSB PDB (https://www.pdbus.org/). GUI-based "AutoDock Tools" were used to prepare and execute the docking studies. Kollman atom charges, solvation parameters, and polar hydrogens were added to the protein, and this information was used for docking studies. As the ligands used are not peptides, Gasteiger charges were assigned only to the protein, and nonpolar hydrogens were merged. Based on the literature and predicted active regions, a grid box was assigned around the active sites using the AutoGrid application. The 3D structures of quercetin were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). Then, we minimized the energy of the downloaded compound through Chem3D and converted it into mol2 format. The small molecular compound was imported into AutoDock Tools software, added with atomic charge, and assigned an atomic type. All flexible keys are rotatable by default. Finally, the best conformation was retained in pdbqt format for utilization in further docking studies. Docking calculations were performed using AutoDock 4.2 to compute the free energy of binding in the protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of $60 \times 60 \times 60$ Å grid points and 0.375 Å spacing were generated using the AutoGrid program. The AutoDock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method. The initial position, orientation, and torsions of the ligand molecules were set randomly, and all rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å and quaternion and torsion steps of 5 were applied.

2.8. Molecular Dynamics. The molecular dynamics (MD) simulation of docked complexes was performed using Desmond version 2020. Here, an OPLS3e force field was used to initiate the MD simulation, and the system was solvated using the TIP3 water model. The neutralization of the system was performed by adding counter ions. Energy minimization of the entire system was performed using OPLS3e because it is an all-atom type force field. The geometry of water molecules, the bond lengths, and the bond angles of heavy atoms were restrained using the SHAKE algorithm. Simulation of the continuous system was executed by applying periodic boundary conditions, and long-range electrostatics were maintained by the particle mesh Ewald method. The equilibration of the system was performed using an NPT ensemble with temperature at 300 K and pressure at 1.0 bar. The coupling of temperature-pressure parameters was performed using the Berendsen coupling algorithm. After preparation of the system, the production run was performed for 100 ns with a time step of 1.2 fs, and trajectory recording was performed every 100 ps summing up to the recording of 1,000 frames. The root mean square deviation (RMSD) was calculated for the backbone atoms and was analysed graphically to understand the nature of protein-ligand interactions. The root mean square fluctuation (RMSF) for every residue was calculated to understand the major conformational changes in the residues in comparison between the initial state and dynamic state.

2.9. Statistical Analysis. The results are presented as the mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software Inc.) and ImageJ software (National Institutes of Health, USA). The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. *R* software was also used to perform statistical analyses. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Active Compounds and Targets of CR. Given that herbal medicine is characterized by the use of multicomponent and multitarget therapeutic drugs, it is necessary to select active compounds with satisfactory pharmacokinetic properties for further research. After retrieval in TCMSP, a total of 104 compounds from CR were obtained. Based on the ADME model, we selected 18 active compounds that meet the requirements of both oral bioavailability (OB) ≥ 30% and

Table 1: Active compounds of CR and their major parameters.

No.	Name	OB	DL
MOL003044	Chryseriol	35.85	0.27
MOL000354	Isorhamnetin	49.6	0.31
MOL003542	8-Isopentenyl-kaempferol	38.04	0.39
MOL000358	β-Sitosterol	36.91	0.75
MOL000359	Sitosterol	36.91	0.75
MOL004027	1,4-Epoxy-16-hydroxyheneicos-1,3,12,14,18-pentaene	45.1	0.24
MOL004053	Isodalbergin	35.45	0.2
MOL004058	Khell	33.19	0.19
MOL004059	Khellol glucoside	74.96	0.72
MOL010489	Resivit	30.84	0.27
MOL004068	Rosenonolactone	79.84	0.37
MOL004071	Hyndarin	73.94	0.64
MOL004074	Stigmasterol glucoside_qt	43.83	0.76
MOL004077	Sugeonyl acetate	45.08	0.2
MOL000422	Kaempferol	41.88	0.24
MOL000449	Stigmasterol	43.83	0.76
MOL000006	Luteolin	36.16	0.25
MOL000098	Quercetin	46.43	0.28

drug-likeness (DL) ≥ 0.18 (Table 1). Information on compound-related targets was also obtained from TCMSP. Ultimately, the 18 active compounds and 225 corresponding targets constitute 496 compound-target connections. The official gene symbols of targets were obtained from the UniProt database for further investigation.

3.2. Potential Anti-MDD Targets of CR. To further predict the potential targets of CR against MDD, a drug-compound-target-disease network was established to analyse the mechanism. The GeneCards database was used to retrieve MDD-related therapeutic targets. As a result, 10,026 MDD-related targets were obtained, and 1440 targets that met the requirement of a relevance score ≥ 10 were selected. A Venn diagram (Figure 1(a)) showed the overlap of a total of 106 significant targets between CR and MDD (C&M), indicating that 106 potential targets were involved in the anti-MDD effect of CR. The 15 compounds corresponding to 106 targets were regarded as candidate effective components of CR. Next, we applied Cytoscape software to construct a drug-compound-target-disease network. As shown in Figure 1(b), 15 drug-compound, 494 compound-target, and 106 target-disease connections were created in a network. The network map displays the synergistic effect of multiple ingredients of CR as they converge on multiple targets. To preliminarily screen key targets in the C&M network, 106 potential targets were collected for further analysis.

3.3. PPI Analysis of C&M Targets. The STRING database was used to establish the PPI network of C&M targets. A total of 106 target genes of C&M were submitted to the STRING website, and 1543 connections that represent the interaction between two targets were generated (Figure 2(a)). To screen core targets thoroughly, the frequency of each node and the combined score between two nodes were calculated. A bar plot (Figure 2(b)) shows the top 30 enriched targets, which may represent the most likely targets of CR against MDD. The interaction nodes of higher degree include AKT1, IL6, VEGFA, and TP53, indicating that these nodes are associated with more proteins and may play pivotal roles in the antidepressant effect of CR.

3.4. GO and KEGG Enrichment Analysis. To clarify the anti-MDD mechanism of major active compounds of CR, GO and KEGG enrichments were conducted based on 106 C&M targets. Table 2 provides details of GO and KEGG enrichment analysis. Figure 3(a)–3(d) display the top 5 enriched GO (BP, MF, and CC terms) and KEGG pathways. The results demonstrate that some items are closely related to the nervous system, such as "regulation of neurotransmitter levels," "intrinsic component of postsynaptic membrane," "integral component of postsynaptic membrane," "ammonium ion binding," and "neurotransmitter receptor activity," indicating that CR displays antidepressant effects through multiple biological processes and signalling pathways. Interestingly, we noticed that "response to lipopolysaccharide" ranks top in the BP category, and the "AGE-RAGE signalling pathway in diabetic complications" ranks top in KEGG pathway enrichment. It has been reported that LPS and diabetes are both risk factors in MDD due to metabolic dysfunction [34, 35]. TCM states that CR disperses stagnated liver qi to relieve depression [36]. Additionally, the liver was verified to play significant roles in LPS and glucose metabolism [37, 38]. Based on these facts, we hypothesized that CR displays antidepressant effects by regulating liver functions to combat LPS and glucose metabolic disorders. As a consequence, we collected enriched "response to lipopolysaccharide" and "AGE-RAGE signalling pathway in diabetic complications" gene sets for further investigation.

3.5. Identification of DEGs. To confirm our hypothesis, we identified the DEGs of liver tissue between the normal group and the MDD group. We obtained the microarray data set GSE94988, which includes 3 control samples, 3 mild depression

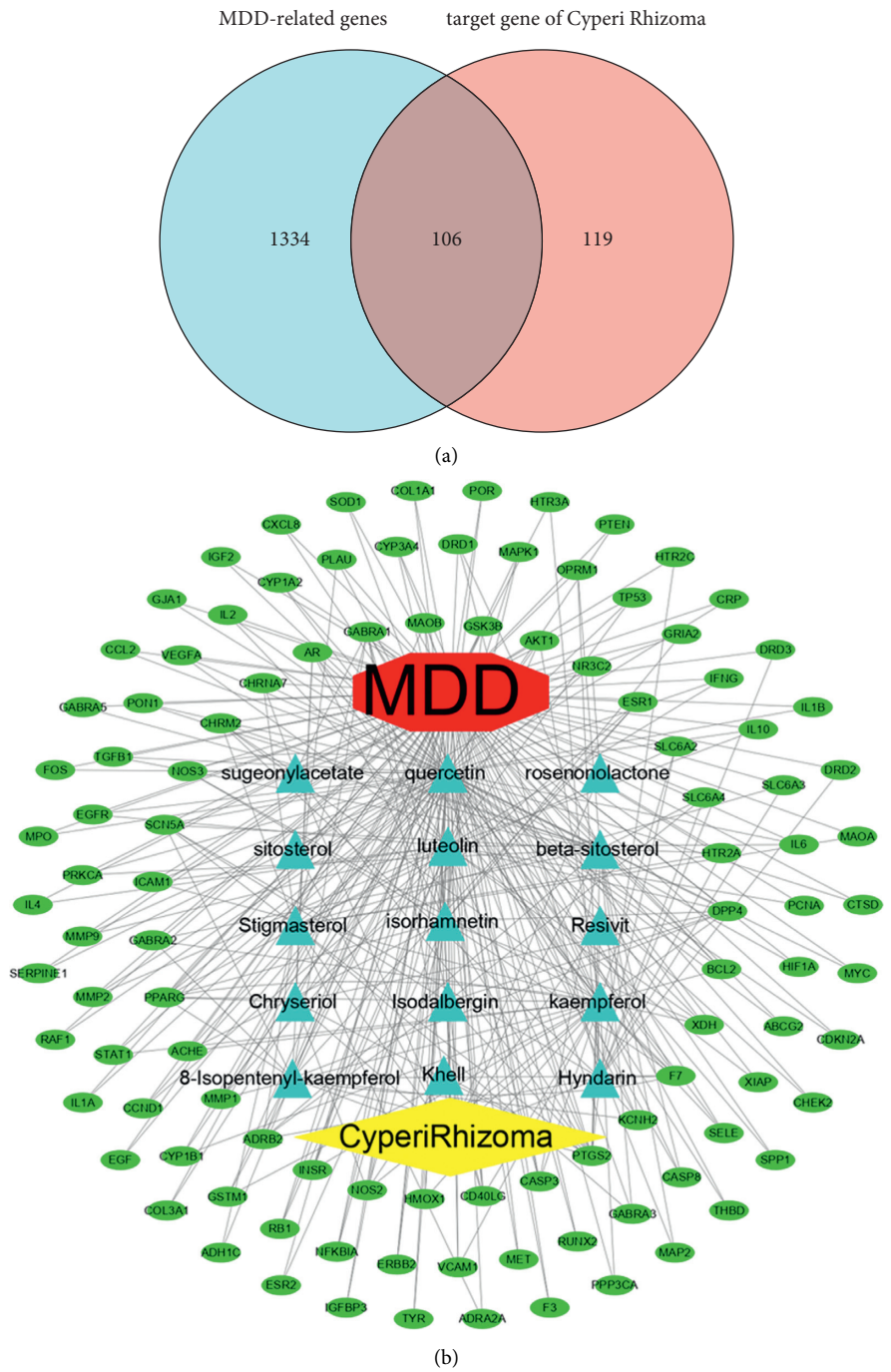
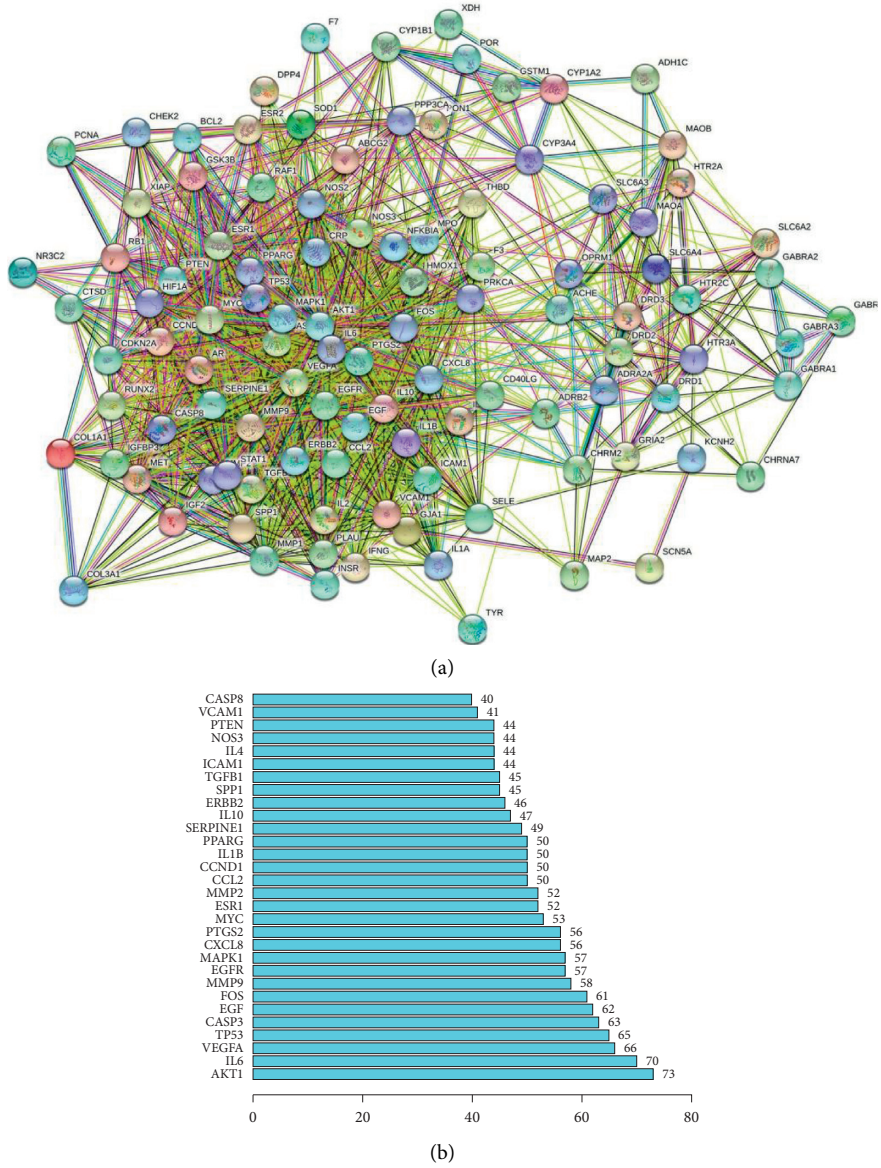


FIGURE 1: Screening of potential effective ingredients and anti-MDD targets of CR. (a) Venn diagram of MDD-related targets and potential targets of CR. (b) The drug-compounds-targets-disease network. The red octagon represents disease. The yellow rhombus represents the drug. The blue triangles represent active compounds. The green ovals represent target genes.

samples, and 3 major depression samples. All the 9 samples have spontaneous diabetes. Three control samples and 3 MDD samples were selected to analyse and identify the DEGs. Before analysis, the original data were preprocessed and normalized based on the *R* software affy package. Figure 4(a) indicates good normalization of the 6 samples. Quality control of the samples was assessed by principal component analysis (PCA) and manifold approximation and projection (UMAP), which can evaluate the intragroup data repeatability and display the relationships between the groups of samples that were compared. PCA results (Figure 4(b)) demonstrated that the first two principal components, principal component 1 (PC1) and principal component 2 (PC2), accounted for 28.1% and 19.7% of the explained variation, respectively. In PCA and UMAP plots, samples were scattered between the CTL and MDD groups, indicating significant differences between the two groups. Thus, subsequent analysis may produce more meaningful results. Compared with control samples, a total of 324



(a)

(b)

Figure 2: Preliminary screening of key C&M targets. (a) PPI network of C&M targets. Each node represents the C&M targets. Each line represents the interaction between two targets. (b) Bar plot of top 30 enriched targets in the PPI network.

DEGs were identified in MDD samples using the criteria of $P < 0.05$ and $|\log2FC| > 1$. These 324 DEGs included 142 downregulated genes and 182 upregulated genes. Volcano diagrams of DEGs are presented in Figure 4(c). The heatmaps in Figure 4(d) display the top 20 downregulated and upregulated genes. These DEGs may be potential targets of the liver to mediate MDD in diabetic rats.

3.6. Potential Targets of CR on LPS and Glucose Metabolism to Combat MDD. To determine the most likely targets of CR in combating MDD through modulating the liver response to LPS and glucose metabolism, we focused on the overlaps between 26 "response to lipopolysaccharide" genes, 24 "AGE-RAGE signalling pathway in diabetic complications" genes, and 324 DEGs. A Venn diagram (Figure 4(e)) displays the overlap of 2 significant targets, namely, SERPINE1 and IL1B, indicating that PAI-1 (SERPINE1) and IL-1B (IL1B) may be potential targets of CR regarding LPS and glucose metabolism to combat MDD. SERPINE1 and IL1B were also listed in the top 30 enriched targets in the PPI network (Figure 2(b)), indicating their central regulatory roles in the antidepressant effect of CR. PAI-1 and IL-1B expression levels

Table 2: Details of top 5 GO and KEGG enrichment terms.

Ontology	ID	Description	GeneRatio	BgRatio	P value	p. adjust	Q value
BP	GO: 0032496	Response to lipopolysaccharide	26/106	330/ 18670	$1.12e-22$	$4.32e-19$	$1.78e-19$
BP	GO: 0002237	Response to molecule of bacterial origin	26/106	343/ 18670	$3.01e-22$	$5.81e-19$	$2.39e-19$
BP	GO: 0031667	Response to nutrient levels	28/106	499/ 18670	$2.05e-20$	$2.64e-17$	$1.09e-17$
BP	GO: 0001505	Regulation of neurotransmitter levels	24/106	354/ 18670	$2.03e-19$	$1.96e-16$	$8.08e-17$
BP	GO: 0010038	Response to metal ion	24/106	364/ 18670	$3.88e-19$	$2.67e-16$	$1.10e-16$
CC	GO: 0045121	Membrane raft	20/106	315/ 19717	$3.61e-16$	$5.23e-14$	$3.46e-14$
CC	GO: 0098857	Membrane microdomain	20/106	316/ 19717	$3.84e-16$	$5.23e-14$	$3.46e-14$
CC	GO: 0098589	Membrane region	20/106	328/ 19717	$7.88e-16$	$7.14e-14$	$4.73e-14$
CC	GO: 0099055	Integral component of postsynaptic membrane	12/106	117/ 19717	$1.48e-12$	$1.00e-10$	$6.65e-11$
CC	GO: 0098936	Intrinsic component of postsynaptic membrane	12/106	122/ 19717	$2.45e-12$	$1.33e-10$	$8.81e-11$
MF	GO: 0030594	Neurotransmitter receptor activity	14/106	117/ 17697	$8.72e-15$	$3.77e-12$	$2.32e-12$
MF	GO: 0070405	Ammonium ion binding	11/106	75/17697	$7.45e-13$	$1.61e-10$	$9.93e-11$
MF	GO: 0005126	Cytokine receptor binding	15/106	286/ 17697	$1.61e-10$	$2.32e-08$	$1.43e-08$
MF	GO: 0002020	Protease binding	11/106	128/ 17697	$2.85e-10$	$3.08e-08$	$1.90e-08$
MF	GO: 0005125	Cytokine activity	13/106	220/ 17697	$6.83e-10$	$5.90e-08$	$3.64e-08$
KEGG	hsa04933	AGE-RAGE signalling pathway in diabetic complications	24/101	100/8076	$4.48e-25$	$1.06e-22$	$3.16e-23$
KEGG	hsa05219	Bladder cancer	15/101	41/8076	$4.75e-19$	$5.60e-17$	$1.67e-17$
KEGG	hsa05215	Prostate cancer	16/101	97/8076	$3.66e-14$	$2.88e-12$	$8.60e-13$
KEGG	hsa05418	Fluid shear stress and atherosclerosis	18/101	139/8076	$6.42e-14$	$3.79e-12$	$1.13e-12$
KEGG	hsa05167	Kaposi sarcoma-associated herpesvirus infection	20/101	193/8076	$1.70e-13$	$8.03e-12$	$2.40e-12$

were evaluated by GSE94988 data analysis. As shown in Figure 4(f), PAI-1 was significantly upregulated in the MDD group ($P < 0.01$), whereas IL-1B was not significantly different ($P > 0.05$). Hence, we selected PAI-1 as the most likely target of CR to combat MDD, and the corresponding active compound was quercetin.

3.7. Molecular Docking between the Target and Compound. Molecular docking between the active compound quercetin and the potential target PAI-1 was performed to explore protein-ligand interactions. The results showed that the binding energy between quercetin and the PAI-1 protein was −7.13 kcal/mol, indicating a strong binding effect (less than −5 kcal/mol). The complex formed by the docked compound and protein was visualized by PyMOL 2.1 software to obtain the binding mode. According to the binding mode (Figure 5(a)), the amino acid residues bound between the compound and protein pocket can be clearly seen. The active pocket of the PAI-1 protein is mainly composed of TYR-79, LEU-75, SER-41, ARGG-76, TYR-37, ASP-95, HIS-143, THR-94, LYS-122, TRP-139, and SER-119 amino acids. Quercetin directly interacts with PAI-1. The amino acid residues involved in the interaction include TYR-79, ASP-95, SER-41, and PHE-117. Quercetin is a pentahydroxyflavone with five hydroxy groups placed at the 3-, 3′-, 4′-, 5-, and 7-positions that can form strong hydrogen bonds and hydrophobic interactions with the pocket amino acids of proteins. For example, quercetin can form hydrogen bonds with SER-41 and PHE-117 amino acids. The hydrogen bond distances are 2.0 Å and 2.3 Å, respectively, which are much smaller than the 3.5 Å distance of the traditional hydrogen bond. Strong binding plays an important role in anchoring small molecules in the protein pocket. In addition, the benzene ring of quercetin also interacts with amino acids TYR-79 and ASP-95 in the active pocket of the protein, forming a strong π-π conjugate interaction, which plays an important role in stabilizing small molecules. In conclusion, these interactions can improve the stability of quercetin in the PAI-1 protein pocket and form a stable complex with the target protein. Thus, the compound is a potential active small molecule targeting PAI-1.

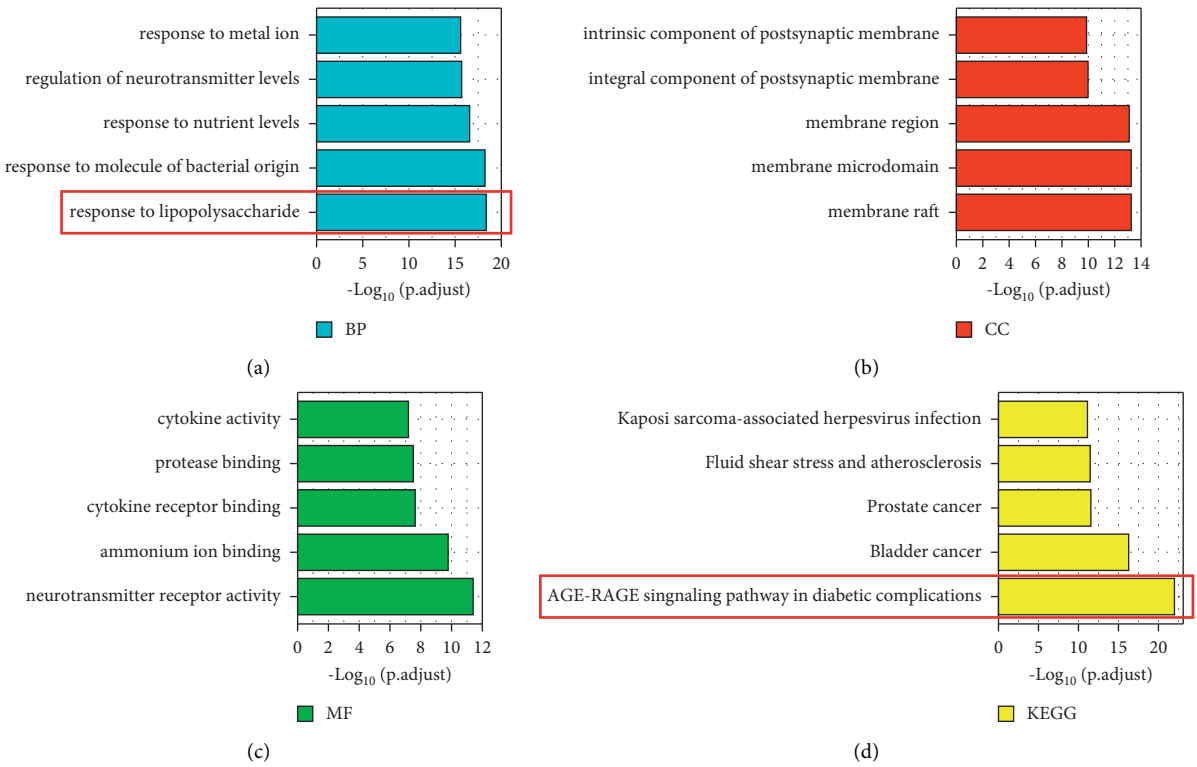


Figure 3: The 5 most representative items identified by GO and KEGG enrichment. (a) Blue columns represent the BP category of GO. (b) Red columns represent the CC category of GO. (c) Green columns represent the MF category of GO. (d) Yellow columns represent the KEGG pathway enrichment. The columns that are significant and selected for further research were framed.

3.8. Molecular Dynamic Analyses. To further study the interaction between the small molecule quercetin and the target protein PAI-1, we used molecular dynamics to simulate the protein-small molecule complex for 100 ns (Figure 5(b)). The stability of RMSD reactive protein and small molecules was evaluated. The greater the RMSD was, the more unstable the complex was. The average RMSD of PAI-1 and the quercetin complex was <2.0 Å, which reaches equilibrium in approximately 10 ns. This result reflects the good combination and stability of the protein and small molecule complex. In addition, the RMSD of small ligand molecules fluctuates slightly near the first 10 ns, which may result from the matching of appropriate conformations after continuous collision with active sites in the protein pocket. Protein-ligand interactions can be monitored throughout the simulation. These interactions can be categorized by type and summarized, as shown in the plot above. Protein-ligand interactions are categorized into four types: hydrogen bonds, hydrophobic, ionic bonds, and water bridges. Figure 5(c) shows that the small molecule quercetin exhibits good interactions with multiple amino acids in the protein pocket. For example, quercetin has a strong hydrogen bond interaction with THR-93 and ASP-95, and quercetin has an 80% chance of forming a stable hydrogen bond with these two amino acids in the whole molecular dynamics simulation process, indicating that these two hydrogen bonds play an important role in anchoring small molecules in the protein pocket. In addition, quercetin exhibits strong hydrophobic interactions with ARG-76 and TYR-79, especially TYR-79, which plays an important role in stabilizing small molecules.

4. Discussion

Currently, the growing number of MDD-induced suicides has become a serious social issue. Although the pathogenesis of MDD is not clearly understood, the last decade has witnessed a step forwards in the diagnosis and treatment of MDD [39]. Accumulating evidence indicates that MDD results from multisystem disorders, including disorders of the nervous system, immune system, and endocrine system [40]. These systems form a complex network through neurotransmitters, endocrine hormones, and cytokines to mediate MDD initiation and development [41]. As a consequence, anti-inflammation and metabolic regulation are promising strategies for MDD treatment. LPS, a bacterial metabolite, is one of the main causes of systemic low-grade inflammation. Exposure to LPS gives rise to a series of mental disorders, such as MDD, cognitive impairment, and social withdrawal [34]. Therefore, LPS is often used to establish animal models of inflammation or MDD. When reaching the blood circulation following intestinal permeability change, LPS causes liver and brain inflammation via a cytokine cascade, which subsequently leads to liver changes, obesity, and metabolic syndrome to mediate MDD [11]. Since the liver is responsible for the clearance of LPS in blood, the enhanced response of the liver to LPS may contribute to the attenuation of inflammation and MDD [42]. Moreover, correlations between LPS and diabetes have been demonstrated. Alison et al. found that type 2 diabetic patients had higher circulating LPS levels (125.4% ↑) than

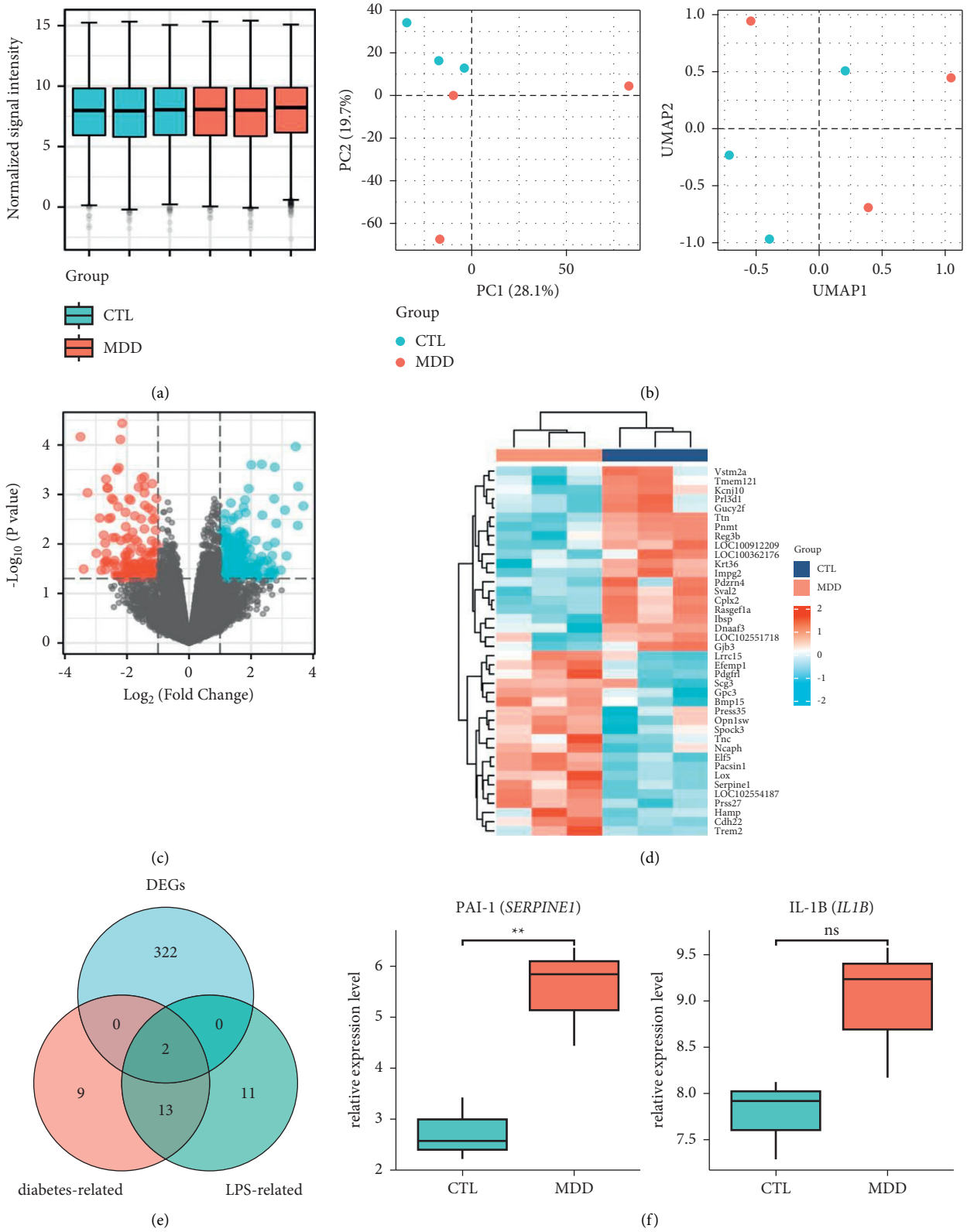


Figure 4: Identification of potential targets of CR in combating MDD through modulating the liver response to LPS and glucose metabolism. (a) Normalization of 3 control samples and 3 MDD samples. (b) Principal component analysis (PCA) and uniform manifold approximation and projection (UMAP) before DEG analysis. PCA and UMAP were applied to 3 MDD liver tissues (red) and 3 CTL liver tissues (blue) characterized by the gene expression of all probes on Affymetrix Rat 230 2.0 Array. (c) Volcano diagrams of MDD-related DEGs. Red dots represent upregulated DEGs, and green dots represent downregulated DEGs. (d) Heatmaps of MDD-related DEGs. The colour shift from blue to red indicates a trend from low to high expression, respectively. (e) Venn diagram of DEGs, LPS-related genes, and diabetes-related genes. (f) Relative expression levels of PAI-1 and IL-1B in the MDD group compared with the control group. $**P < 0.01$ vs. control.

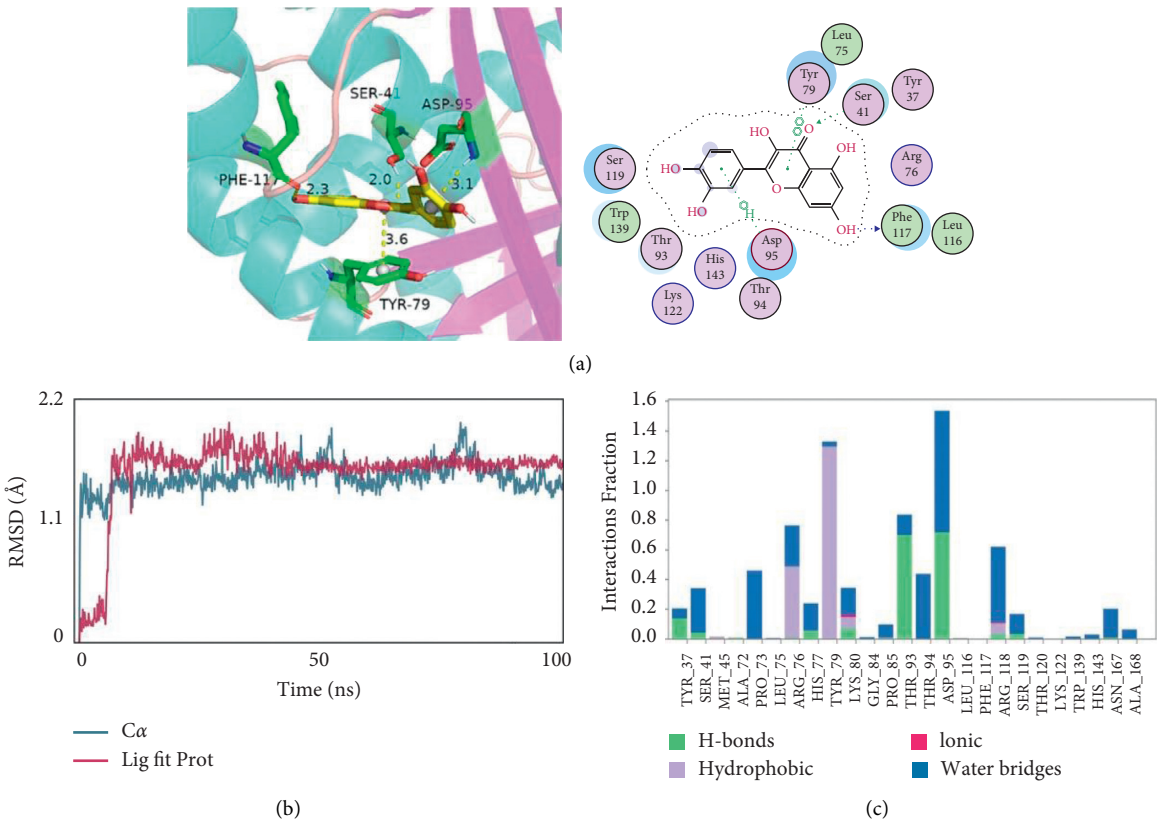


Figure 5: The interactions between quercetin and PAI-1. (a) The binding mode of quercetin with PAI-1. The yellow structure is quercetin, and the green structure represents the binding site of PAI-1. (b) RMSD plot during molecular dynamics simulations of PAI-1 with quercetin. (c) The residues of PAI-1 that interact with quercetin.

healthy people [43], indicating that diabetes is also a risk factor for inflammation-induced MDD. In fact, patients with diabetes are more susceptible to developing depression than the general population [44]. The pathophysiological mechanisms of diabetes-induced MDD include hyperglycaemia, excess glucocorticoids, inflammation, and insulin resistance [45]. In our present study, the potential antidepressant targets of CR were enriched and identified through GO and KEGG analyses. We found that the regulation of LPS and glucose metabolism may be involved in the antidepressant action of CR. Moreover, AGE-RAGE signalling has been a well-studied cascade involved in various diseases, especially diabetes [46]. Abnormal glucose metabolism increases the activation of NAPDH oxidase and the production of reactive oxygen species (ROS) [47]. AGEs exert deleterious effects in diabetes via interaction with RAGE, thus inducing ROS formation [48]. The combination of AGEs and RAGE elicits oxidative stress, resulting in inflammatory and fibrotic reactions. Therefore, oxidative stress mediated by AGE-RAGE signalling has been recognized as a promising therapeutic target for inflammation-related disorders [49]. Since systemic low-grade inflammation has become the key risk factor for depression, combating AGE-RAGE signalling may represent a potential therapeutic strategy for depression treatment. As a consequence, CR may exert its antidepressant effect by regulating AGE-RAGE signalling in glucose metabolism.

Cyperi Rhizoma, the rhizome of *Cyperus rotundus* L., has been extensively used as medicine and food in Asian countries for centuries [15]. With therapeutic effects on menstrual or emotional disturbances in women, CR has been prescribed in various TCM formulae, such as Xiang-Su-San, Xiang-Fu-Si-Wu decoction, and Chaihu-Shu-Gan-San, to treat neurological disorders [50, 51]. TCM theory states that CR relieves depression and anxiety by dispersing stagnated liver qi to soothe the liver. By studying the mechanism of the anti-MDD effect of CR, we demonstrated that 106 targets corresponding to 15 compounds were involved. The complex connections between CR and MDD indicated that multiple possible mechanisms participate in this process, necessitating further research. This finding also demonstrated that TCM has multiple ingredients, multiple targets, and synergetic effects. The practice of active compound screening from TCM is an important strategy for drug discovery [52]. Natural products exert their pharmacological activity through various novel mechanisms [53]. The exploration of their targets may help us better understand the pathogenesis of multiple diseases. In this research, we attempted to elucidate the active compounds and their molecular targets of CR against MDD. Based on the association between liver function and the metabolism of LPS and glucose, we hypothesized that the anti-MDD effect of CR may be mediated by an altered response of the liver to LPS and glucose metabolism. To verify this hypothesis, we selected DEGs between normal and MDD liver tissue in rats with

spontaneous diabetes and found that PAI-1 may represent a potential target of the active compound quercetin in CR. This research provided new insights into MDD treatment from the perspective of the liver. Correlations between MDD and liver disease have been reported. The rate of depression in chronic liver disease is higher than that of the general population [54]. Every third patient with liver cirrhosis or hepatitis shows depressive symptoms [11]. A crucial link between MDD and liver disorder seems to be inflammatory processes. PAI-1 is an inflammatory marker and is mainly synthesized in the liver [28]. Therefore, targeting PAI-1 to combat inflammation may be a useful strategy in the treatment of MDD. Molecular docking and molecular dynamics analyses also confirmed the strong binding of quercetin to PAI-1, indicating that quercetin may represent a promising agent for MDD treatment. However, whether PAI-1 is the precise target of quercetin and the downstream signalling of PAI-1 requires further research.

Some studies demonstrated that elevated PAI-1 levels were found in hippocampal tissues and blood in depressed mice and patients [21, 55], indicating that high PAI-1 levels contribute to the pathogenesis of depression. In contrast, Party et al. found that PAI-1 deficiency induces a depressive-like phenotype, which is associated with reduced serotonin and dopamine levels [56]. This result revealed that the lack of PAI-1 is a factor of predisposition to MDD. According to these facts, we can see that abnormal PAI-1 expression is closely related to depression. The strategy of targeting PAI-1 to combat MDD involves restoring normal PAI-1 expression levels. Excessive inhibition or activation of PAI-1 may both result in adverse outcomes. Given that quercetin is the active compound in Cyperi Rhizoma that combats depression through targeting PAI-1, we predict that quercetin treatment alone may cause a more potent inhibitory effect on PAI-1 than CR treatment. However, safety concerns exist, given that the precise target of quercetin is unknown, and excessive inhibition of PAI-1 may cause side effects. Therefore, quercetin treatment may be more potent but not necessarily more effective than CR treatment. Moreover, there may be other mechanisms for the antidepressant effect corresponding to other active compounds of CR.

In summary, this research may provide evidence for the antidepressant effects of CR through soothing the liver. This study serves as an example for explaining TCM theory through modern pharmacological methods. To emphasize its clinical value and expand its clinical application, further exploration is needed.

5. Conclusion

In conclusion, we are the first to report that quercetin is the active compound of CR that acts against MDD by targeting PAI-1 to enhance the liver response to LPS and glucose metabolism, which may shed light on the modern pharmacological mechanism of CR against MDD through soothing the liver. We also demonstrated that PAI-1 is a promising target and that quercetin is a promising agent for MDD treatment, but these findings require further in-depth study.

Data Availability

The data supporting the conclusions of this article are included within the article.

Conflicts of Interest

The authors declare there are no conflicts of interest.

Authors' Contributions

Yuhe Lei, Mingquan Du, and Ge Zhang contributed equally to this work.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (no. 82074382) and Natural Science Foundation of Guangdong Province (no. 2021A1515011232).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2021, Article ID 2809004, 15 pages
https://doi.org/10.1155/2021/2809004

Research Article

Exploring the Potential Mechanism of Chuanxiong Rhizoma Treatment for Migraine Based on Systems Pharmacology

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Received 12 July 2021; Revised 29 November 2021; Accepted 9 December 2021; Published 28 December 2021

Academic Editor: Junqing Huang

Migraine is a disease whose aetiology and mechanism are not yet clear. Chuanxiong Rhizoma (CR) is employed in traditional Chinese medicine (TCM) to treat various disorders. CR is effective for migraine, but its active compounds, drug targets, and exact molecular mechanism remain unclear. In this study, we used the method of systems pharmacology to address the above issues. We first established the drug-compound-target-disease (D-C-T-D) network and protein-protein interaction (PPI) network related to the treatment of migraine with CR and then established gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. The results suggest that the treatment process may be related to the regulation of inflammation and neural activity. The docking results also revealed that PTGS2 and TRPV1 could directly bind to the active compounds that could regulate them. In addition, we found that CR affected 11 targets that were more highly expressed in the liver or heart but were the lowest in the whole brain. It also expounds the description of CR channel tropism in TCM theory from these angles. These findings not only indicate that CR can be developed as a potential effective drug for the treatment of migraine but also demonstrate the application of systems pharmacology in the discovery of herbal-based disease therapies.

1. Introduction

Currently, the medical community considers migraine to be a chronic neurobiological disorder, which is characterized by a long period of unilateral headache (from 4 hours up to 72 hours), recurrent attacks, and other features such as nausea, or photo or sound phobias [1]. In addition, the prevalence of the disease is much higher in women than in men [2]. From the point of view of modern medicine, the pathophysiology of migraine has not been fully elucidated, and its pathogenesis can be divided into vascular theory, neuron theory, inflammatory mediator theory and "microbiota-gut-brain axis" theory [3–6].

Traditional Chinese medicine has been used to treat migraine for more than 2,000 years. Among historical figures, Cao Cao, who was an emperor in the Three Kingdoms period, suffered from migraine symptoms recognized by later doctors. Migraine belongs to the traditional Chinese medicine category "head wind" "head wind," and differentiated by "external feeling" and "internal injury," commonly two big categories. Treatment is frequently with dispelling wind, liver, phlegm, or line stasis [7]. Compared with Western medicine, Chinese medicine has many different antimigraine compounds and targets [8]. Therefore, Chinese medicine has accumulated rich experience in the treatment of migraine [9–11]. Chuanxiong Rhizoma (CR), which is derived from the root of *Ligusticum chuanxiong* Hort, is a traditional Chinese herbal medicine. It belongs to *Ligusticum L.* in the Umbelliferous family and is widely found in Sichuan, Yunnan, Guizhou, Guangxi, and other

provinces in China. CR has been recorded in "Shen Nong's Herbal Classic" and "Compendium of Materia Medica" and is described as "pungent and warm." In the Chinese Pharmacopoeia, CR can be used to promote the flow of qi and blood circulation, wind-expelling, and pain alleviation. It is often used to treat migraine, rheumatism, and irregular menstruation.

Although CR can effectively treat migraine, its active compounds, drug targets and exact molecular mechanisms remain unclear. It is gratifying that in recent years, systems pharmacology has been used to study the therapeutic effects and therapeutic targets of active compounds contained in traditional Chinese medicine and biology. Its concept of "network targets, multicompounds" is the most suitable tool for exploring the therapeutic effects of herbal medicine at the molecular level [12, 13]. This novel research model can be used to explain and promote the development of evidence-based medicine and new drug discovery based on herbs. Using a network-based approach, systems pharmacology can systematically determine the actions and mechanisms of drugs used to treat complex diseases at the molecular, cellular, tissue, and biological levels. This strategy has been widely used in the study of *Atractylodes macrocephala* Koidz., Radix *Puerariae*, *Zanthoxylum bungeanum* Maxim., and *Citri Reticulatae* Pericarpium [14–17].

In this study, we used systems pharmacology to explore whether CR has a therapeutic effect on migraine and to elucidate its potential mechanism of action. The flowchart of this study is shown in Figure 1.

2. Materials and Methods

2.1. Plant Materials and Sample Preparation. Pure distilled water was purchased from Watsons (Hong Kong, China). Formic acid was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). HPLC grade acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). The raw material of CR was purchased from herbal medicine markets located in Zhangshu City, Jiangxi Province. These samples were identified by professor Qianfeng Gong, Jiangxi University of Traditional Chinese Medicine. The voucher specimens were deposited at the herbarium of the Jiangxi University of Traditional Chinese Medicine.

Accurately weighed powder (1.0 g) was placed into a 50 mL flask, and each sample was extracted with 30 mL of ethanol in an ultrasonic water bath at room temperature for 1 h. The extraction solutions of the sample were centrifuged for 15 min at 12000 rpm. Finally, 2 μL of the CR filtered supernatants were injected for UHPLC-QTOF-MS/MS analyses.

For UHPLC-TOF-MS/MS analysis, the UHPLC analyses were conducted on a Shimadzu system (Kyoto, Japan), combined with a LC-3AD solvent delivery system, a SIL-30ACXR auto-sampler, a CTO-30AC column oven, a DGU-20A3 degasser, and a CBM-20A controller. The analytical column was a Welch UHPLC C18 (100 mm × 2.1 mm, 1.8 μm). The column oven temperature was set at 40°C. The mobile phases consisted of water containing 0.1% formic acid (solvent A) and acetonitrile (solvent B). The flow rate was set at 0.3 mL/min. The binary gradient was applied with linear interpolation as follows: 0.01 min, 2% B; 3 min, 8% B; 11 min, 16% B; 19 min, 18% B; 26 min, 30% B; 32 min; 60% B; 42 min, 95% B; 42.1 min, 2% B; 45 min, 5% B.

The QTOF-MS/MS detection was operated on a Triple TOF^{TM} 5600+ system (AB SCIEX, Foster City, CA, USA). The electrospray ionization was applied in both the negative and positive mode with the following parameters: ion spray voltage, −4500 V/5500V; ion source temperature, 550°C; curtain gas, 35 psi; nebulizer gas (GS 1), 55 psi; heater gas (GS 2), 55 psi; and declustering potential (DP), 100 V. The mass ranges were set at m/z 50–1250 Da for the TOF-MS scan and 50–1250 Da for the TOF-MS/MS experiments. In the IDA-MS/MS experiment, the collision energy (CE) was set at 35 eV, and the collision energy spread (CES) was (±) 15 eV for the QTOF-MS/MS detection. The MS/MS data was analyzed by PeakView® 1.2 software (AB SCIEX, Foster City, CA, USA).

2.2. Collection of the Candidate Compounds of CR. We searched six databases, including the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; http://lsp.nwu.edu.cn/tcmsp.php), the Chinese Academy of Sciences Chemistry Database (http://www.organchem.csdb.cn), the TCM Database@Taiwan (http://tcm.cmu.edu.tw), the Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine (TCMIP; http://www.tcmip.cn), Symptom Mapping (SYMMAP, http://www.symmap.org), and the Traditional Chinese Medicine Integrated Database (TCMID; http://www.megabionet.org/tcmid) to collect the candidate compounds of CR as comprehensively as possible. In addition, we also reviewed the relevant literature to gather a more comprehensive collection of CR compounds. Finally, we summarized the compounds and established a database of CR for this study.

2.3. Screening of Active Compounds. Since the compounds of traditional Chinese medicine are very complex and in order to better select compounds with high potential to become drugs for subsequent targeted research, we screened the compounds in the database that we had established previously. In this study, two parameters, "oral bioavailability" (OB) and "drug likeness" (DL), were used to perform the screening process.

OB is defined as the percentage of a drug capable of invading a primitive culture and that is not modified enough to enter the human circulatory system [18, 19]. OB is usually regarded as an objective and important index to evaluate the internal quality of drugs [20]. The OB of compounds is proportional to their likelihood for clinical use.

DL refers to the similarity between compounds and known drugs [21, 22]. Compounds with DL properties may not necessarily already be drugs but have the potential to be drugs. Such compounds usually include drug-like small molecules or drug-like compounds. Here, we use the

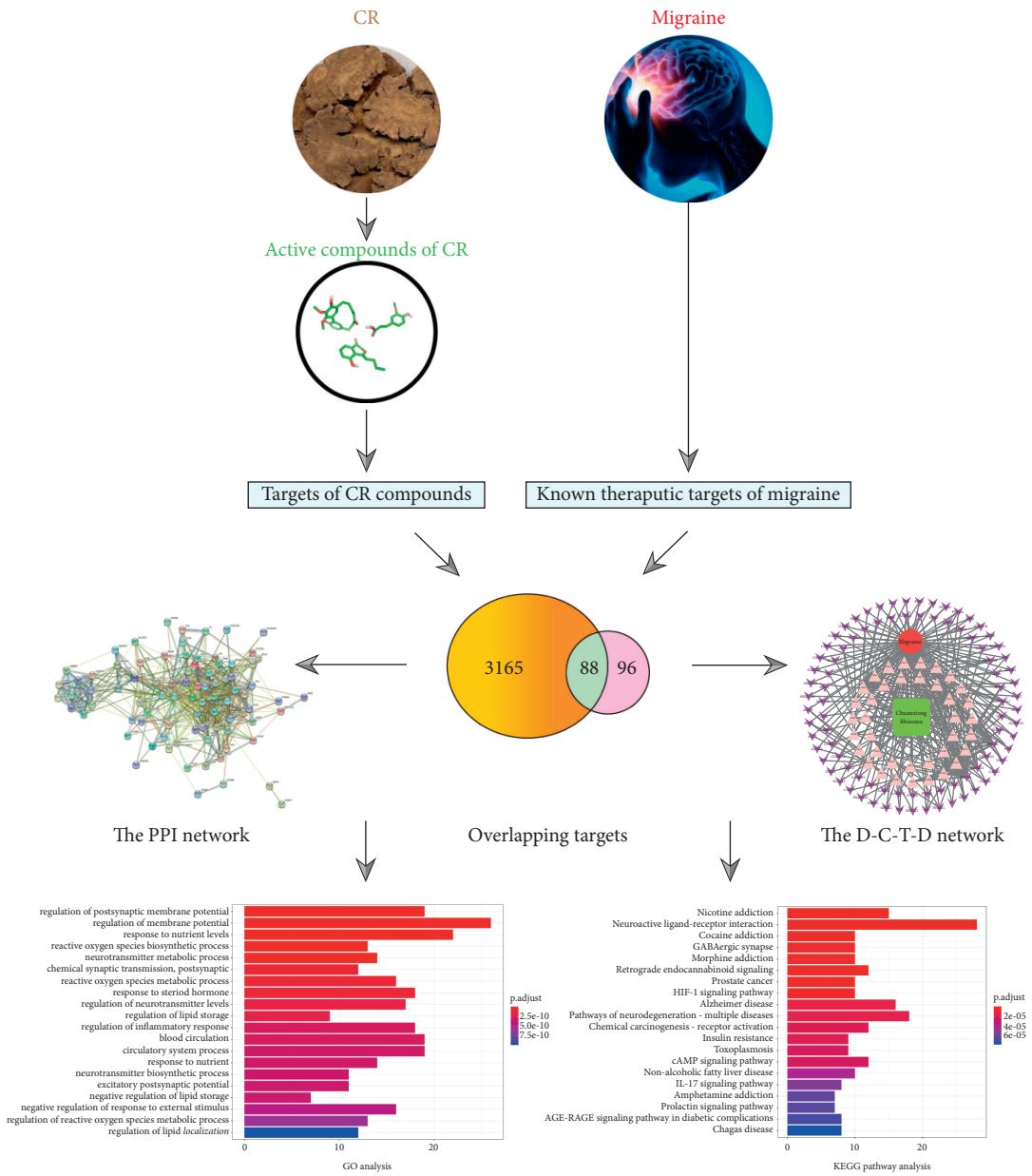

Figure 1: Flowchart of a systems pharmacology-based strategy to investigate the pharmacologic mechanisms of *Chuanxiong* Rhizoma for the treatment of migraine.

classical Tanimoto coefficient to calculate the DL index of the compounds contained in CR; the formula is as follows:

$$T(\alpha,\beta) = \frac{\alpha \times \beta}{\alpha^2 + \beta^2 - \alpha \times \beta}. \quad (1)$$

α represents the molecular properties of CR compounds based on computing from Dragon software (http://www.talete.mi.it/products/dragon_description.htm), and β for all of the drugs comes from the average molecular properties in the DrugBank database (http://www.drugbank.ca) [23].

Most of the compounds in traditional Chinese medicine have weak pharmacological properties, so they are difficult to combine with specific targets on cells significantly. Therefore, molecules with OB ≥ 15% or DL ≥ 0.10 are generally considered to have stronger pharmacological effects in this kind of study, and, therefore, researchers select these as the active compounds for focused analysis [24–26]. Therefore, in this study, we applied the same principle to further screen the candidate compounds in order to ultimately obtain the active compounds.

2.4. Prediction of the Relevant Targets of CR Active Compounds. Traditional Chinese medicine is characterized by multicompounds and multitarget modes of action. Therefore, it is particularly important to predict targets that can be affected by active compounds. Based on the experience accumulated in our previous studies, we ultimately chose the ligand-based screening method for the prediction of this part [14].

2.5. Acquisition of Targets for Migraine. The migraine-related targets were from the Therapeutic Target Database (TTD), the Human Phenotype Ontology (HPO), the Integrative Pharmacology-Based Research Platform of Traditional Chinese Medicine (TCMIP), Online Mendelian Inheritance in Man (OMIM), and GeneCards (the Human Gene Database (http://www.genecards.org/) comprehensive collection in five databases).

2.6. Drug-Compound-Target-Disease (D-C-T-D) Network Construction. The intersection of the predicted drug-related and disease-related targets was chosen to obtain the Venn diagram of the overlapping targets. Next, complex information networks based on the interactions of drugs (CR), active compounds, overlapping targets, and disease (migraine) were constructed. Finally, Cytoscape 3.7.1 software was used to visualize and analyze the drug-compound-target-disease (D-C-T-D) network.

2.7. Protein-Protein Interaction (PPI) Network Construction. The STRING online database (https://string-db.org/) was used to obtain PPI data of the previous overlapping targets in the network. The object was selected as "*Homo sapiens*," and the others were kept as defaults. Finally, the PPI relationship network was established by Cytoscape 3.7.1 software, and topology analysis was carried out. In addition, the BIOGPS database (https://biogps.org) was used for analysis to identify the high expression of the targets in some major organs.

2.8. Enrichment of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes Pathways (KEGG). GO analysis and KEGG pathway enrichment were performed using Bioconductor (R) V3.8 bioinformatics software (http://bioconductor.org/). GO items ($p.adjust \leq 0.05$) were collected for functional annotation clustering. The KEGG database was used for pathway enrichment analysis to verify statistically significant gene function categories ($p.adjust \leq 0.05$).

2.9. Computational Validation of Compound-Target Interactions. We hope to determine the interaction between the active compounds and their targets and explore their binding patterns. Therefore, three active compounds and two targets were selected, and a total of four compound-target interactions were used to verify molecular docking. Docking studies were conducted using AutoDock Vina [27], and input files required by AutoDock programs were prepared using AutoDockTools [28]. The size of the grid box in AutoDock Vina remained $40 \times 40 \times 40$ for X, Y, and Z, and the energy range remained the default setting. The X-ray crystal structures of PTGS2 and TRPV1 were obtained from the RCSB protein database (PDB) (http://www.rcsb.org). The PDB entry codes for these proteins were 5F19 and 6L93, respectively. The program makes calculations based on the different binding energies of each ligand and yields nine possible conformations. We then selected the best model based on binding affinity and molecular contact. The calculation of molecular contact was carried out by the program CONTACT provided in the CCP4 package [29]. The docking complex was analyzed and plotted using PyMol (http://www.pymol.org).

3. Results

3.1. Chemical Structure Identification of CR. An effective and systematic UHPLC-QTOF-MS/MS method was established to screen and identify the constituents of CR. As a result, a total of 33 compounds were efficiently found and identified from an extract of CR. A representative total ion chromatographic (TIC) is shown in Figure 2. The identified 33 compounds are exhibited in Table 1.

3.2. Collection the Candidate Compounds of CR. By searching six databases combined with the literature search, we ultimately established a database of CR compounds for the present study (Supplementary Table S1). A total of 248 candidate compounds were included.

3.3. Screening of Active Compounds. In order to screen for active compounds with high potential for CR, we used two classical absorption, distribution, metabolism, and excretion (ADME) parameters, OB and DL, to screen our subdatabase. At the same time, we noted that although some compounds did not conform to the above rules, they may also have therapeutic effects on the human body. Therefore, for this reason but also to be able to study this issue more fully, we nevertheless treated them as active compounds, even though they did not conform to the screening rules. For example, although ferulic acid does not conform to the above rules, we attach great importance to it because it is the standard compound for CR in the Chinese pharmacopoeia and has strong biological activity [30]. Studies have shown that it can regulate various inflammatory responses by inhibiting the production of interleukin 8 (IL-8), thus producing anti-inflammatory effects [31]. It shows strong antioxidant activity by scavenging free radicals [32, 33]. In addition, it inhibits vascular smooth muscle cell proliferation induced by angiotensin II [34]. In summary, through this part of the work, we ultimately screened and obtained 38 active compounds of CR that we considered, as shown in Table 2.

3.4. Prediction of the Relevant Targets of CR Active Compounds. The relevant target information of CR active compounds was collected from the above six databases. After the UniProt database was converted into standard names and redundant items were deleted, 38 active compounds and 184 targets relevant for CR were obtained (Supplementary Table S2).

3.5. Acquisition of Targets for Migraine. We collected targets related to migraine from the above five disease databases. After removing the redundancy, a total of 3253 known

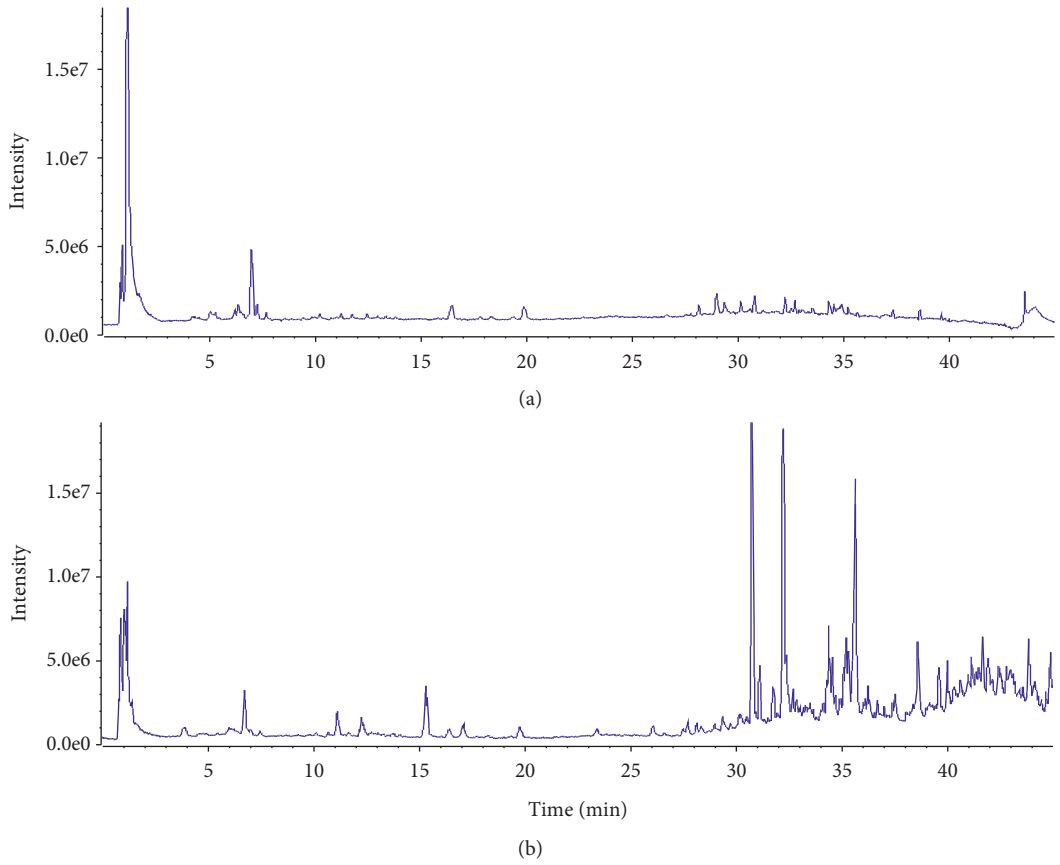

Figure 2: The total ion chromatograms (TICs) from the analysis of a crude extract of CR (a) in negative mode and (b) in positive mode.

Table 1: MS data of (±) ESI- QTOF-MS/MS spectra and the identification of the Chuanxiong Rhizoma extract.

No.	Formula	Precursor ion ()	Error (ppm)	tR (min)	MS/MS (abundance ratio)	Identity
1(−)	$C_7H_{12}O_6$	191.0562 ()	0.2	1.15	173.0440, 127.0403, 93.0352, 85.0306	Quinic acid
2(−)	$C_7H_6O_3$	137.0252	5.8	5.132	108.0230, 108.0230	4-Hydroxybenzoic acid
3(−)	$C_{13}H_{16}O_9$	315.0719	−0.8	4.19	153.0109,152.0107, 109.0297, 108.0223	Protocatechuic acid-3 -glucoside
4(−)	$C_{16}H_{18}O_9$	353.0874	−1.1	5.05	191.0549, 179.0337, 173.0457, 161.0238, 135.0445	Neochlorogenic acid
5(−)	$C_{16}H_{18}O_9$	353.0874	−1.1	6.99	191.0554, 179.0344, 173.0449, 161.0241, 135.0453	Chlorogenic acid
6(−)	$C_{16}H_{18}O_9$	353.0873	−1.5	7.27	191.0555, 179.0342, 173.0451, 161.0243, 135.0450	Cryptochlorogenic acid
7(−)	$C_{22}H_{28}O_{14}$	515.1404	−0.5	5.28	353.0871, 341.0887, 191.0548, 179.0336, 173.0440, 161.0238, 135.0449	Chlorogenic acid-glucoside
8(−)	$C_{22}H_{28}O_{14}$	515.1402	−0.8	6.37	353.0877, 341.0882, 323.0765, 191.0550, 179.0336, 173.0438, 161.0232, 135.0441	*iso*-Chlorogenic acid-glucoside
9(−)	$C_{15}H_{18}O_9$	341.0875	−1.0	5.51	179.0337, 135.0444	Caffeic acid 3-glucoside
10(−)	$C_{15}H_{18}O_9$	341.0875	−1.0	5.51	179.0336, 135.0447	Caffeic acid 4-glucoside
11(−)	$C_{23}H_{30}O_{14}$	529.1566	0.6	6.19	367.0998, 193.0483, 191.0538, 178.0248, 173.0438, 134.0368	Feruloylquinic acid-glucoside
12(−)	$C_{23}H_{30}O_{14}$	529.1562	−0.2	6.77	355.0989, 337.0346, 193.0493, 178.0262, 173.0450, 134.0365	*iso*-Feruloylquinic acid-glucoside
13(−)	$C_{16}H_{20}O_9$	355.1028	−1.7	6.63	193.0488, 178.0255, 149.0596, 134.0364	Feruloyl-glucose
14(−)	$C_8H_8O_4$	167.0352	1.4	7.01	123.0447, 79.0561	1-*p*-CoQA*
15(−)	$C_{17}H_{20}O_9$	367.1036	0.5	7.27	193.0491, 191.0542, 173.0472, 149.0239, 134.0370	3-O-Feruloylquinic acid
16(−)	$C_{17}H_{20}O_9$	367.1029	−1.4	10.22	193.0492, 191.0545, 173.0440, 134.0361	5-O-Feruloylquinic acid
17(−)	$C_9H_8O_4$	179.0351	0.7	7.24	135.0444, 134.0365	Caffeic acid
18(−)	$C_{10}H_{10}O_4$	193.0508	0.6	11.24	353.0884(14.9), 335.0774(1.8), 191.0563(100), 178.267, 149.0605, 134.0371	Ferulic acid

Table 1: Continued.

No.	Formula	Precursor ion ()	Error (ppm)	tR (min)	MS/MS (abundance ratio)	Identity
19(−)	$C_{25}H_{24}O_{12}$	515.1190	−0.9	16.46	353.0863, 335.0762, 191.0548, 179.0334, 173.0436, 161.0231, 135.0443	3,5-Dicaffeoylquinic acid
20(−)	$C_{25}H_{24}O_{12}$	515.1190	−0.9	19.34	353.0862, 335.0756, 191.0538, 179.0334, 173.0431, 161.0246, 135.0441	4,5-Dicaffeoylquinic acid
21(−)	$C_{25}H_{36}O_{12}$	549.2184	1.2	19.87	503.2132, 311.0974, 293.0872, 251.0751, 221.0654, 191.0551, 149.0448	*trans*-4,5-di-CQA
22(−)	$C_{12}H_{14}O_3$	205.0870	−0.3	28.99	161.0964, 131.0496, 106.0429	Senkyunolide H
23(−)	$C_{12}H_{12}O_3$	203.0713	−0.1	30.15	173.0232, 160.158, 145.0287, 132.0312	Senkyunolide B
24(−)	$C_{12}H_{16}O_3$	207.1026	−0.3	27.74	163.1117, 161.0965	Senkyunolide K
25(+)	$C_{12}H_{16}O_2$	193.1221	−0.9	30.76	147.1163, 105.693, 91.0540, 77.0387	Senkyunolide A
26(+)	$C_{12}H_{14}O_2$	191.1065	−0.9	32.24	173.0956, 128.0618, 115.0537, 91.0541, 77.0388	3-N-Butylphthalide
27(+)	$C_{24}H_{28}O_4$	381.2054	−1.6	35.64	191.1064, 173.0961, 149.0597, 135.0440	Levistilide A
28(+)	$C_{12}H_{12}O_2$	189.0908	−1.1	32.40	171.0803, 152.0619, 128.0620, 115.0546	3-Butylidenephthalide
29(+)	$C_{12}H_{18}O_4$	227.1273	−2.4	12.25	209.1160, 191.1034, 153.0542, 105.0693, 77.0391	Senkyunolide J
30(+)	$C_{12}H_{16}O_4$	225.1119	−1.0	15.32	207.1031, 189.0909, 133.0651, 91.0544	Senkyunolide I
31(+)	$C_{12}H_{14}O_4$	223.0962	−1.3	26.60	205.0865, 149.0233, 121.0281	Senkyunolide D
32(+)	$C_{12}H_{16}O_3$	209.1169	−1.4	12.26	163.1111, 153.0543, 91.0541, 77.0387	Senkyunolide G
33(+)	$C_{12}H_{16}O_4$	207.1014	−0.9	15.31	189.0906, 146.0728, 133.0648, 91.0549, 77.0396	Senkyunolide F

*Positive mode: +; negative mode: −

Table 2: The active compounds of CR.

No.	Name	CAS no.	OB (%)	DL	References	No.	Name	CAS no.	OB (%)	DL	References
CR01	α-Cubebene	17699-14-8	16.73	0.11	[35]	CR20	Aromadendrene oxide	85710-39-0	65.10	0.14	[36]
CR02	Linoleic acid	60-33-3	41.90	0.14	[37]	CR21	(−)-Cedrene	469-61-4	51.14	0.11	[38]
CR03	(−)-Globulol	489-41-8	85.51	0.12	[39]	CR22	(Z)-9-Octadecenoic acid methyl ester	112-62-9	31.90	0.16	[40]
CR04	Mandenol	544-35-4	42.00	0.19	[41]	CR23	Alexandrin	474-58-8	20.63	0.62	[42]
CR05	Methyl linoleate	112-63-0	41.93	0.17	[39]	CR24	Angelicin	83-46-5	36.91	0.75	[43]
CR06	3-Methylchrysazin	481-74-3	18.64	0.21	[42]	CR25	Folacid	59-30-3	68.96	0.71	[43]
CR07	Adenocard	58-61-7	18.82	0.10	[44]	CR26	Oleic acid	112-80-1	33.13	0.14	[45]
CR08	(+)-Aromadendrene	489-39-4	55.74	0.10	[46]	CR27	Cetostearic acid	57-10-3	19.30	0.10	[47]
CR09	Methanoazulene	50894-66-1	52.87	0.10	[48]	CR28	Octadecanoic acid	57-11-4	17.83	0.14	[37]
CR10	α-Selinene	473-13-2	31.81	0.10	[49]	CR29	Methyl hexadecanoate	112-39-0	18.09	0.12	[41]
CR11	β-Cubebene	13744-15-5	32.16	0.11	[50]	CR30	Isoledene	95910-36-4	49.01	0.10	[39]
CR12	Chuanxiongol	87421-30-5	22.19	0.10	[51]	CR31	Ferulic acid	1135-24-6	Not applied	Not applied	[52]
CR13	Myricanone	32492-74-3	40.60	0.51	[53]	CR32	Clionasterol	83-47-6	Not applied	Not applied	[47]
CR14	Perlolidin	29700-20-7	65.95	0.27	[54]	CR33	Choline	62-49-7	Not applied	Not applied	[55]
CR15	Senkyunolide D	94530-82-2	79.13	0.10	[37]	CR34	Chrysophanic acid	491-59-8	Not applied	Not applied	[42]
CR16	1-Acetyl-beta-carboline	50892-83-6	21.14	0.10	[56]	CR35	Biocolina	67-48-1	Not applied	Not applied	[55]
CR17	Espatulenol	6750-60-3	82.33	0.12	[57]	CR36	Xiongterpene	50627-73-1	23.77	0.42	[58]
CR18	Wallichilide	93236-64-7	42.31	0.71	[53]	CR37	Senkyunolide I	94596-28-8	Not applied	Not applied	[59]
CR19	Pedatisectine C	103805-66-9	25.82	0.12	[60]	CR38	Retinol	68-26-8	Not applied	Not applied	[61]

targets for migraine treatment were collected (Supplementary Table S3).

3.6. Analyses of Drug-Compound-Target-Disease (D-C-T-D) Network. Figure 3(a) shows that 3253 targets for migraine and 184 targets for CR had 88 overlaps. That is, the 88 overlapping targets may be the key for migraine treatment by CR. The 88 overlapping targets are detailed in Supplementary Table S4.

Chinese medicine has multicompounds and multitargets. To illustrate this feature, we attempted to use these active compounds and outstanding targets. To this end, we used Cytoscape software to build the drug-compound-target-disease (D-C-T-D) network for visualization, as shown in Figure 3(b). The green square node represents the drug (CR), the red round node represents the disease (migraine), 38 pink triangle nodes represent the active compounds in CR, and 88 purple arrow nodes represent the overlapping targets between CR and migraine, which constitute the drug-compound-target-disease (D-C-T-D) network. The centralization and heterogeneity of the network were 0.666 and 1.901, respectively. This network indicates the potential relationship between compounds and targets, which suggests the potential pharmacological mechanism of CR or compounds in the treatment of migraine. The node with the highest degree of connection with other compounds or targets represents the hub in the whole network or, in other words, potential compounds or targets. Here, we use two parameters to help us judge the importance of these nodes: the degree (for connection to the node number of edges) and middle degree of intermediate (betweenness centrality, BC) [62]. For example, the connection degree of the highest compounds is CR32 (clionasterol, degree = 24). CR26 (oleic acid) and CR36 (Xiongterpene) also had high degrees of 22 and seven, respectively. These results suggest that a single compound can act on multiple targets at the same time, suggesting that the active compounds in CR can achieve the goal of treating migraine through multiple targets. Generally, BC can measure the importance of nodes in the network, which can help us find more important nodes [63]. Therefore, if the degree value of some nodes is not high and the BC value is more prominent, then we think that the node is also more important in the network. Generally, there is a positive correlation between degree and BC. However, everything has two sides. Although the degree value of CR31 (ferulic acid, degree = 2, BC = 0.00135) was lower, its BC value was higher than that of other compounds to the same degree. This suggests that we should pay more attention to ferulic acid, which is the quality control compound of the Chinese pharmacopoeia for CR. It has strong biological activity and can not only resist inflammation but also show strong antioxidant activity [64–66]. It can downregulate IL-1β, IL-6, and TNF-α. Moreover, it can decrease the NLRP3 inflammasome and regulate NF-κB signal transduction, ultimately inhibiting inflammation [67]. For target analysis, PTGS2, PTGS1, and CHRM2 were separately linked to 24, 15, and 10 compounds, respectively. These findings indicate that different compounds can regulate the same target in a cooperative way. These analyses support the view that CR, as a treatment for migraine, has multiple compounds acting on multiple targets. Details of the active compounds of CR and overlapping targets that play key roles are shown in Supplementary Table S5.

3.7. Analyses of Protein-Protein Interaction (PPI) Network. In order to further explore the possible relationship between the overlapping targets, which can help us better analyze the therapeutic mechanism of CR for migraine, we constructed a protein-protein interaction network (PPI network) composed of 88 nodes and 683 edges, as shown in Figures 4(a) and 4(b) (the first 35 targets are intercepted for display). In this PPI network, the degree of the target is proportional to its importance. The results can also provide us with targets worthy of our attention. Details of the PPI network are shown in Supplementary Table S6. As shown in Figure 4(b), we found that targets related to inflammation, such as IL-6, TNF-a, PTGS2, and IL-10, play the more important roles.

To determine the effect of CR on vital organs in the treatment of migraine, we conducted a more in-depth study on the expression of some targets in vital organs, as shown in Figure 4(c). We found that the expression of core targets in various organs of the human body is relatively different. For example, AR was the highest in liver, at 45.6. However, it is much lower in other organs. In the heart, the expression of AR is only 5.40, but it is much higher than the expression in the whole brain, kidney, and lung. The expression of the 11 targets shown in Figure 4(c) in the liver or heart was much higher than that in the other three organs. However, the only exception was PTGS1, which had its highest expression in the lungs. It is, however, worth noting that the expression of this target in the liver and heart was also relatively high. Surprisingly, almost all of the targets in humans were most highly expressed in the liver and in the heart. This also means that CR specifically affects the liver and heart in the treatment of migraine.

3.8. Analyses of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes Pathways (KEGG). To further understand the biological characteristics of 88 key overlapping targets of CR, GO enrichment analysis was performed on the assumed targets to clarify the related biological processes ($P < 0.01$), as shown in Figure 5(a). The results show that the antiobesity effect of ZBM involves several biological processes, including regulation of postsynaptic membrane potential (GO:0060078), regulation of membrane potential (GO:0042391), response to nutrient levels (GO:0031667), and reactive oxygen species biosynthetic process (GO: 1903409). Details of the GO enrichment analysis are shown in Supplementary Table S7.

In addition, to further identify potential pathways involved in CR treatment of migraine, we performed KEGG pathway enrichment analysis on these 88 targets. In the end, a total of 98 enrichment pathways associated with CR treatment for migraine were identified, and 20 pathways with higher confidence are presented in Figure 5(b) ($P < 0.01$). Details of the KEGG pathway enrichment

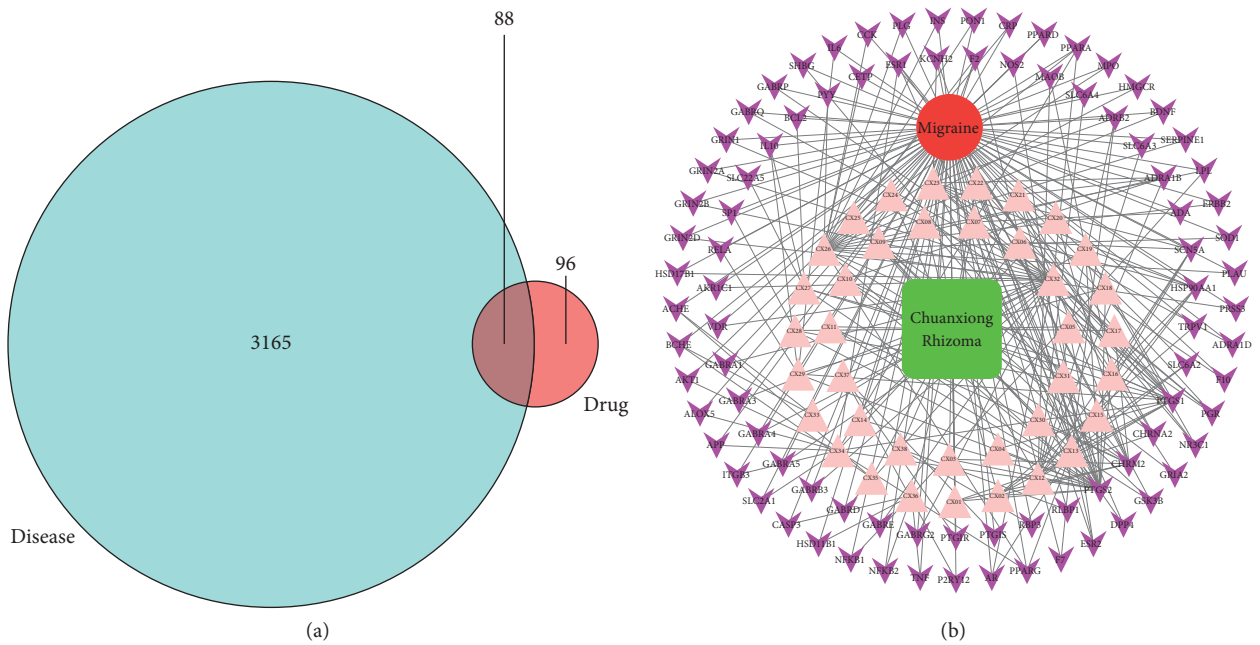

Figure 3: (a) Eighty-eight overlapping targets between the disease and drug. (b) D-C-T-D network. The green square node represents the drug (CR), the red round node represents the disease (migraine), 38 pink triangle nodes represent the active compounds in CR, and 88 purple arrow nodes represent the overlapping targets between CR and migraine.

analysis are shown in Supplementary Table S8. After analyzing the results, we found that the enriched targets were related to a variety of signalling pathways, mainly neuro-related and inflammation-related pathways. This is particularly manifested in neuroactive ligand-receptor interactions (hsa04080) and the IL-17 signalling pathway (hsa04657). These pathways may be the key pathways responsible for CR in the treatment of migraine. This analysis may provide a new way to explore the mechanism of CG in the treatment of migraine.

3.9. Computational Validation of Compounds-Targets Interactions. It is well known that the binding strength of ligands to receptors is determined by the number of covalent bonds between them and their binding affinity [68]. In order to explore the possible binding mechanism between the active compounds and the predicted targets, we used molecular docking technology. Here, we explored the potential binding modes of the active compounds to PTGS2 and TRPV1, as shown in Figure 6. To verify the robustness of our model, we used the classic PTGS2 inhibitor aspirin and the TRPV1 inhibitor capsazepine as positive controls (as shown in Figures 6(a) and 6(d)). Our results showed that chuanxiongol (Figure 6(b)), myricanone (Figure 6(c)), and the target inhibitor aspirin bind to the same site as PTGS2. Similarly, ferulic acid (Figure 6(e)) and the target corresponding inhibitor capsazepine were combined with TRPV1 in the same pocket. Based on the above results, we believe that the strong interaction between these active compounds and their targets (PTGS2 and TRPV1) is the basis for their effective biological activities. Therefore, from the perspective of computer simulation, these results demonstrate the potential ability of active compounds to treat migraine by affecting their related targets and further verify our prediction results in the D-C-T-D network.

4. Discussion

Traditional Chinese medicine always has the characteristics of multicompounds and multitargets when it plays a therapeutic role. It is a great challenge to evaluate the therapeutic efficacy of traditional Chinese medicine given many active compounds. In recent years, systems pharmacology has been an ideal pharmacological research tool for the treatment of diseases with traditional Chinese medicine. We used a knowledge-based and a computing-based strategy to build the network and perform more in-depth research. Systems pharmacology is helpful for discovering the relationship among traditional Chinese medicine, diseases, and molecular targets on the basis of networks and to understand the molecular mechanisms behind therapeutic effects as deeply as possible. In the present study, we first identified the active compounds in CR and their related targets, then obtained the known targets for migraine treatment, and lastly established the D-C-T-D network.

The D-T-C-D network highlighted a total of 88 targets, which may be the key targets for CR in the treatment of migraine. Among these 88 targets, many are related to inflammation, such as IL-6, IL-10, and TNF-α [69–71]. Similarly, TRPV1 is closely related to neural activity [72]. In fact, the KEGG pathway enrichment analyses of the 88 targets also highlight the importance of the inflammation module, suggesting that CR may treat migraine through an anti-inflammatory pathway. This conclusion is also supported by the existing literature [73]. In fact, some studies

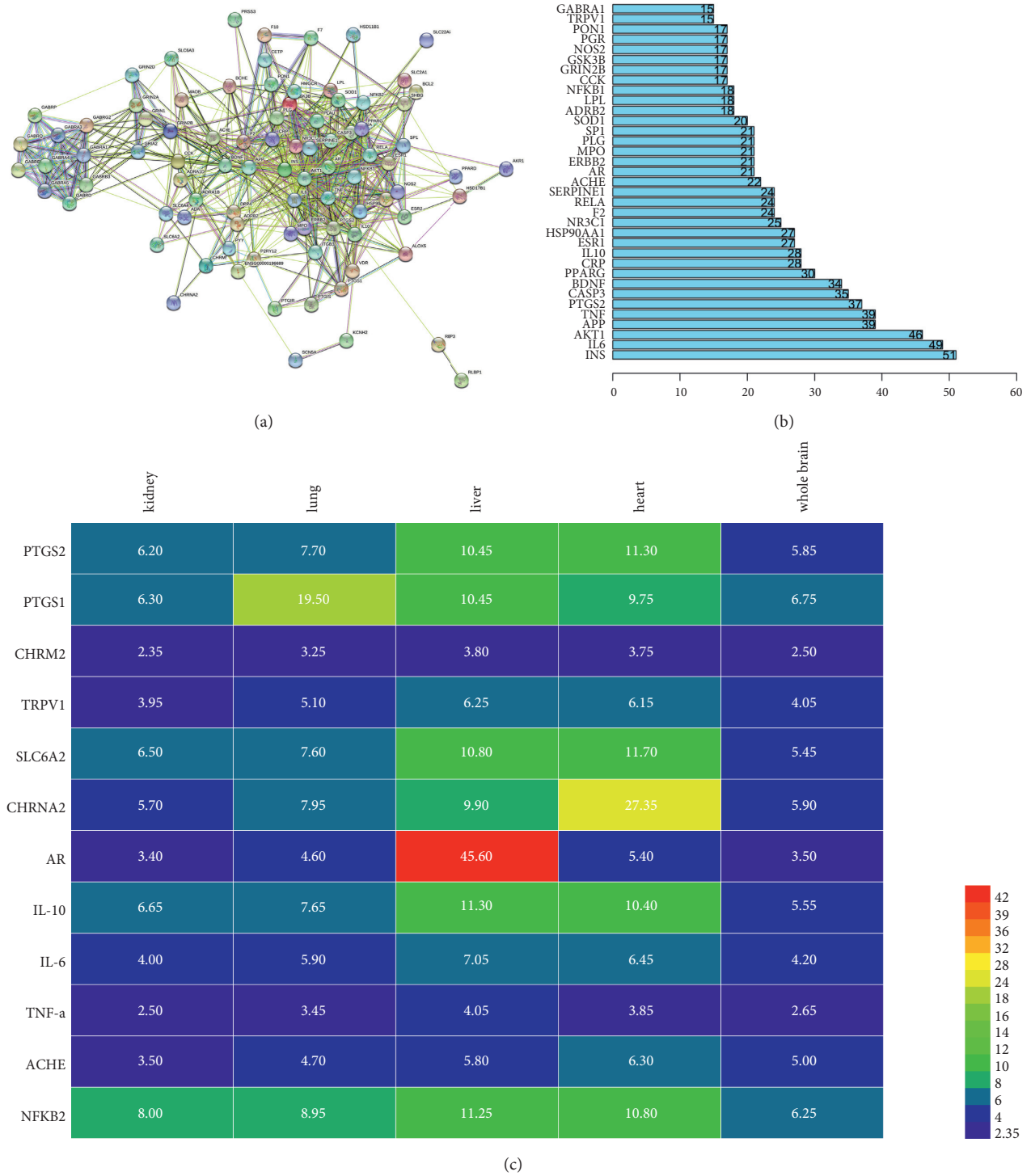

Figure 4: (a) The PPI network. (b) The number of targets that can influence each other in the PPI network. (c) The expression of the core targets in vital organs.

have shown that migraine is closely related to neurogenic neuroinflammation [74, 75]. However, due to the lack of finding standard markers of central nervous system (CNS) inflammation, such as changes in BBB integrity or glial activation or leukocyte infiltration, researchers do not believe that CNS inflammation is involved in migraine attack. Therefore, TRPV1 is also a target of great interest to us. It belongs to the transient receptor potential (TRP) channel family and is a nonselective cation channel [76]. It is mainly expressed in primary afferent sensory neurons, which detect and integrate chemical and thermal stimulation signals to induce pain, convert them into action potentials, upload this information to the central nervous system, and ultimately make the body feel pain or uncomfortable [77]. For example,

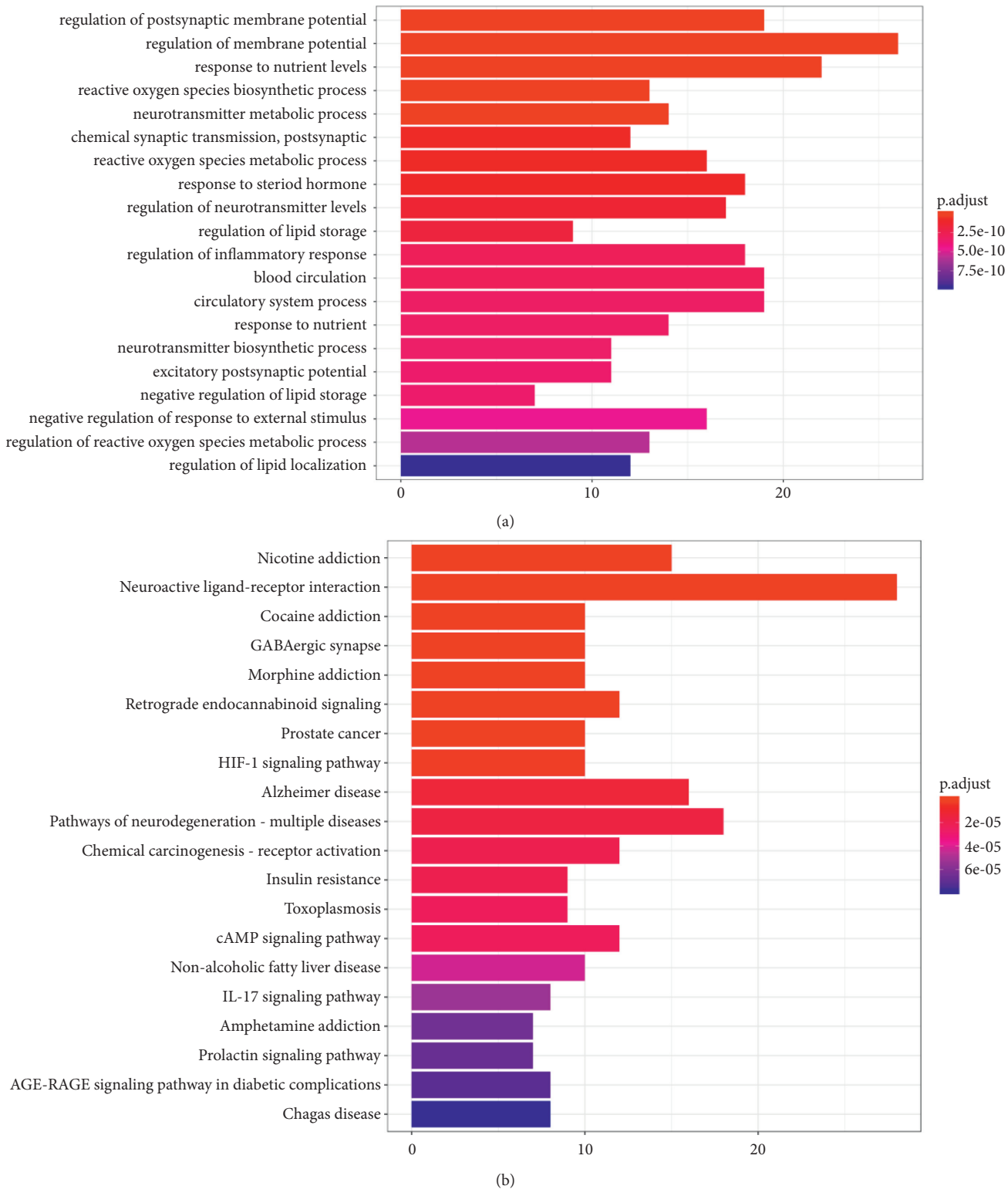

Figure 5: (a) GO enrichment analyses. The x-axis represents significant enrichment in the counts of these terms. The y-axis represents the categories of "biological process" in the GO of the targets ($P < 0.01$). (b) KEGG pathway enrichment analyses. The x-axis represents the counts of the target symbols in each pathway; the y-axis represents the main pathways ($P < 0.01$).

capsaicin can activate TRPV1 channels, cause calcium influx, and lead to excitation of primary sensory neurons; long-term use leads to neuron desensitization, which blocks the transmission of pain. Furthermore, TRPV1 blockers can also block the initial pathway of pain afferents, providing a new avenue for the clinical treatment of pain [78]. Studies

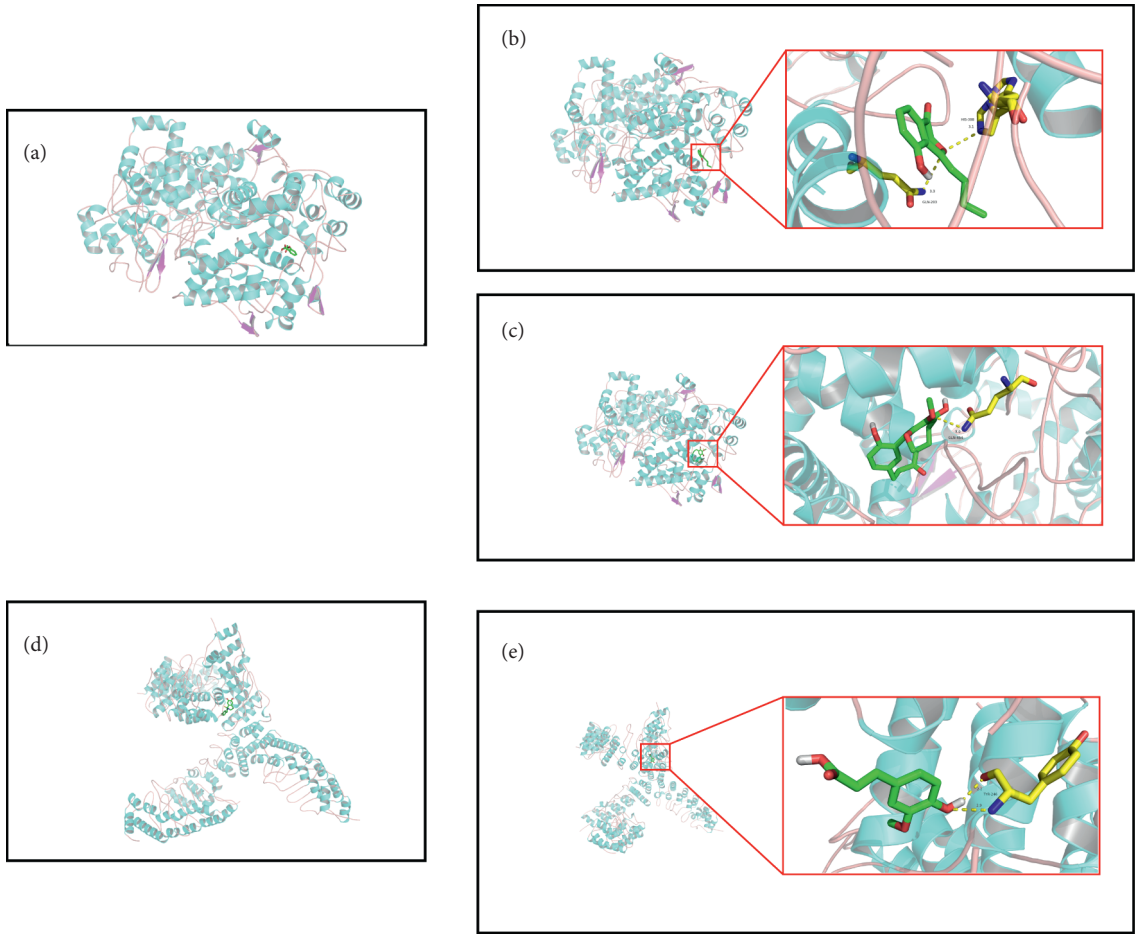

Figure 6: Binding studies of active compounds with PTGS2 and TRPV1 interactions. (a) Aspirin with PTGS2; (b) chuanxiongol with PTGS2; (c) myricanone with PTGS2; (d) capsazepine with TRPV1; and (e) ferulic acid with TRPV1. Molecules are represented by the ball and bar model, hydrogen bonds are represented by dotted lines, and distances are represented by angstroms. Atoms C, O, and N are green, red, and blue, respectively.

speculate that antagonizing TRPV1 is a promising treatment approach and should receive more attention in future studies and in the development of antimigraine drugs [79, 80]. We think this target is very interesting because in traditional Chinese medicine theory, CR is a drug with the effect of Xin and San. After taking CR, the human body will have a reaction similar to that with pepper, namely, sweating. We believe that this is also the embodiment of TRPV1 macrocontrol.

In this study, we also discussed the channel tropism of CR through Biogps. The theory of traditional Chinese medicine holds that traditional Chinese medicine acts on the whole human body, but the organs that produce curative effects are the focus. In addition, we found that CR affected 11 targets that were more highly expressed in the liver or heart but least expressed in the whole brain. This is a very interesting finding. Migraine is a kind of brain disease, and CR can treat it by acting on the liver and heart. We think that this is a very new and appropriate explanation for the holistic concept of TCM treatment. Of course, more evidence is needed to verify this explanation. Furthermore, we also speculated on the potential mechanism of CR in treating migraine based on the above study, which will be verified in the future.

In conclusion, we systematically explored the mechanism of CR in the treatment of migraine. Our results may provide some unique insights for the treatment of migraine in TCM.

Data Availability

The data used to support the findings of our study are included within the manuscript or within the supplementary information files.

Disclosure

Xianhua Wen and Yuncheng Gu are co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Authors' Contributions

Xianhua Wen and Yuncheng Gu contributed to this work equally. Xianhua Wen, Songhong Yang, Feipeng Gong, Xuping Liu, and Lingyun Zhong conceived and designed the study. Beili Chen and Qianfeng gong were responsible for the writing of the paper. Junmao Li and Songhong Yang were responsible for the chemical structure identification of CR. Wenting Wu and Hengli Tong were responsible for the audit and proofreading of active ingredients. All the authors participated in drafting of the manuscript and revising it before final submission.

Acknowledgments

The authors are thankful to Jiangxi University of Chinese Medicine and Jiangxi Provincial People's Hospital Affiliated to Nanchang University and Tiantai County Food and Drug Testing Center for help in conducting this study. This work was supported by the Jiangxi Provincial Drug Administration Project (2020JS10), Jiangxi Provincial Natural Science Foundation (20212BAB216013), and Jiangxi Provincial Drug Administration Project (2019GL02).

Supplementary Materials

Supplementary Table S1: active compounds of CR). A total of 248 candidate compounds were included. Supplementary Table S2: relevant targets of CR active compounds. Supplementary Table S3: targets for Migraine. Supplementary Table S4: overlapping targets between disease and drug. Supplementary Table S5: details of the active compounds of CR and overlapping targets. Supplementary Table S6: the details of the PPI network. Supplementary Table S7: details of the GO enrichment analysis. Supplementary Table S8: details of the KEGG pathway enrichment analysis. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2021, Article ID 6138854, 10 pages
https://doi.org/10.1155/2021/6138854

Research Article

Mechanism of Action of Dengzhan Shengmai in Regulating Stroke from an Inflammatory Perspective: A Preliminary Analysis of Network Pharmacology

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Received 22 July 2021; Accepted 19 September 2021; Published 31 October 2021

Academic Editor: Junqing Huang

Stroke is a complicated disease with an increasing incidence and a very high mortality rate. A classical Chinese herbal medicine, Dengzhan Shengmai (DZSM), has shown to have therapeutic effects on stroke; however, its chemical basis and molecular mechanism are still unclear. In this study, a systems biology approach was applicable to elucidate the underlying mechanism of action of DZSM on stroke. All the compounds were obtained from databases, and pendant-related targets were obtained from various data platforms, including the TCM Systematic Pharmacology (TCMSP) database, TCM Integrated Database (TCMIP), High Throughput Experimental Reference Database (HERB), Comparative Toxicogenomics Database (CTD), SwissTargetPredicition, and SymMap, The Human Gene Database (GENECARD) and Comparative Toxicogenomics Database (CTD) were used for stroke disease target data, followed by network pharmacology analysis to predict the potential effect of DZSM on stroke. Animal experiments were intended to validate the underlying mechanisms. A total of 846 chemical components were compiled for the targets of DZSM drug, and quercetin, kaempferol, and Wuweizisu C are the highest chemical components compiled from DZSM. Overlapping with 375 disease-specific targets and 149 core targets, the core targets include TNF, IL-6, ALB, and AKT1, which are shown to regulate the disease process from an anti-inflammatory perspective. 198 enrichment messages were obtained by KEGG enrichment analysis, and we believe that the role of the AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, and IL-17 signaling pathway is more important. Based on rat experiments, we also demonstrated that DZSM could effectively modulate the inflammation level of brain infarct tissues and effectively alleviate behavioral characteristics. Grouped together, our study suggests that the combination of network pharmacology prediction and experimental validation can provide a useful tool to describe the molecular mechanisms of DZSM in Chinese medicine (TCM).

1. Introduction

Stroke is a syndrome of localized cerebral arterial vascular blockage, resulting in hypoxia and ischemia of brain tissue in the area of vascular innervation, causing corresponding neurological dysfunction, which is the main cause of long-term disability and reduced quality of life in later life [1, 2]. Some studies have shown that ischemic stroke patients have varying degrees of spontaneous recovery of neurological function over time, which is dominant with appropriate rehabilitation and drug therapy [3–7]. It is generally believed that the inflammatory response is an important cause of

neural remodeling, mainly including vascular regeneration, axonal sprouting, and synaptic remodeling [8–11], and inflammation levels can affect axon generation, while axonal sprouting and synaptic remodeling can promote the establishment of neural circuits and compensate for the innervation of damaged areas; therefore, regulation of inflammation levels is crucial to promote neural remodeling in stroke [12, 13]. Therefore, regulation of inflammation levels is critical to promote neurological remodeling after stroke.

Chinese medicine has been extensively used as an alternative therapy for stroke in China [14], in which proprietary Chinese medicine has the advantages of multitarget, multipathway, and multilinkage. Ginseng is the most commonly used Chinese patent medicine for the treatment of stroke, which consists of Radix et Rhizoma Ginseng, Radix et Rhizoma Ginseng, Radix et Rhizoma Macrocephala, and Radix et Rhizoma Wu Wei Zi and has the effect of benefiting Qi and nourishing Yin, invigorating blood, and strengthening the brain. It is utilized to treat the symptoms of stroke by removing blood stasis and with ginseng, maitong, and wu wei zi to form the famous formula sang wei sheng to benefit qi and nourish yin. Benefiting qi and nourishing yin means that when qi is healthy, blood moves and when yin is sufficient, the brain and kidney are combined, so that brain nerve function is normal and nerve function is improved and restored after stroke.

Modern pharmacological studies have shown that *Erigeron breviscapus* has anti-inflammatory, antioxidant, anticoagulant, and vascular protective effects [15, 16]. Clinical practice has confirmed that DZSM capsules and its active ingredients can effectively suppress the level of inflammation after ischemic stroke and contribute to neurological recovery and cognitive function improvement in patients [17–20]. Recent studies have found that the active ingredients of DZSM capsules can treat chronic brain tissue ischemia and hypoxia leading to neurosynaptic changes by regulating the levels of inflammatory factors [21]. Therefore, it is of major clinical significance to clarify the mechanism of DZSM on ischemic stroke.

2. Materials and Methods

2.1. Screening of Active Ingredients. The HERB database, Swiss database, TCMSP database, CTD database, and SYMMAP database were utilized to predict the ingredients of DZSM.

2.2. Prediction of Drug-Related Targets. The TCMSP, HERB SWISSTARGET, CTD, and SYMMAP database were used to predict the targets of DZSM, and then, the targets were predicted in the Uniport online protein database (Uniprot), to find the corresponding standard gene name, so as to obtain the related target of active ingredients of DZSM.

2.3. Prediction of Disease-Related Targets. Using the GENECARDS database and CTD database to obtain the relevant content of stroke and delete the repetitive target gene, we can obtain the relevant targets of stroke.

2.4. Finding Common Targets of Drugs and Diseases. By mapping the target information of DZSM with that of stroke, we can obtain the overlapped target Venn map and overlapped target information.

2.5. Construction of the Active Ingredient Target Network. The active components and overlapping genes of DZSM can be sorted out by using Cytoscape 3.7.1 software, and the network of active components and overlapping genes of DZSM can be drawn.

2.6. Construction of the Drug Disease Overlapping Target Protein Interaction Network (PPI). The overlapping targets of DZSM and stroke were imported into the string data analysis platform for calculation, and PPI could be constructed according to the relationship between the targets.

2.7. Enrichment Analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway. The overlapping target names of DZSM and stroke were transformed into Entrez gene ID by R language, and then, the KEGG-related information was obtained by analyzing and calculating with the KOBAS database. Then, the P value was used as the reference value to screen the related targets, and then, the R language was used to analyze the related content of pathway enrichment analysis.

2.8. Animals. Adult-specific pathogen-free SD rats (males, 250–300 g) were bought from the Experimental Animal Resource Center of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2020058). They were housed at $20 \pm 1^{\circ}$C, 40–60% humidity, with 12 h light/dark cycles. The procedure was carried out in accordance with the Guide for the Care of Laboratory Animals. The animal protocol was subject to approval by the Institutional Animal Care and Use Committee of Tianjin University of Traditional Chinese Medicine.

2.9. Experimental Groups. Rats were randomly divided into four groups: the control group, MCAO group, MCAO + low DZSM dose treatment group (113.4 mg/kg administered by gavage), and MCAO + high DZSM dose C group (226.8 mg/kg administered by gavage). Vehicle rats underwent the same procedure without carotid artery obstruction.

2.10. Focal Cerebral Ischemic Stroke Model. Rats were initially anesthetized with 5% isoflurane and then maintained with 1.5–2% isoflurane, 30% oxygen, and 70% nitrous oxide. The body temperature of the rats was maintained at 37°C (±1.0°C) using a thermostatic heating pad. After cutting the surface skin with a scalpel, the anterior soft tissue of the rat's neck was gradually and bluntly separated, and nylon thread

was tied from the proximal and distal ends of the external carotid artery, with a dead knot at the distal end and a live knot at the proximal end, and the wall of the external carotid artery blood vessel was gently cut with vascular scissors, and a matching thread plug was inserted, and the thread plug was sequentially passed through the external carotid artery and the common carotid artery to the internal carotid artery, and the insertion of the thread plug was stopped when there was appropriate resistance to the thread plug, and the position of the Marker was observed and tied. When there was appropriate resistance to the wire plug, the insertion of the wire plug was stopped, the position of the Marker was observed, and the wire plug was tied to prevent it from falling off. The sham-operated group was modeled in the same way but without blocking the middle cerebral artery blood flow.

2.11. Model Evaluation. The stability of the model was evaluated by performing magnetic resonance scans on rats in the modeling and sham-operated groups that met the neurological function criteria 24 hours after the MCAO model was performed to determine the percentage of brain infarct area in rats. Rats were anesthetized with 10% chloral hydrate (preparation: 10 g dissolved in 100 mL saline) at a dose of 0.4 g/kg. After the rats were unconscious, they were fixed in a rat coil for scanning in the prone position while maintaining an anal temperature of $37 \pm 0.5^{\circ}$C. Six rats in each group were randomly selected for magnetic resonance scanning. Magnetic resonance diffusion-weighted imaging (DWI) was used to detect acute cerebral infarction. Axial T2WI and DWI scans were performed, and the T2WI sequence was used to calculate the total area of the lesion, while the DWI sequence was used to calculate the area of the infarct core lesion, and the difference between the two was the area of the ischemic semidark zone.

Parameter settings: T2WI: repetition time 4300 ms, echo time 100 ms, layer thickness 1.5 mm, and matrix 70×70; DWI: repetition time 3800 ms, echo time 125 ms, layer thickness 1.5 mm, and matrix 80×80. Total area of lesion = total area of healthy brain tissue−area of normal brain tissue on the affected side, and outline the area of core lesion, area of ischemic semidark zone = total area of the lesion−area of the core lesion.

2.12. Physical Exercise. We recorded the weight change of the rats during the treatment period and conducted various behavioral evaluations of the rats' status in the balance beam experiment: a 2 m-long balance beam supported by an iron frame at each end (60 cm high), with a plastic cage at the end, was used as the end point of each experiment. Hanging wire experiment: a wire 50 cm long and 0.15 cm in diameter was fixed horizontally to a stand 37 cm above the ground, as described in our previous study, and the rats were placed in the middle of the wire rope and observed for 30 seconds each time, with the average score of 3 trials per animal recorded for each trial. Muscle strength experiments: tests were also performed at baseline, 3 and 7 days postoperatively. In a quiet environment, the rats were placed in a net with a mesh size of 2.3 cm × 2.3 cm, and the number of times the rats accidentally entered the net by their front paws within 2 minutes while walking in the net was counted as follows: (number of forepaw missteps measured on the contralateral side of the lesion−number of forepaw missteps on the ipsilateral side of the lesion)/total number of steps. Rats were needed to undergo acclimatization 3 days prior to modeling before operation.

2.13. Inflammatory Factor Assay. Inflammatory factor levels in infarcted tissue were measured using commercial ELISA kits at a 1 : 1 dilution (50 μL) of each rat peri-infarct brain tissue. Intra- and interassay variation was <4–6% and <8–10%, respectively, with detectable concentrations ranging from 0.066–1024 ng/ml. Peri-infarct brain tissue concentrations of proinflammatory cytokines, including TNF-α, were measured with ELISA kits.

2.14. Statistical Analysis. Data are expressed as mean ± SEM. Statistical analyses were performed using Graphical Board Prism version 4.0 software. Differences between groups were assessed by an unpaired *t*-test or ANOVA followed by Tukey's posttest. For correlation analysis, Pearson's correlation analysis and Spearman's correlation analysis served.

3. Results

3.1. Screening of Effective Components and Determination of Action Targets of DZSM. The TCMSP database has a total of 30 components of DZSM, of which 12 belong to ginseng, 10 to lampblackberry, and 8 to northern *Schisandra*. It is to be noted that these herbs have some components in common. All of them met the requirements of OB ≥ 40% and DL index ≥0.2. After eliminating the overlap, they were further analyzed as candidate bioactive components, and the details are shown in Table 1. Since the information was not included in the TCMSP database, a search using the TCMIP database yielded information on 35 related components. Among all the components, quercetin, kaempferol, and wuweizisu C were shown to be the three components with the highest degree values, respectively. Modern pharmacological studies have shown that both have anti-inflammatory effects.

DZSM compound indicators were obtained by searching the TCMSP and TCMIP online databases, combining the data of TCMSP, Herb, SwissTargetPrediction database, CTD, and SYMMAP and using the relevant target prediction techniques in each platform to screen the predicted targets of the abovementioned active ingredients and eliminate duplicate targets, finally obtaining a total of 744 herbal prediction targets.

We searched the GENECARD, CTD, and TCMIP databases and screened for known target genes related to stroke. We combined target genes from each database, removed duplicate disease targets, and retrieved 372 disease targets. We combined 744 predicted herbal targets to obtain information on 91 specific targets for DZSM for stroke. We constructed a component-target network diagram based on the retrieved data, as shown in Figure 1.

Table 1: Chemical composition of Wuweiz, Renshen, and Dengzhanxixin selected from the TCMSP.

Mol ID	Molecule name	Drug	OB (%)	DL
MOL002879	Diop	Ginseng	43.59	0.39
MOL000449	Stigmasterol	*Ophiopogon japonicus*	43.83	0.76
MOL003648	Inermin	Ginseng	65.83	0.54
MOL000422	Kaempferol	Ginseng/*Erigeron breviscapus*	41.88	0.24
MOL005308	Aposiopolamine	Ginseng	66.65	0.22
MOL005314	Celabenzine	Ginseng	101.88	0.49
MOL005320	Arachidonate	Ginseng	45.57	0.2
MOL005321	Frutinone A	Ginseng	65.9	0.34
MOL005356	Girinimbin	Ginseng	61.22	0.31
MOL005360	Malkangunin	Ginseng	57.71	0.63
MOL005384	Suchilactone	Ginseng	57.52	0.56
MOL000787	Fumarine	Ginseng	59.26	0.83
MOL004624	Longikaurin A	*Schisandra*	47.72	0.53
MOL005317	Deoxyharringtonine	*Schisandra*	39.27	0.81
MOL008956	Angeloylgomisin O	*Schisandra*	31.97	0.85
MOL008957	Schizandrer B	*Schisandra*	30.71	0.83
MOL008968	Gomisin-A	*Schisandra*	30.69	0.78
MOL008974	Gomisin G	*Schisandra*	32.68	0.83
MOL008978	Gomisin R	*Schisandra*	34.84	0.86
MOL008992	Wuweizisu C	*Schisandra*	46.27	0.84
MOL000098	Quercetin	*Erigeron breviscapus*	46.43	0.28
MOL000392	Formononetin	*Erigeron breviscapus*	69.67	0.21
MOL000816	Ergosta-7,22-dien-3-one	*Erigeron breviscapus*	44.88	0.72
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	*Erigeron breviscapus*	42.36	0.21
MOL002712	6-Hydroxykaempferol	*Erigeron breviscapus*	62.13	0.27
MOL002914	Eriodyctiol	*Erigeron breviscapus*	41.35	0.24
MOL005922	Acanthoside B	*Erigeron breviscapus*	43.35	0.77
MOL007963	1-Hydroxy-2,3,5-trimethoxy-xanthone	*Erigeron breviscapus*	101.06	0.3
MOL007984	Δ5,22-Stigmastadien-3-ol	*Erigeron breviscapus*	43.83	0.76

Prediction results of potential targets of DZSM in the treatment of stroke.

3.2. PPI Network Construction. 91 cross-target genes mentioned above were imported into the STRING online platform to obtain protein-protein interaction data columns, construct PPI network graphs, and analyze the data for topological heterogeneity. In order to more accurately understand the potential protein targets of DZSM-regulated stroke, the topology analysis data were screened, and targets with TOP32° values were selected as the core targets of DZSM-regulated diseases, and the topology data were imported into Cytoscape 3.7.1 to construct the core target network graph. As shown in Figure 2, the core targets of PPI are TNF,IL-6,ALB,AKT1,VEGFA, and CREB1, which play a key role in the regulation of stroke by DZSM (Figure 2).

3.3. Enrichment Analysis of the KEGG Pathway. In order to identify overlapping genes associated with stroke, we performed an enrichment analysis of the KEGG pathway to elucidate the associated signaling pathway. The *y*-axis represents the signaling pathway, and the *x*-axis indicates the number of genes enriched for the term. The redder the color, the smaller the p.adjusted value (FDR); it also indicates higher confidence and importance. Conversely, the more blue the color, the larger the value of p.adjust. The results suggest that the mechanism of action of DZSM in regulating stroke involves many signaling pathways.

Through enrichment analysis, we identified the functions of active ingredients in directly or indirectly regulating a number of pathways associated with the treatment of stroke, including TNF, cAMP, IL-17, and MAPK signaling pathways (Figure 3).

3.4. MCAO Model Stability Evaluation. Magnetic resonance scans show normal brain tissue and no lesions in the sham-operated rats. The percentage of the ischemic semidark zone area was $(16.48 \pm 1.29)\%$ in the MCAO group, $(16.18 \pm 2.07)\%$ in the low-dose group, and $(15.00 \pm 1.40)\%$ in the high-dose group. The rats in the modeling group were significantly different from the rats in the sham-operated group ($P < 0.001$), and no significant differences were observed between the groups in the modeling group ($P > 0.05$).

3.5. DZSM Can Effectively Reverse the Physiological Changes Caused by MCAO. For correlation analysis, a total of 24 rats were subjected to MCAO. A decrease in body weight was found in the MCAO rats, compared to the normal group of animals. Also, with DZSM treatment, there were signs of weight reversion. Given the potent stimulating effects of DZSM on alleviating neurological deficits, DZSM may be involved in motor recovery. Rats in high dose of DZSM showed significantly better motor recovery, which was carried out at days 7 after MCAO. The

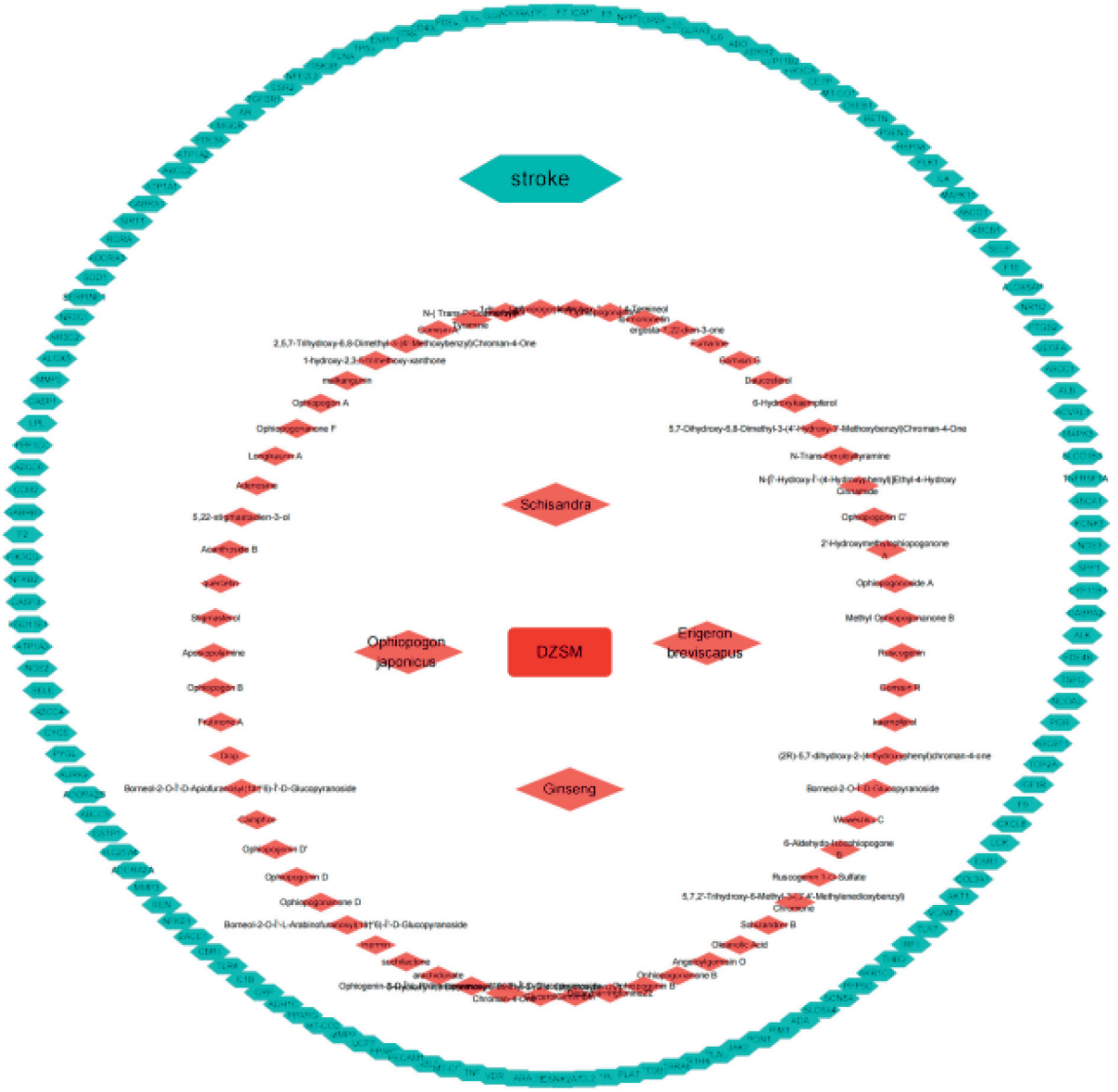

Figure 1: Chemical composition core target network diagram of DZSM. The red nodes represent the chemical components in DZSM, and the green nodes represent the core targets of the drug to treat the disease.

results of the foot-fault test and hanging wire test showed that the motor abilities of rats were aggravated after surgery while improving following high dose of DZSM treatment ($P < 0.05$) (Figure 4).

3.6. DZSM Modulates the Inflammatory Response Brought about by MCAO. Analysis of inflammatory factor levels assayed using DZSM administration in MCAO-modeled individuals reduced the levels of TNF-α in peri-infarct brain tissue, which indicates that the inflammatory response signal brought about by MCAO is inhibited by DZSM treatment. The inhibition of inflammatory factors from DZSM may slow down this process (Figure 4).

4. Discussion

Stroke is a complex disease, an acute cerebrovascular disease, and a group of diseases that cause damage to brain tissue due to sudden rupture of blood vessels in the brain or failure of blood to flow to the brain due to blockage of blood vessels [22] including ischemic and hemorrhagic strokes. It has become the first cause of death in China and the main cause of disability among Chinese adults. TCM consists of multiple compounds, and TCM may have a wide range of pharmacological and multitarget and pathway pharmacological activities that may be beneficial to the treatment of stroke [23]. On the other hand, this property of TCM may be, therefore, difficult to investigate the underlying

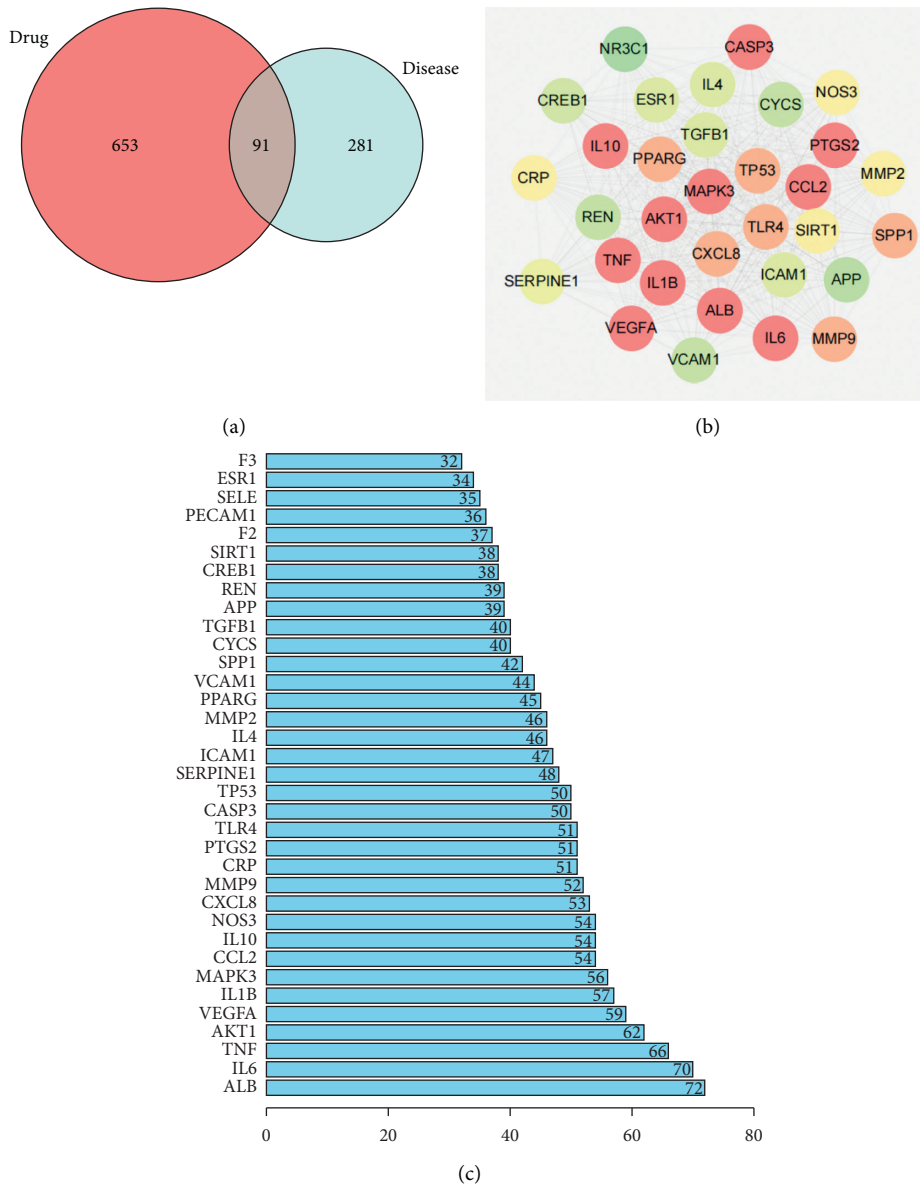

Figure 2: (a) Overlapping target Venn diagram, stroke and DZSM co-overlap; (b) PPI network analysis of the core target. The colors in the PPI network diagram represent degree statistical trends, from green to red, and degree values from low to high; and (c) the target degree values in the overlapping target PPI network were counted, and TOP 32 was plotted from the highest to lowest.

mechanisms in depth. A network pharmacology approach that integrates systems biology and silicon techniques could provide a direction for the mechanistic study of complex TCM. In the current study, we used this approach to elucidate the pharmacological mechanism of stroke alleviation by DZSM.

Among the active ingredients of DZSM, the compounds with the highest number of connected targets are quercetin and kaempferol. Pharmacological studies have revealed that quercetin and kaempferol have been shown to exert neuroprotective effects in stroke patients through anti-ischemia, anti-free-radical oxidation, and inhibition of inflammatory responses [24–27].

The 91 targets acquired by combining the component-target protein PPI with the disease target protein PPI are the targets corresponding to the chemical components in DZSM and also the targets related to stroke, so these 91 targets are the core targets of DZSM for the treatment of stroke, and the targets interact with each other through 400 interactions to influence the treatment of the disease.

The core targets of DZSM for stroke are TNF, AKT1, VEGFA, and IL-1β, which are highly correlated. It has been demonstrated that TNF-α protein is abundantly expressed in brain tissue during ischemia and hypoxia and plays an important neurotoxic role in the pathogenesis and pathology of ischemic stroke by inducing the release of potent vasoactive substances, leading to vasoconstriction, reducing local cerebral blood flow, and increasing capillary permeability to promote the development of cerebral ischemia and edema [28]. VEGFA is a member of the VEGF family of vascular

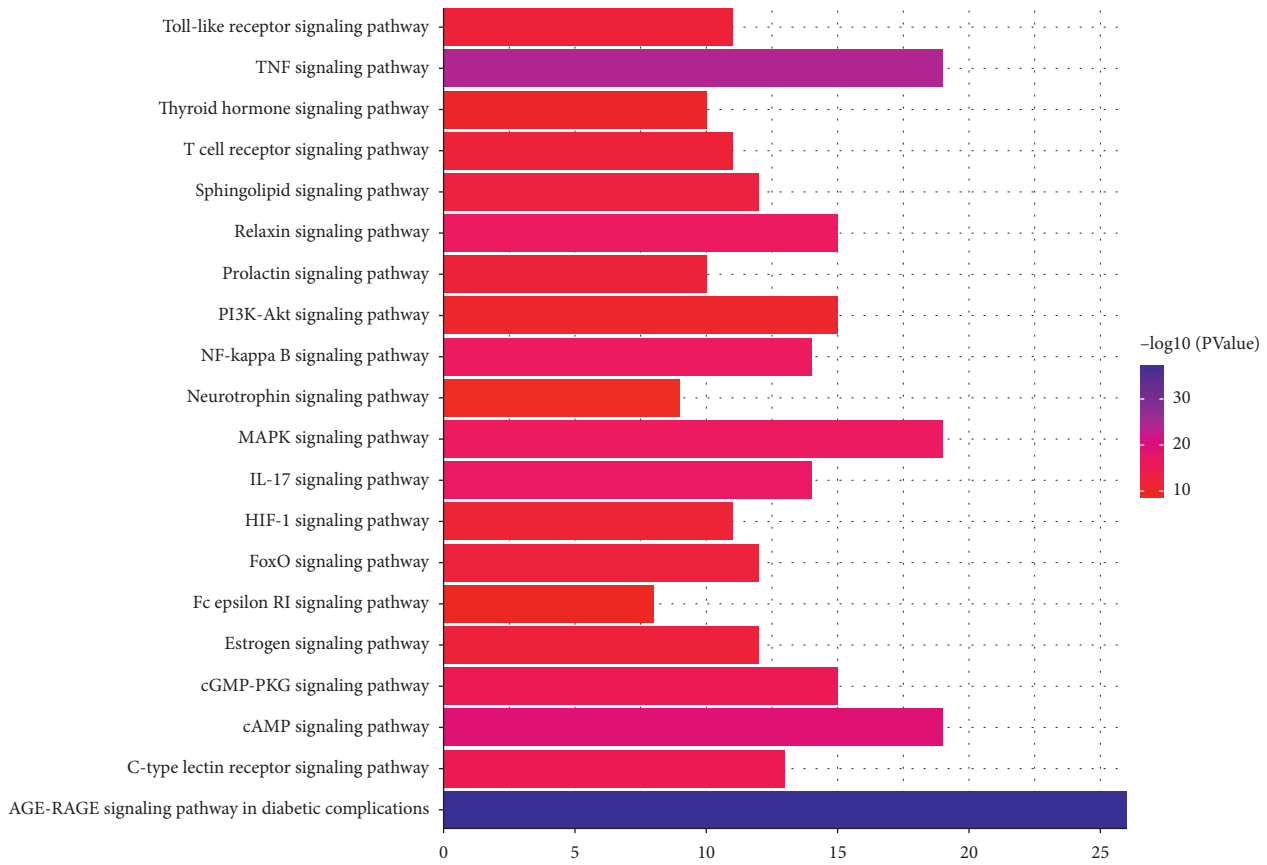

Figure 3: Enrichment analysis of the KEGG pathway. The colors in KEGG enrichment represent *P* value trends from red to blue with low to high correlation values.

endothelial growth factors, which plays an important role in the process of angiogenesis after cerebral ischemia, and the administration of VEGFA to the MCAO rat model can increase neuroprotective effects. VEGFA can increase the density of biological microvessels in the ischemic semidark zone and promote angiogenesis after administration [29].

Based on enrichment analysis of the KEGG pathway in the core targets, it is clear that the AGE-RAGE signaling pathway, IL-17 signaling pathway. TNF signaling pathway, and cAMP signaling pathway are significantly enriched. Previous studies have shown that AGEs are irreversible end products formed by glycosylation of macromolecules, and one of their receptors, RAGE, belongs to the immunoglobulin superfamily [30]. In pathological states such as inflammatory response, ischemia-reperfusion injury, and hypoxia, the AGE-RAGE axis is activated [31]; on the one hand, the transcription factor NF-κB is activated and AP-1 is inhibited, stimulating the activation of inflammatory response factors and coagulation factors leading to an expanded inflammatory response, increased endothelial cell damage, reduced synthesis of proangiogenic factors such as VEGF, and inhibition of vascularization; on the other hand, increased NF-κB expression can upregulate RAGE, forming a positive feedback loop that further aggravates the abovementioned situation [32]. The pathophysiology of ischemic stroke is very complex, and the ensuing inflammatory and immune responses can aggravate the disruption of the blood-brain barrier and the development of brain edema, leading to secondary brain injury [33]. Astrocytes are the main source of IL-17, and the previous literature has shown that IL-17-mediated neurological responses exacerbate neurological injury after ischemic stroke, but recent studies have shown that IL 17A mediates cortical astrocytes, alleviates ischemic injury, and thus, affects neurological outcome in rats with ischemic stroke [34, 35]. cAMP is one of the important intracellular second messengers, which play a cerebral protective role by activating the PKA signaling pathway, mediating cAMP response element-binding protein CREB, and regulating the formation of neuronal regenerative synapses [36].

We made an MCAO rat model and tested it by neurological function score, and staining and motor function confirmed the successful model construction. Basing on this model, ischemia may extend to the entire vascular region of MCAO and lead to focal metabolic disturbances of the infarct, selective neuronal necrosis, and brain edema.

The Zea-Longa scale is the current standard for measuring neurological deficits in rats. We examined the locomotor behavior of rats in the MCAO group, sham-operated rats, and rats in different administrative groups using various behavioral methods. Consistent with previous findings, after focal cerebral ischemia, rats showed a

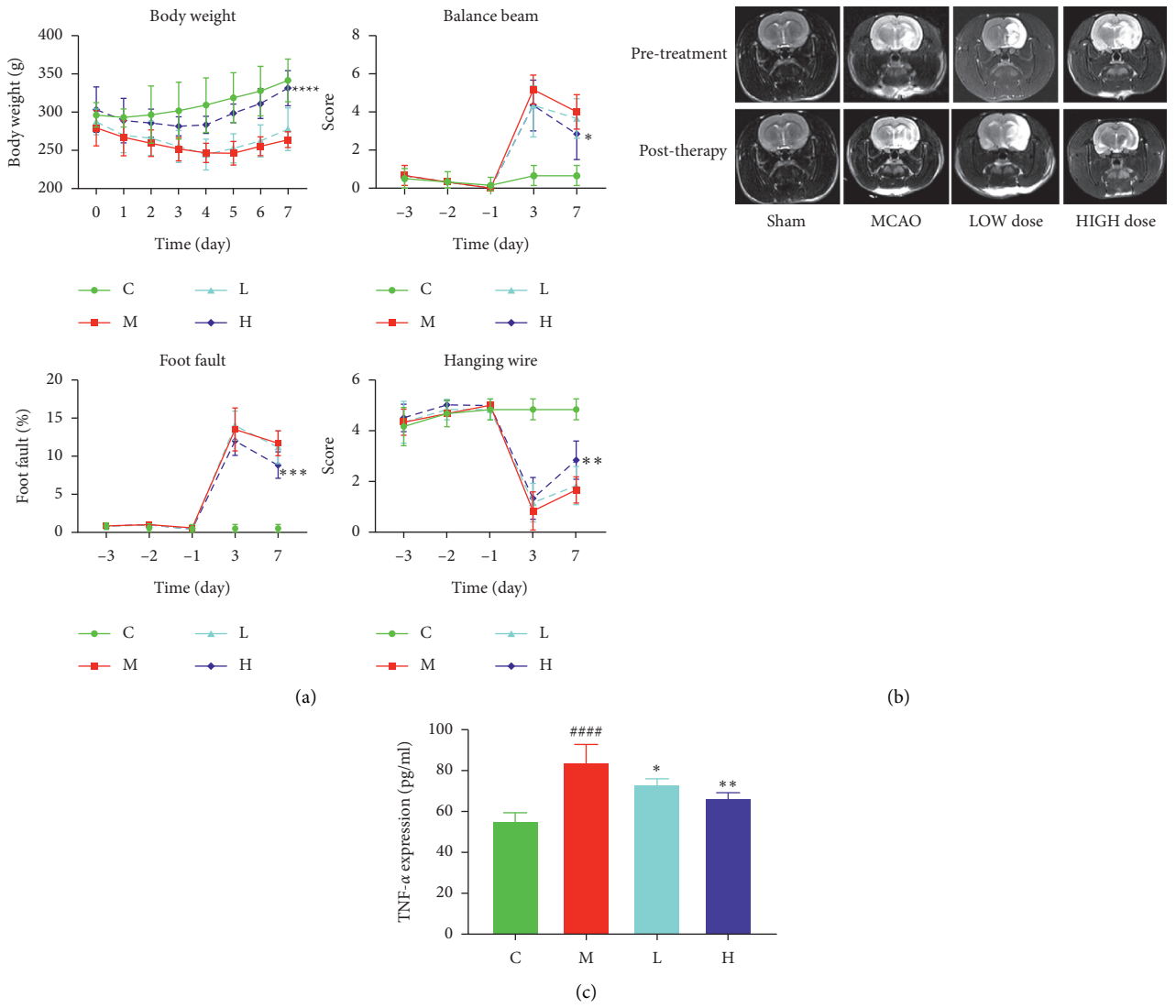

Figure 4: (a) Weight changes of rats in 7 days, balance beam experiment, foot-fault experiment, and hanging wire experiment; (b) model stability evaluation; and (c) detection of TNF-α in brain tissue by ELISA. Compared with the control group, $P < 0.001$; the low-dose group compared with the model group, $P < 0.05$; and the high-dose group compared with the model group, $P < 0.01$.

significant decrease in balance beam time and a response of shortened suspension time, which was closely related to the size of the cerebral infarct and tended to occur in association after drug administration.

Given the effective stimulatory effect of DZSM in alleviating neurological deficits, DZSM may be involved in motor recovery. Rats with high doses of DZSM exhibited significant motor recovery, which was performed on day 7 after MCAO. The results of the balance beam, hanging wire test, and step-error test showed that the locomotor ability of the rats was aggravated after MCAO surgery and had a palliative effect after the administration of DZSM, where it improved after high-dose DZSM treatment. Also, inflammatory factors of cerebral infarct tissues were found to be significantly reduced and altered by DZSM treatment. This indicates that DZSM has a modulating effect on the improvement of the behavioral ability of rats after surgery.

Overall, here, the relationship and mechanism of DZSM as a multicomponent and multitarget drug interacting with stroke was systematically described using a network pharmacology approach. First, the relevant components, key targets, and enrichment pathways of DZSM were identified through a systematic analysis. These findings are valuable because understanding the biological functions of DZSM is important for the development of antistroke drugs, and our study provides insight into the application of TCM for the treatment of strong stroke, but the specific molecular biological mechanisms need further intensive study.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any conflicts of interest.

Authors' Contributions

Yiqi Yan, Chao Sun, and Xiaoting Rong conceived and designed the study. All the other authors completed the experiment and the paper work together. Yiqi Yan, Chao Sun, and Xiaoting Rong contributed equally to this work.

Acknowledgments

The authors are thankful to Tianjin University of Traditional Chinese Medicine for the help in conducting this study. This work was supported by the Scientific Research Project of the Tianjin Municipal Health Commission, Tianjin Administration of Traditional Chinese Medicine, Integrated Traditional Chinese and Western Medicine (grant no. 2019117), and 2019 Annual Graduate Students Innovation Fund, School of Integrative Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, China (grant no. ZXYCXLX201918).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2021, Article ID 9888607, 12 pages
https://doi.org/10.1155/2021/9888607

Research Article

Exploration of Ziziphi Spinosae Semen in Treating Insomnia Based on Network Pharmacology Strategy

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Received 22 July 2021; Accepted 4 October 2021; Published 28 October 2021

Academic Editor: Junqing Huang

Ziziphi Spinosae Semen (ZSS) is a common natural medicine used to treat insomnia, and to show clearly its method of action, we managed and did an in-depth discussion. Network pharmacology research is very suitable for the analysis of multiple components, multiple targets, and multiple pathways of Traditional Chinese Medicine (TCM). According to the relevant theory, we first carefully collected and screened the active ingredients in ZSS and received 11 active ingredients that may work. The targets going along with these active components were also strongly related to insomnia targets, 108 common genes were identified, and drug-compound-gene symbol-disease visualization network and protein-protein interaction network were constructed. Forty-eight core genes were identified by PPI analysis and subjected to GO functional analysis with KEGG pathway analysis. The results of GO analysis pointed that there were 998 gene ontology items for the treatment of insomnia, including terms of 892 biological processes, 47 cellular components, and 59 molecular functions. It mainly shows the coupling effect and transport mode of some proteins in the biological pathways of ZSS in the treatment of insomnia and explains the mechanism of action through the connection between the target and the cell biomembrane. KEGG enrichment analyzed 19 signaling pathways, which were collectively classified into seven categories. We have identified the potential pathways of ZSS against insomnia and obtained the regulatory relationship between core genes and pathways and know that the same target can be regulated by multiple components at the same time. The results of molecular docking also prove this conclusion. We sought to provide a new analytical approach to explore TCM treatments for diseases using network pharmacology analysis tools.

1. Introduction

Insomnia refers to the symptoms of unable to get commonly and regular sleep or difficulty falling asleep, insufficient sleep time, lack of deep sleep, or even sleepless nights. Sleep deprivation is a potential factor for a variety of risk sicknesses. General medicine-based treatments such as cognitive behavioral therapy for insomnia have limitations, and better alternatives have also been searched for [1]. There are many links between insomnia and mental health, of which anxiety and depression may have a bidirectional effect with insomnia, and the problem can no longer be ignored by people [2]. Such people are prone to dizziness and brain swelling, generalized weakness, explosive temper, and irritability in the morning, which have been very harmful to the body for a long time in the past. With the fierce development of social competition, people's life rhythm has become faster. The prevalence and recurrence rates of insomnia remain high, and it has become one of the hot issues in the field of public health.

The effective drugs commonly used clinically are benzodiazepines, but they also have obvious side effects, even increasing the death rate of the elderly [3, 4]. As an alternative to treating insomnia, Chinese medicine has achieved

good therapeutic effects and has gradually attracted the attention of clinicians [5]. Ziziphi Spinosae Semen (ZSS) is the dried mature seed of Rhamnaceae plant *Ziziphus jujuba* Mill.var *spinosa* (Bunge) Hu ex H. F. Chou; according to TCM theory, ZSS is a gentle temperament drug that can nourish the heart and benefit the liver, tranquilize the mind, converge sweat, and generate fluid [6]. ZSS is a relatively easy-to-obtain natural medicine. It is found in many Chinese medicine prescriptions for calming and tranquilizing the nerves, and it is most commonly used to treat insomnia [7].

Chinese herbal medicine has been used to treat diseases in China for thousands of years. Drugs regulate the immune system or nervous system by eliminating pathogenic factors and strengthening vital energy, so that various organs of the human body can operate normally and maintain the balance between the body and nature. Traditional Chinese herbs, on the other hand, are often multicomponent, multitarget, and multicenter of action. This paper also explains the treatment of insomnia by ZSS with the help of tools and analysis methods (Figure 1).

2. Materials and Methods

2.1. Screening of Active Compounds. To get the compounds in ZSS directly and effectively, we used the most commonly used specialized Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (http://lsp.nwu.edu.cn/tcmsp.php); this platform relatively comprehensively covers the vast majority of TCM compounds' described information, while including the links between related targets and diseases [8, 9]. All ingredients about ZSS were first collected as candidates with the Chinese name "Suanzaoren" as the search term. Afterwards, we introduced these limiting parameters of oral bioavailability (OB) and drug-likeness (DL) according to the classical active ingredient screening rules [10], in which OB refers to the percentage of unmodified drugs that enter the circulatory system after oral administration and can effectively represent the availability of drugs, and OB $\geq$ 30% of compounds is considered as one of the screening rules for active ingredients [11]. While DL refers to the similarity of compounds to clinically used drugs, the greater DL value represents the possibility of compounds becoming drugs, and molecules with DL $\geq$ 0.18 are carefully thought about to have better pharmacological effects and are also one of the most commonly used active ingredient screening rules [12], so we started obeying this rule. In addition, as a drug often used by Chinese clinicians, the active substances of traditional Chinese medicine must meet the relevant provisions of the Chinese Pharmacopoeia when prescribing [13]. We also added the active ingredients specified in the Chinese Pharmacopoeia as active compounds.

2.2. Identification of Targets and Gene Symbols Associated with ZSS Compounds. The SMILES structural formulas of the compounds were obtained with the ZINC 15 database [14] and TCMSP Platform according to the CAS number, and the SMILES formulas were input into the SwissTargetPrediction (STP; http://www.swisstargetprediction.ch) [15]. We use the information given to extract the protein targets of each ZSS-active compounds and simplify the obtained data to get gene symbols.

2.3. Acquisition of Insomnia Gene Targets. In order to ensure that the genes we obtain that are associated with the disease are more comprehensive, we used "Insomnia" as a keyword to search on these two reliable database platforms, respectively, from the GeneCards database (https://www.genecards.org/) and the Online Mendelian Inheritance in Man (OMIM) database (http://www.omim.org/). GeneCards database automatically integrates data from about 125 network-derived genes including genome, transcriptomics, and proteomics, with very powerful functions [16, 17]. The OMIM database can question almost any data on genetic diseases and provide linkage relationships known about causative genes [18, 19]. After summarizing the obtained disease information, it is then screened in detail. Eventually, we obtained genes associated with insomnia.

2.4. Drug-Compound-Gene-Disease (D-C-G-D) Network Construction. We first obtained overlapping targets by crossing ZSS-related targets with insomnia-related targets by Venn diagram and then built a visual comprehensive network (D-C-G-D) based on the interaction between drugs (ZSS), compounds, gene symbols, and diseases (insomnia) by Cytoscape software (version 3.8.0) and made the corresponding schematic.

2.5. Protein-Protein Interaction (PPI) Network Construction. Discovering and annotating the interaction relationships of all functional targets in proteins enable system-level learning and understanding of the functions of proteins, and protein-protein interaction data were obtained from the STRING database (https://string-db.org/). STRING is commonly used to filter and evaluate relevant data for functional genomics, setting the organism species to *Homo sapiens* (human) before retrieval [20] and then organizing the received data.

2.6. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment. Gene Ontology (GO) enrichment analyses was performed with R software (version 4.0.2), and the entries enriched were determined by the Bioconductor database (http://bioconductor.org/). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using ClueGO plug-in in Cytoscape [21].

2.7. Computational Validation of Compound-Target Interactions. As a feasible and modern verification method, molecular docking is a method for drug design through the characteristics of receptors and the interaction mode between receptors and drug molecules and is widely used in the field of drug binding to protein receptors [22]. We selected

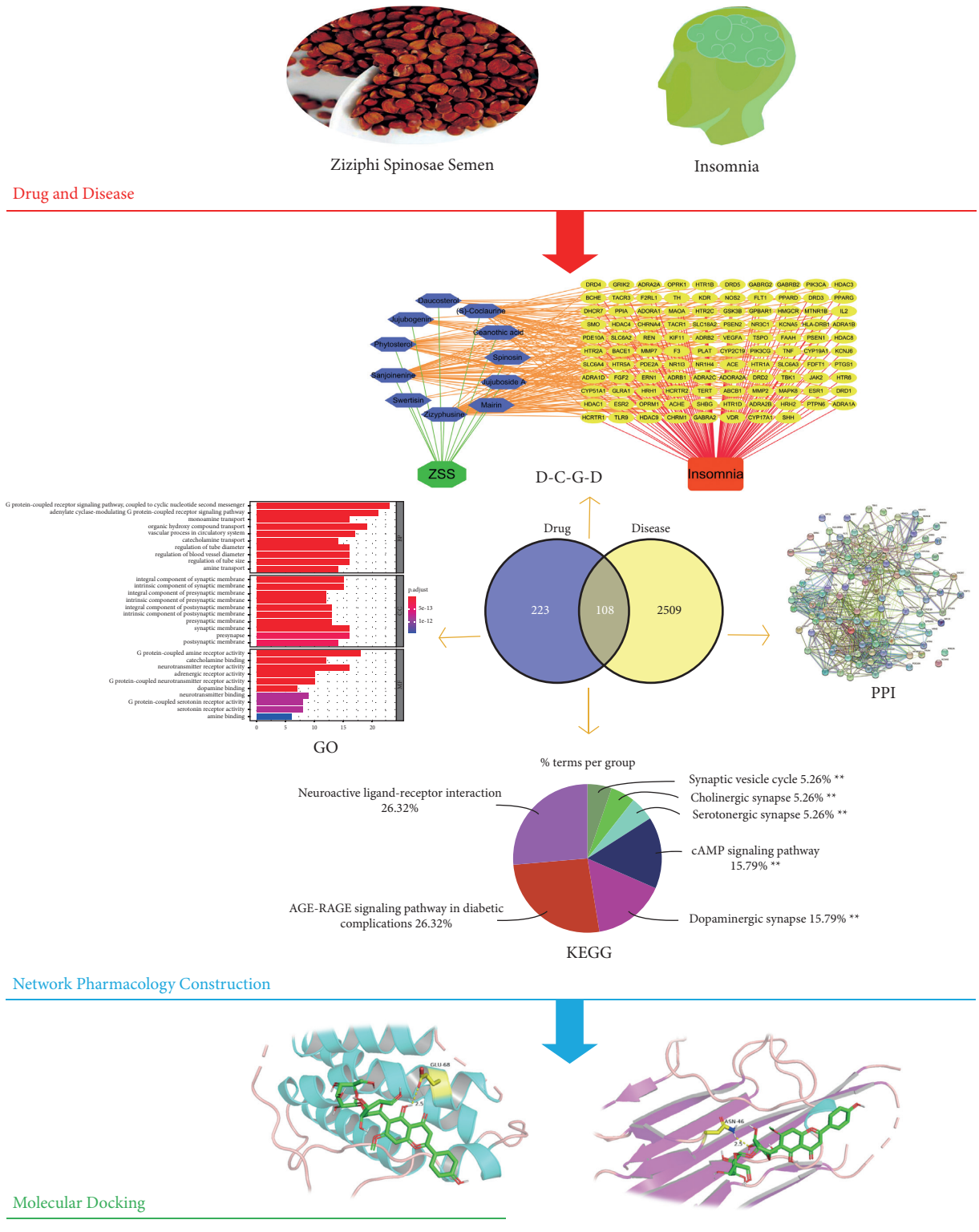


Figure 1: A comprehensive strategy diagram for the study of the mechanism of ZSS acting on insomnia.

three active ingredients for docking with their corresponding two targets, for a total of four component-target interactions to validate the prediction results. The protein crystal forms matching up to all targets were obtained from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org). We chose the appropriate protein crystal overall principle to be structurally intact and stable and obtained X-ray crystal structures of TNF (TNF-alpha) and IL-2 (interleukin-2), which have a PDBID of 5 UUI and 1 M4B, respectively. In addition, to further confirm the reliability of the docking results, we also docked these two proteins with their positive inhibitors curcumin and upadacitinib. After processing

targets and compounds using PyMOL software (version 1.3), docking work was performed using AutoDock Vina software (version 1.1.2), and the presentation files were processed by AutoDockTools.

3. Results

3.1. Screening of Active Compounds. We collated the collected data and compared the obtained component names, CAS numbers, and structures one by one to remove some inaccurate information. Nine active ingredients that met the above conditions were obtained by screening according to the previous parameters of OB ≥ 30% and DL ≥ 0.18. In addition, two components, jujuboside A and spinosin, were included in the provisions of Chinese Pharmacopoeia 2020. Studies have shown that saponins and flavonoids are the two most important active components in ZSS [23, 24], the most important of which are jujuboside A and spinosin. Jujuboside A can inhibit cancer cell growth through various mechanisms such as cell cycle arrest, proliferation inhibition, stem cell inhibition, and promotion of aging and can contaminate antitoxins [25, 26], and our analysis results show that there are many intersections of targets associated with it and insomnia targets. Spinosin exerts neuroprotective effects by inhibiting oxidative damage and has a significant antidepressant effect, and there is a great association between spinosin and insomnia [27, 28]. Although the kinetic values of these two components are relatively low, they have very excellent biological activity and are closely related to our study. Finally, all eligible compounds were combined and we obtained 11 fractions as final active compounds (Table 1).

3.2. Identification of Targets and Gene Symbols Associated with ZSS Compounds. After collecting related proteins from the STP database and simplifying (probability>0), we obtained 11 components in the ZSS and 331 known target symbols associated with them (Supplementary Table S1).

3.3. Acquisition of Known Therapeutic Gene Targets for Insomnia. We merged all target data obtained from the GeneCards and OMIM databases for insomnia-related targets and removed duplicated genes from them, collecting 2617 known insomnia therapeutic targets (Supplementary Table S2).

3.4. D-C-G-D Network Construction. According to Venn diagram (Figure 2(a)), we can visually see the cross genes common to drugs and diseases, of which 331 drug gene symbols and 2617 disease gene symbols have 108 overlaps. The D-C-G-D network (Figure 2(b)) also clearly shows how ZSS treats insomnia by acting between components and targets, and the details of the D-C-G-D network are shown in Supplementary Table S3.

3.5. PPI Network Construction. According to the results received by STRING platform, we got the action relationship map among 108 overlapping genes (Figure 3(a)), including two unconnected free genes, which may be important targets of ZSS to treat insomnia. After removing the free genes, we calculated the topological index node degree of 106 genes, which largely points to the degree of association of this gene with other genes. The higher node degree indicates that this gene may be more important, and the details of the specific gene name and its corresponding node degree are given in Supplementary Table S4. In addition, we calculated the mean value of 106 overlapping gene degrees to be 15.05, selected all genes with value greater than the mean value [34], and generated 48 core targets of relatively more important networks with other gene interactions (Figure 3(b)). Moreover, to better highlight the importance of key targets, after processing these related targets using Cytoscape software, it displayed 48 genes with higher values in the inner loop of Figure 3(c). The deeper the blue color and the larger the nodes, the higher the node degree; the line between them represented the interaction. These core targets may be the key genes of ZSS in the treatment of insomnia.

3.6. GO and KEGG Pathway Enrichment. GO enrichment analysis ($p < 0.01$) of 48 core target genes was classified and enriched according to three modules: biological process (BP), cellular component (CC), and molecular function (MF), with 998 GO terms enriched, of which the BP term accounted for a relatively high proportion, with 892 GO terms, mainly showing the coupling role and transport mode of some proteins in biological pathways, such as G protein-coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger (GO:0007187), adenylate cyclase-modulating G protein-coupled receptor signaling pathway (GO:0007188), monoamine transport (GO:0015844), and organic hydroxy compound transport (GO:0015850). CC is rich in 47 GO terms and analyzes that these core genes are closely related to those cell biofilms, mainly involving a variety of synaptic membranes, such as integral component of synaptic membrane (GO:0099699), intrinsic component of synaptic membrane (GO:0099240), and integral component of presynaptic membrane (GO:0099056). Similarly, we enriched 59 GO terms for MF, and in MF analysis, we could understand which receptor activities the core genes would affect and the forms of partial protein and target binding, mainly G protein-coupled amine receptor activity (GO:0008227), neurotransmitter receptor activity (GO:0030594), and catecholamine binding (GO:1901338). To present the GO enrichment results more directly, we based on p. Adjust intercepts for the top 10 terms from small to large for an abbreviated presentation (Figure 4), respectively, and the detailed results of the specific GO item analysis are given in Supplementary Table S5.

KEGG pathway enrichment analysis can help us further figure out the potential pathways of ZSS against insomnia; after all core genes are entered and after applying p value significance selection criteria, 44 genes from all clusters are systematically selected and 19 representative pathways are selected ($p < 0.01p < 0.01$), as shown in Figure 5(a), and these pathways are divided into 7 categories by signaling pathway functional clustering analysis, of which neuroactive ligand-

Table 1: Final active compounds selected as the details of ZSS in this study.

No.	Molecule name	MF	MW	OB (%)	DL	CAS number	References
1	Ceanothic acid	C30H46O5	486.76	33.41	0.77	21302-79-4	[29]
2	(S)-coclaurine	C17H19NO3	285.37	42.35	0.24	486-39-5	[30]
3	Daucosterol	C35H60O6	576.80	36.91	0.75	474-58-8	[31]
4	Jujubogenin	C30H48O4	472.78	34.96	0.62	54815-36-0	[32]
5	Phytosterol	C29H50O	414.79	36.91	0.75	949109-75-5	[31]
6	Sanjoinenine	C29H35N3O4	489.67	67.27	0.79	107446-80-0	[31]
7	Swertisin	C22H22O10	446.44	31.83	0.75	6991-10-2	[33]
8	Zizyphusine	C20H24NO4	342.45	41.52	0.55	107446-79-7	[29]
9	Mairin	C30H48O3	456.78	55.37	0.78	472-15-1	[31]
10	Jujuboside A	C58H94O26	1207.52	8.03	0.02	55466-04-1	[30]
11	Spinosin	C28H32O15	608.60	6.31	0.72	72063-39-9	[33]

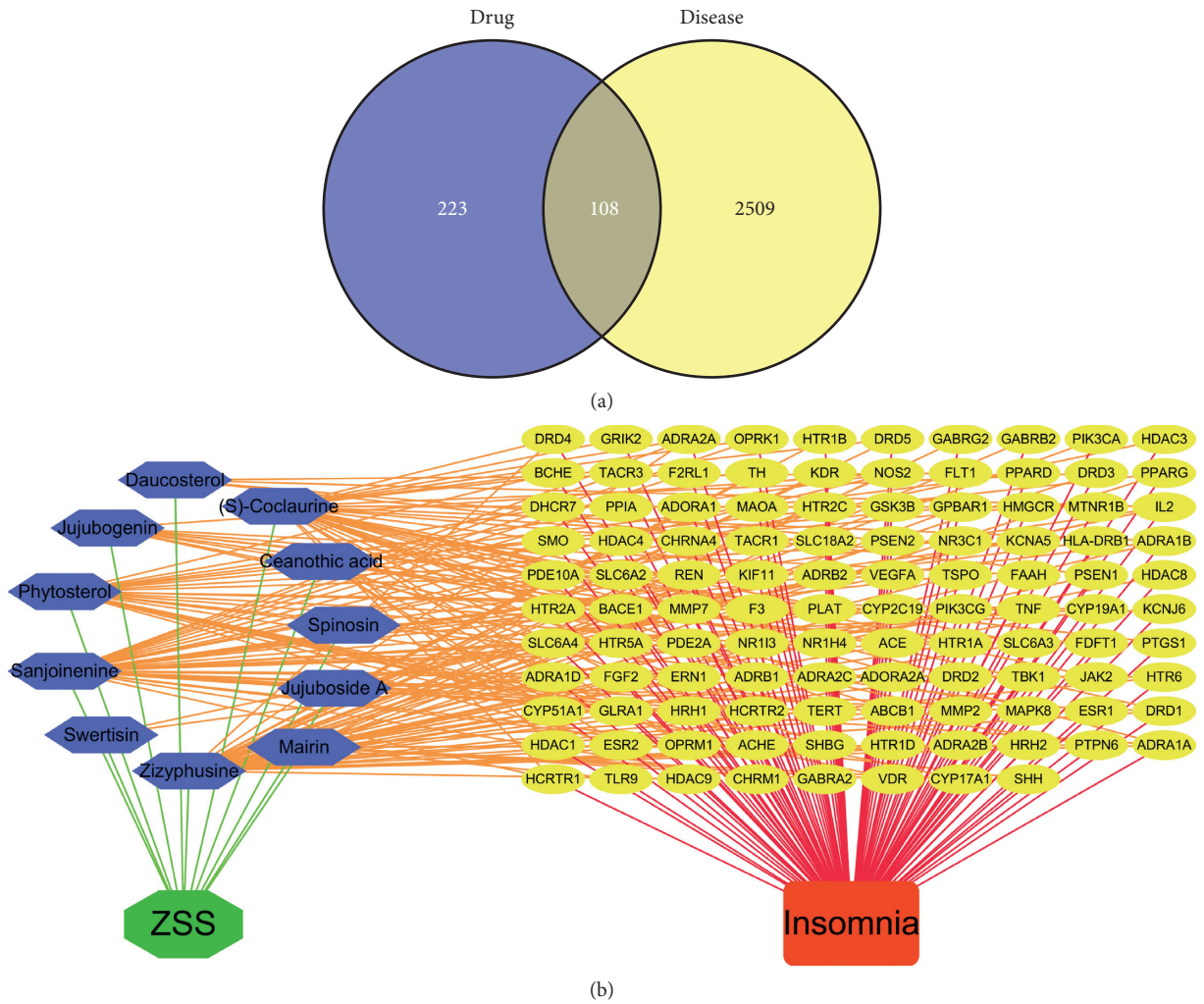


Figure 2: (a) Venn diagram of related targets of ZSS and insomnia. (b) D-C-G-D network. Green and red nodes indicate ZSS and insomnia, respectively. 11 blue nodes represent active ingredients in ZSS, 108 yellow nodes represent overlapping gene symbols between disease and drug, with edges indicating that nodes can interact, red edges indicate the action of insomnia with genes, green edges indicate the interaction of ZSS with active ingredients, and orange edges indicate the interaction of active ingredients with genes.

receptor interaction (KEGG:04080), AGE-RAGE signaling pathway in diabetic complications (KEGG:04933), dopaminergic synapse (KEGG:04728), cAMP signaling pathway (KEGG:04024), synaptic vesicle cycle (KEGG:04721), serotonergic synapse (KEGG:04726), and cholinergic synapse (KEGG: 04725) each represent a class of biological pathways (Figure 5(b)). Some of these pathways have been shown to be highly connected with insomnia, such as key

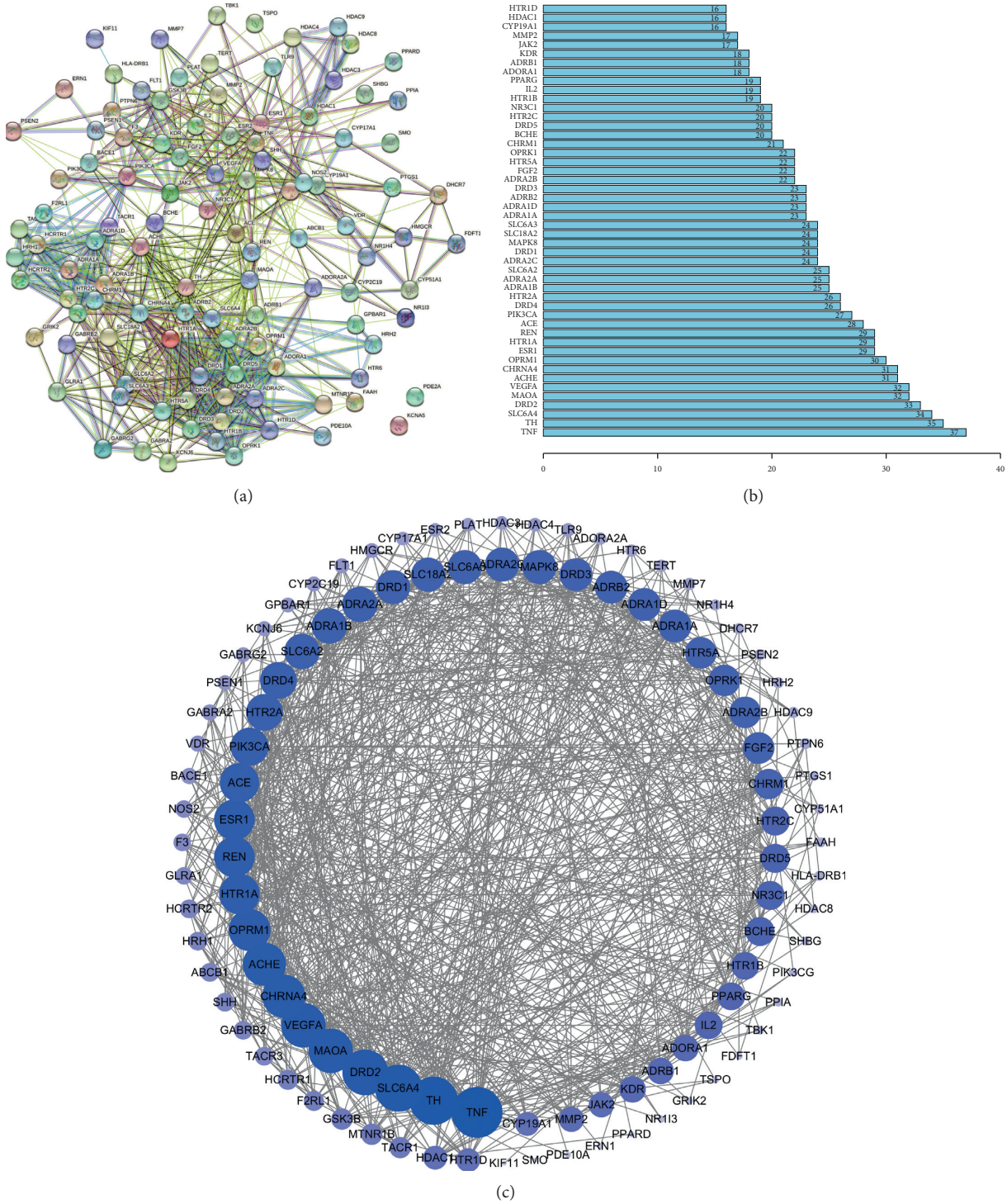


Figure 3: (a) The PPI network obtained from the STRING database platform. (b) The core gene degree barplot diagram. (c) The PPI network arranged according to degree value.

protein pathways that upregulate neuroactive ligand-receptor interaction signaling that can significantly improve insomnia [35], and some pharmacological treatments for psychiatric disorders also use selective blockade of presynaptic dopamine receptors in the frontal cortex, enhancing dopaminergic transmission, which is consistent with the results of our analysis of modulation of the dopaminergic synapse signaling pathway [36]. All details of the KEGG pathway enrichment analysis are described in Supplementary Table S6.

3.7. Computational Validation of Ingredient-Target Interactions. Based on the above prediction and analysis, we selected three classical components, swertisin, jujuboside A,

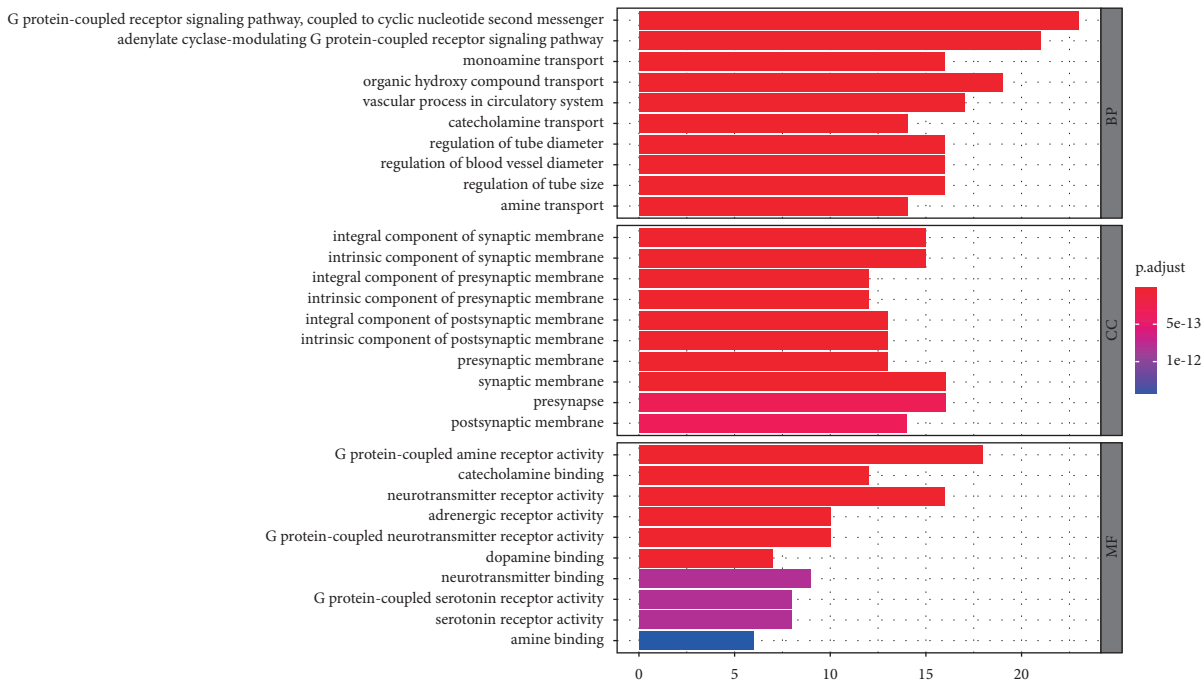
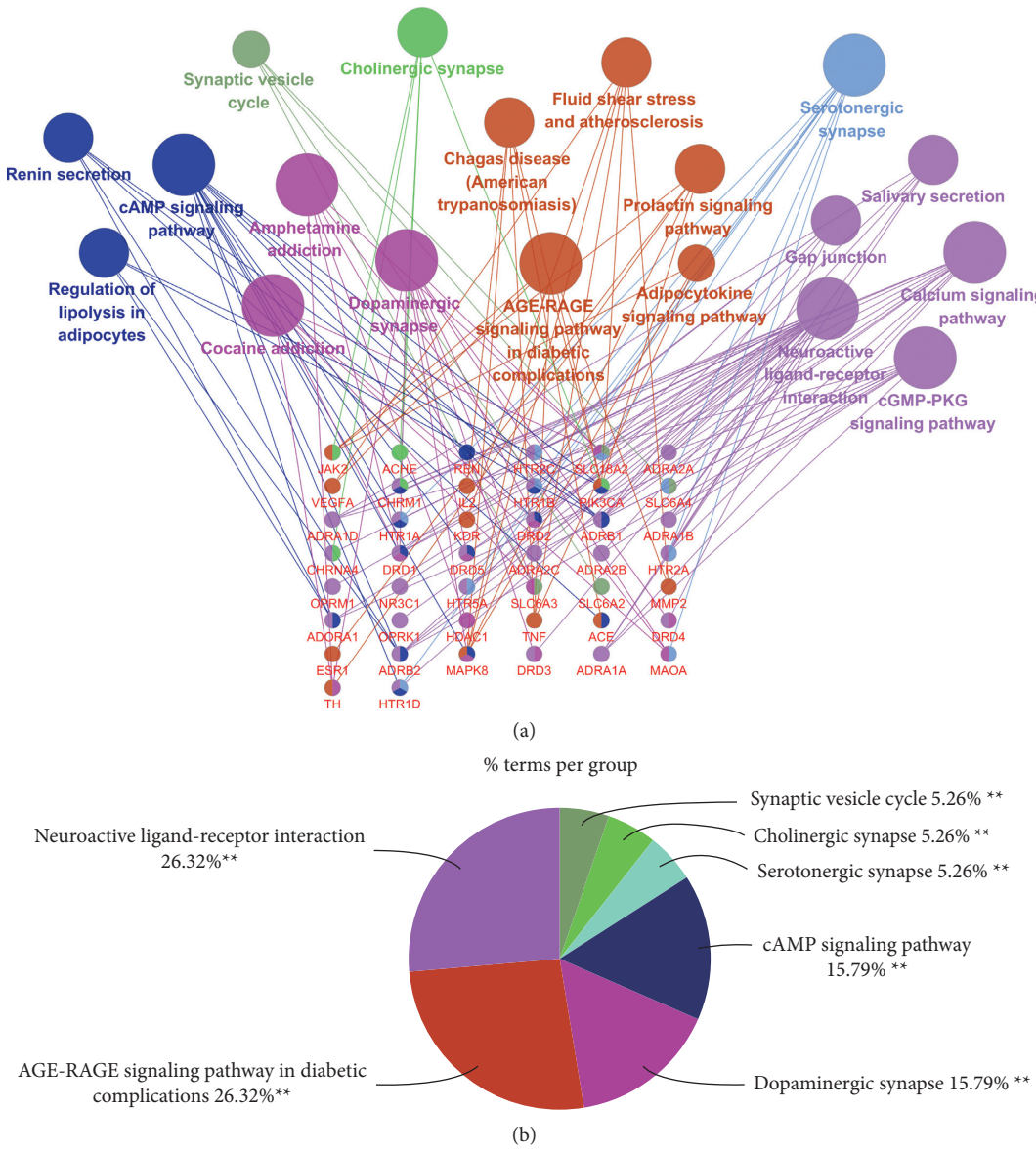


FIGURE 4: Bar graph of GO function enrichment of overlapping targets. The *Y*-axis represents GO terms. The *X*-axis indicates the number of genes enriched in this pathway. The redder the color, the smaller the p.adjust value; it also indicates the reliability and importance. The bluer the color, the greater the p.adjust value.

and spinosin, in ZSS for molecular docking with two important proteins, TNF and IL-2, to validate our analysis results. According to the docking results, we know that the binding energy of swertisin to TNF is −6.4 kcal/mol, the binding energy of jujuboside A to IL-2 is −7.8 kcal/mol, and the binding energy of spinosin to TNF and IL-2 is −6.9 and −5.9 kcal/mol, respectively. The lower binding energy between molecules represents that the stronger force binding energy between them (<0 kcal/mol) is carefully thought about being conducive to the binding reaction, which shows that our analysis is accurate and reliable, and the compounds can better act on the related proteins. The binding energy of IL-2 and its inhibitor curcumin is −6.5 kcal/mol, and the binding energy of TNF and the inhibitor upadacitinib is −6.4 kcal/mol. The compound and the inhibitor are docked into the same pocket of the protein, which means our results are more reliable. Figure 6 shows the specific situation of their combination, and the molecule is represented in a ball-stick model of atoms C, O, and N in green, red, and blue, respectively. Hydrogen bonds are indicated by dashed lines, and the numbers above represent distances in angstroms (Å). Among them, two amino acid residues in IL-2, GLN-57 and GLU-68, form multiple binding locations with jujuboside A (Figure 6(a)), while GLU-68 also binds to spinosin through hydrogen bonding (Figure 6(c)). Three amino acid residues in TNF, GLN-47, LYS-90, and GLU-135-48, form multiple hydrogen bonds with swertisin (Figure 6(b)), and another amino acid residue, ASN-46, forms a hydrogen bond with spinosin (Figure 6(d)). It linked the positive inhibitor curcumin to the two amino acids, GLY-27 and ARG-83, on IL-2 (Figure 6(e)), and upadacitinib forms two hydrogen bonds with the amino acid ILE-136 on TNF (Figure 6(f)). Our analysis shows that ZSS can effectively act on multiple targets such as TNF and IL-2 simultaneously through various components such as swertisin, jujuboside A, and spinosin, which makes the drug molecules affect the protein, and this achieves the effect of treating insomnia.

4. Discussion

With the acceleration of people's modern life rhythm, the number of insomnia population showed an increasing trend. Despite developed medical conditions and increasingly diverse means of treating psychiatric disorders, the problem of insomnia still cannot be effectively treated [37, 38]. Monomeric drugs have opened a channel of remission for some patients with neurological diseases, but it is undeniable that the effects of drug resistance and other side effects are not negligible [39, 40]. Most herbs originate from nature with relatively few side effects and adverse effects. ZSS is a pure natural traditional Chinese medicine that has a significant therapeutic effect on insomnia [41, 42], but its application and development have some limitations because of the complex mechanism of action of multiple components and multiple targets of traditional Chinese medicine. In this study, we used network pharmacology theory and related tools to select the active components and related targets of ZSS and linked them with insomnia-related targets to construct a D-C-G-D visual network, which more intuitively showed the specific relationship between drug components and diseases through 108 consensus genes. The results of PPI analysis showed complex interactions between overlapping genes, and 48 core targets were further screened according to the average node degree between them, such as TNF with a



(a)

(b)

Figure 5: (a) Correlation diagram between core targets and KEGG pathway. The smaller the term p.adjust, the larger the node. Nodes with different colors represent different pathway classifications, and the line between pathway and gene represents the interaction between them. (b) Representative classification of pathways and percentage.

high node degree, meaning that one key to ZSS treatment of insomnia may be the inhibition of inflammation *in vivo* [43], which also made our subsequent analysis more prominent.

GO functional analysis makes a simple annotation of gene products, BP enrichment results in the coupling effect and transport mode of proteins in biological pathways, for example, G protein-coupled receptors can mediate the normal function of the brain, of which H3 and H4 receptors are high-affinity receptors in the brain and immune system, respectively, and H3 receptors can regulate histamine and neurotransmitters, which control sleep quality [44]. The results of CC analysis show that intersection proteins or their products take part in the cellular environment, and active biofilm, such as a variety of protrusion membranes, can be utilized as tools to transmit synaptic currents in hypothalamic neurons, triggering arousal and regulating energy metabolism [45]. By MF analysis, we can determine that some protein receptor activities are regulated by drugs, such as neurotransmitter receptor, which is closely related to sleep, of which γ-amino butyric acid receptor plays an important role in controlling different vigilance states in the human body [46]. KEGG pathway analysis effectively helps us to associate genomes with cellular, species, and ecosystem functions. According to the results of our analysis, neuroactive ligand-receptor interaction, cAMP signaling pathway, and one of the key pathways to treat insomnia in ZSS, in the pathway, multiple targets such as ADRA1A, ADRA1B, and ADRA1D can be simultaneously regulated by components such as (S)-cointeraction, sanjoinenine, and zizyphusine, and upregulation of neuroactive ligand-receptor interaction

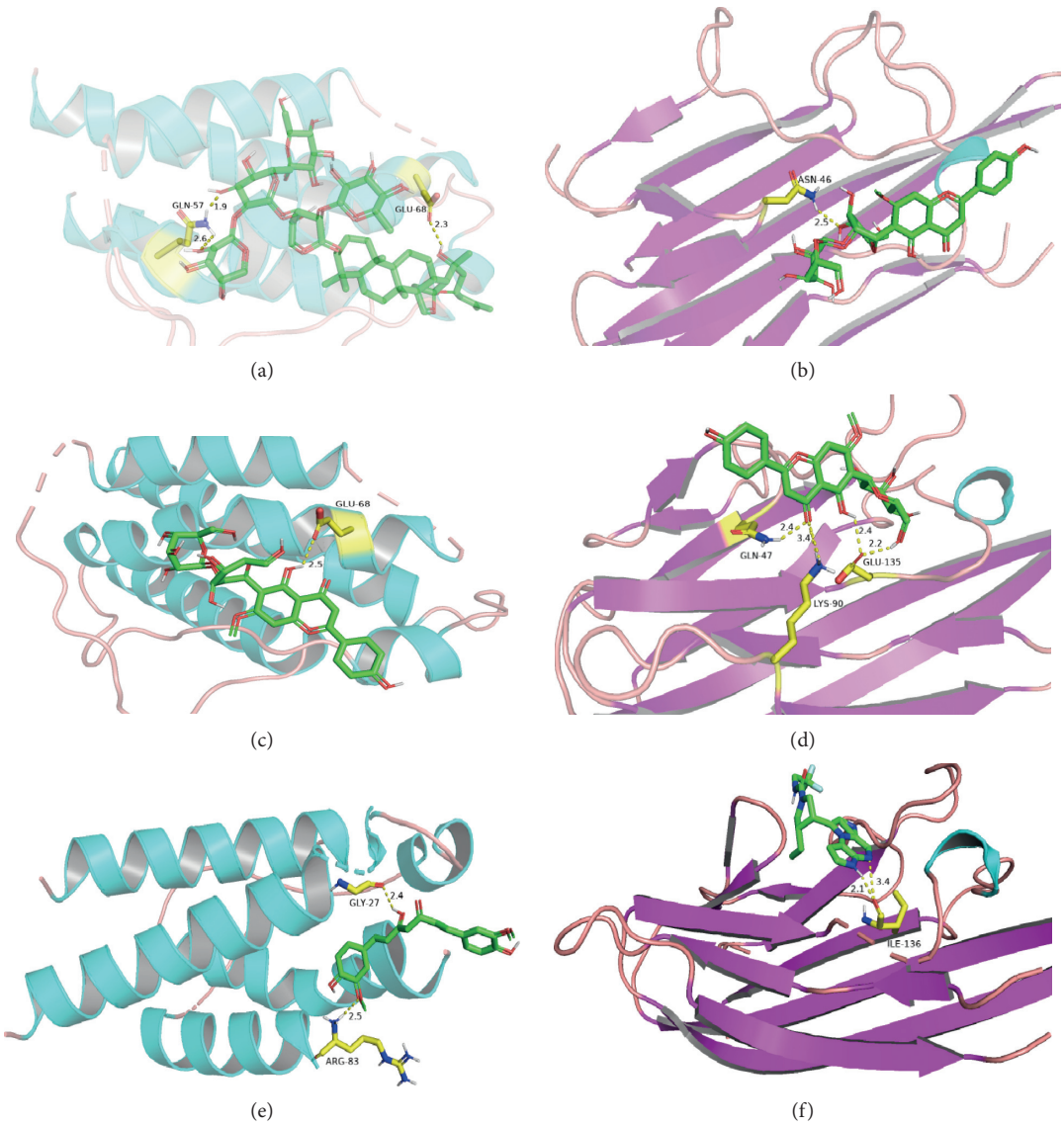


Figure 6: Selected compounds' interactions with the targets. (a) Jujuboside A with IL-2, (b) swertisin with TNF, (c) spinosin with IL-2, (d) spinosin with TNF, (e) curcumin with IL-2, and (f) upadacitinib with TNF. The molecule is represented in a ball-stick model with atoms C, O, and N in green, red, and blue, respectively. Hydrogen bonds are indicated by dashed lines, and the numbers above represent distances in angstroms (Å).

has also been an active process for the treatment of insomnia [35]. Besides identifying the specific pathways involved, cluster analysis of these pathways is also unique to our study, such as the cAMP signaling pathway, renin secretion, and dopaminergic synapse; these three pathways function similarly or are more closely linked during ZSS treatment of insomnia. Some protein kinases in the cAMP signaling pathway phosphorylate some specific proteins associated with insomnia, controlling the molecular and intracellular signaling of sleep and wake states [47]. Renin secretion can affect vital active states, and increased activity of the renin-angiotensin system in the brain elevates fluid intake, blood pressure, and resting metabolic rate renin secretion [48]. In addition, antipsychotics may improve psychomotor and routine by releasing dopamine in the prefrontal cortex, which may also involve the dopaminergic synapse signaling pathway [36]. Classification analysis of signaling pathways reveals population differences between different signaling pathways when specific drugs are used to treat diseases. Based on the prediction results, we finally performed in silico molecular docking validation. The tight binding of a variety of small molecule compounds and amino acids on proteins preliminarily showed that our analysis was reliable, and it was also to affirm the feasibility of traditional Chinese medicine, an active compound in ZSS, in the treatment of insomnia.

Complex diseases often have many symptoms, and sometimes, the use of monomeric components is less effective to treat complex diseases and has significant side effects. TCM has been used to treat diseases for thousands of years, with significant results. Meanwhile, the special philosophical ideas of Chinese medicine and the very complex

composition of TCM limit its further development, and the rise of network pharmacology brings another idea for the study of TCM. By constructing a drug-target-disease network, higher levels of information can be obtained by calculating the mechanism of simultaneous action of multiple components, multiple targets, and multiple pathways, and then using the construction of visual network charts can enable researchers to quickly and clearly know the mechanism of complex components in the treatment of diseases, which is a very practical new research tool. All of our work is dedicated to establishing a new analytical method with the help of network pharmacology, and besides exploring ZSS for the treatment of insomnia, we also hope to apply it in more traditional Chinese medicine research.

Data Availability

In this study, the sources are indicated in the text when the third-party data that may be involved are cited, and all datasets presented in this study are included within the article/supplementary materials and can be open to the public.

Additional Points

Contribution to the Field. Ziziphi Spinosae Semen (ZSS) has a unique effect in the field of insomnia and comforting the nerves, and the curative effect is remarkable. ZSS appears in many Chinese doctors' prescriptions for treating insomnia. It is known as the sleeping fruit of the East. However, at present, there are very few studies on how it works in the human body. With the help of network pharmacology, we conducted an association analysis on the important proteins of jujube seed in the treatment of insomnia for the first time and carefully studied the important biological pathways and cell functions that may be involved. Our work effectively makes the theoretical research of traditional Chinese medicine more closely integrated with the actual clinical application.

Disclosure

No human studies are presented in this article. No potentially identifiable human image or data are presented in this study.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Authors' Contributions

Gong Feipeng, Xie Luxin, and Chen Beili contributed equally to this work and are regarded as co-first authors.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81960716) and the National Key Research and Development Plan (2018YFC1707206 and 2017YFC1702900). The authors are thankful to Jiangxi University of Chinese Medicine for the help in conducting this study.

Supplementary Materials

Table S1: detailed information of related targets and gene symbols of compounds in ZSS. Table S2: the known therapeutic gene targets for insomnia. Table S3: detailed information about the D-C-G-D network. Table S4: overlapping gene symbols and corresponding degree values between disease and drug. Table S5: details of GO enrichment analyses. Table S6: details of KEGG pathway enrichment analyses. (*Supplementary Materials*)

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2021, Article ID 6486287, 11 pages
https://doi.org/10.1155/2021/6486287

Research Article

Based on UPLC-Q-TOF-MS/MS, Systematic Network Pharmacology, and Molecular Docking to Explore the Potential Mechanism of Fructus Aurantii for Major Depression Disorder

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Received 22 July 2021; Accepted 20 September 2021; Published 8 October 2021

Academic Editor: Junqing Huang

Background. Major Depression Disorder (MDD) is a common mental disease that has become one of the world's major medical diseases. Currently, the Fructus Aurantii (FA) has been widely used to treat depression. However, the active substance ingredients and potential mechanisms of the shell antidepression have not yet been clarified. *Method*. First, we used ultraperformance liquid chromatography-quadrupole/time-of-flight tandem mass (UPLC-QTOF-MS/MS) technology to identify the chemical composition of the FA. Then, it is predicted for active ingredients, pharmaceutical disease target screening by DiscoveryStudio 2016 (DS), Metascape, and other databases, PPI network diagram, and FC core pathway. Finally, the system network pharmacology results are verified by molecular contact verification. *Results*. Forty-six compounds in FA were identified, and twelve active ingredients were determined. Various database information, PPI network analysis of 41 intersections, and 20 core targets including DRD2, MTOR, FASP3, and PIK3P1 were integrated. Finally, the MDD treatment is indicated by molecular docking, and the most relevant potential signal pathway is the PI3K-Akt signaling pathway.

1. Introduction

Major Depression Disorder (MDD) is the most common chronic mental disease nowadays, mainly manifested as depression, loss of pleasure, cognitive impairment, panic, lack of initiative, anorexia, insomnia, suicide injury, and other behaviors. Therefore, it is difficult to treat this disease due to its complexity, heterogeneity, and high recurrence [1–4]. Many records related to depression treated using traditional Chinese medicine are available, which divide MDD into six syndrome types: liver depression and spleen deficiency, liver depression and phlegm stagnation, liver depression and qi stagnation, heart and spleen deficiency, heart and kidney disharmony, and qi stagnation and blood stasis [5]. According to the latest report released by the World Health Organization (WHO), there are about 350 million people suffering from MDD in the world, with an average incidence of 4.4%. It is estimated that depression will become the world's largest burden disease by 2030 [6, 7]. At present, the pathogenesis of MDD has not yet been established, and the conventional western medicine uses fluoxetine hydrochloride and sertraline, which are exerting side effects, so it is difficult for them to achieve satisfactory treatment effect. In general, antidepressants cause side effects that ruin the normal daily life, so that patients have poor compliance.

Therefore, in this work, the multicomponent approach used by the traditional Chinese medicine was compared to the traditional treatment of MDD in consideration of the shortcomings of western medicine.

Fructus Aurantii (FA) is the dry immature fruit of the *Citrus Aurantium* L. It often used as a Chinese medicine for regulating qi and used in clinical practice and is one of the components of the most common Chinese medicine prescriptions, including Chaihu Shugan San and Weichang

Anwan [8, 9]. The main active ingredients of the FA include flavonoids, coumarins, volatile oils, and alkaloids, which exert an antidepressant effect [10], lower the cholesterol [11], have antitumor effect [12], promote the gastrointestinal power [13], and have other pharmacological activities. Psychological stress due to MDD can lead to gastric emptying and abnormal gastrointestinal hormone levels; FA not only has antidepressant effects, but also promotes the gastrointestinal power by regulating the hypothalamic-pituitary-adrenal axis, alleviating the discomfort caused by MDD [14]. However, the mechanism of action of FA on MDD is not yet clear. Therefore, this study explored the potential mechanism of FA on MDD using UPLC-QTOF-MS/MS, system network pharmacology, and molecular docking (Figure 1).

The FA is mainly produced in the province of Jiangxi, and it is of the highest quality. Therefore, this experiment used FA as raw material, and the chemical composition was determined by UPLC-QTOF-MS/MS. In addition, the active ingredient, disease target, and the action pathway were investigated to obtain the underlined mechanism used by FA on MDD, which was further verified by molecular docking [15–17].

2. Materials and Methods

2.1. Chemicals, Agents, and Materials. FA was purchased in Yicheng Town, Zhangshu City, Jiangxi Province (Jiangxi, China).

Professor Geifei, who is the head of the Chinese Medicine Resources Discipline Group, at the Jiangxi University of Traditional Chinese Medicine characterized the dry unripe fruit of *Citrus aurantium* L. Twelve pure compounds (purity >98%) such as eriodictyol, 5-demethylnobiletin, naringenin, nobiletin, 3′, 4′, 3, 5, 6, 7, 8-heptamethoxyflavone, auraptene, ferulic acid, umbelliferone, hesperidin, naringin, neohesperidin, and limonin were selected according to the 2015 "*Chinese Pharmacopoeia*" method to process FA and purchased from Sichuan Vicky Biotechnology Co. Ltd. (Sichuan, China). Methanol was purchased from Xilong Scientific Co, Ltd. (Guangdong, China). Acetonitrile (Tedia, USA), formic acid (ACS), and ethanol were purchased from the National Medicine Group Chemical Reagent Co., Ltd. (Shanghai, China). The water used in this work was ultrapure, obtained by the Milli-QB system.

3. UPLC-QTOF-MS/MS

3.1. Preparation of the Standard and Sample Solutions. The impurities were removed from FA, then it was washed, moisturized, and cut into thin slices, and the broken core was sifted out after drying [18]. FA crude powder (10 g) was precisely weighed and placed in a conical bottle with 100 ml 70% ethanol, thoroughly mixed, socked for 0.5 h, and boiled under reflux for 1.5 h, and the filtrate was collected. In the above methods, 80 mL 70% ethanol and 60 ml 70% ethanol were used. Three filtrates were mixed, and the filtrate was condensed and made into extract. Then, 1 g concrete was weighed and placed in 100 ml methanol to dilute it. Finally, the solution was filtered through a 0.22 mm microporous membrane.

Twelve milligrams of each reference compound (eriodictyol; 5-demethylnobiletin; naringenin; nobiletin; 3′, 4′, 3, 5, 6, 7, 8-heptamethoxyflavone; auraptene; ferulic acid; umbelliferone; hesperidin; naringin; neohesperidin; and limonin) was weighed, transferred to 10 ml volumetric flasks, and diluted with methanol to reach the volumetric mark. The solutions were filtered through a 0.22 μm microporous membrane to obtain the standard solutions.

3.2. Ultraperformance Liquid Chromatography-Quadrupole-Time-of-Flight Tandem Mass Conditions. Chemical analysis was conducted on a UPLC- (Nexera X2 LC-30A, Shimadzu Corp., Japan) hybrid triple quadrupole-time-of-flight mass spectrometer (Triple TOF™5600+, AB Sciex, Forster City, FA, USA) connected with an electrospray ionization source (ESI). Acquity UPLC BEH C18 column (2.1 × 100 mm × 1.7 μm) was used to perform the chromatographic separation with a flow rate of 0.3 ml/min at 40°C. A linear gradient program with a mobile phase system including solvent A (100% acetonitrile, v/v) and solvent B (0.01% formic acid in water, v/v) was performed as follows: solvent A at 5% ~20% for 0.01~2 min, 20%~30% for 2~10 min, 30~55% for 10~25 min, 55%~100% for 25~30 min, 100% A for 30~32 min, and 100%~5% for 0.5 min, with isocratic elution performed at 5% for 2.5 min.

The instrumental setting of Q-TOF-MS/MS was the following: ion source gas 1 (GSI) and gas 2 (GS2) were both set to 60 kPa, curtain gas (CUR) was set to 35 kPa, ion spray voltage floating (ISVF) was set to 5500 V, ion source temperature (TEM) was set at 500°C, collision energy (CE) was set at 45 eV, collision energy spread (CES) was set at 45 ± 10 eV, the declustering potential (DP) was set at 100 V, and nitrogen was used as a nebulizer and auxiliary gas. Samples were analyzed in positive ionization modes with a sFAnnin mass-to-charge (m/z) range from 50 to 1,000. Data were collected in information-dependent acquisition (IDA) mode and analyzed by PeakView®1.2 software (AB Sciex).

3.3. Ingredients Identification Analysis. The chemical composition of FA was obtained from existing databases and documents, including TCMSP, SCHINDER, TCMIP, China Knowledge Network, and Geen Medical. The data were sorted out and the FA composition database was established. (+) MS data was imported into PeakView®1.2, "XIC Manager" was used to analyze the 70% ethanol extract of FA, and each chemical composition in the retention time and its corresponding primary and secondary mass spectrum data were obtained. It combined the base peak chart with the reference substance and compared it with the data reported in the relevant references, and the chemical composition was confirmed.

3.4. Identification of the Related Targets of FA Components. In this study, the chemical compounds were imported into Discovery Studio 2016 (DS), through "ADMET Descriptors" to screen the active ingredients. The related targets of FA components were obtained from SwissTargetPrediction (https://www.swisstargetprediction.ch/) and Pharmmapper (https://www.lilab-ecust.cn/pharmmapper/).

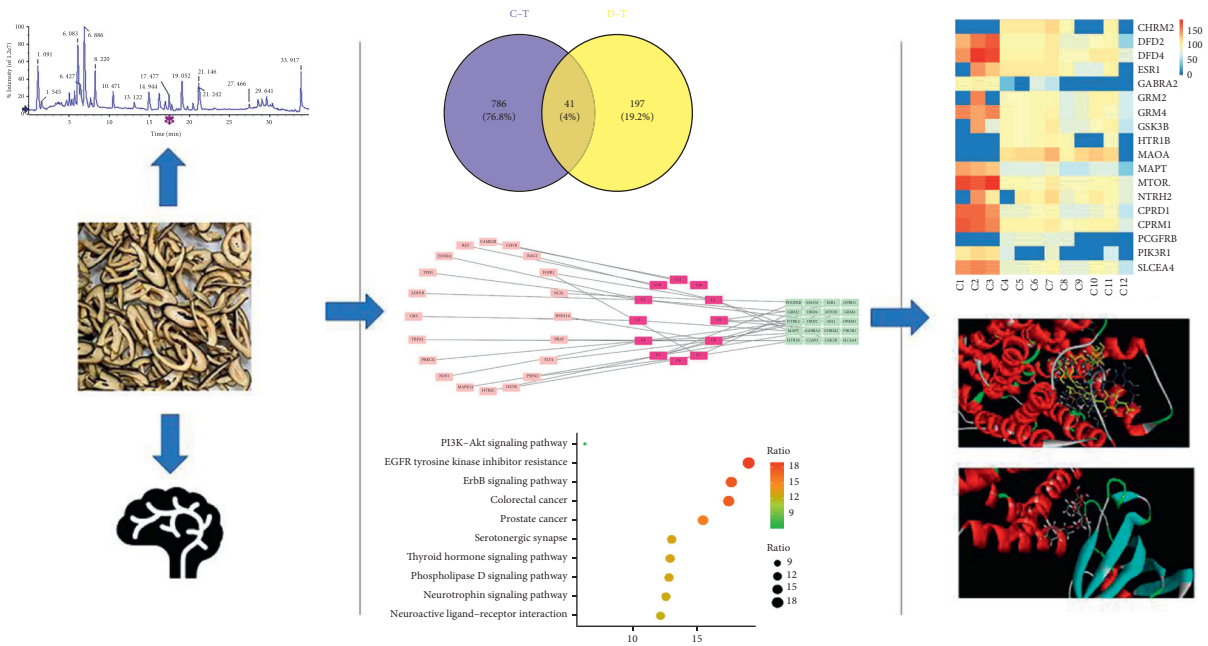


Figure 1: Based on systems pharmacology strategy to study the mechanism of FA in treatment of MDD.

3.5. MDD-Associated Targets Collection. The keyword "depression" was used in the Genecards database (https://www.geneFArds.org/) and Online Mendelian Inheritance in Man (OMIM, https://omim.org/) to identify disease targets associated with MDD.

3.6. Construction of the Protein-Protein Interaction Network. VENNY2.1 software (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to obtain the overlapping targets between FA-related targets and MDD-related targets. Overlapping targets were imported into the Retrieval of Interacting Genes/Proteins (STRING) 11.0 (https://www.string-db.org/), and the interaction results were saved. The interaction resulting file was added to Cytoscape v3.9.0 software, and the Protein-Protein Interaction (PPI) network was obtained.

3.7. Go Function and Genomes (KEGG) Pathway Enrichment Analysis. Metascape is a powerful tool for the analysis of gene function annotation. It applies the bioinformatics analysis method to a large number of genes or proteins, and it uses more than 40 independent databases to annotate genes or proteins, for the enrichment analysis and the construction of the PPI network. WebGestalt was used to perform the enrichment analysis. The software can meet user requirements from different areas, by pathway figure and hierarchical network visualization. Since its release in 2005, it has gradually become one of the most popular software types in the field of biology [19].

Overlapping targets were imported into Metascape (https://www.metascape.org/), "Homo sapiens" was selected as "Organism of interest," and "Pathway" and "KEGG" were selected as "Functional Database." Finally, "genome" was selected as the "Select reference set," and the KEGG pathway analysis was performed.

3.8. I-D-G Network Construction. Related files were established as "core ingredients-core targets," "core ingredients-Secondary targets," "disease-core targets," and "disease-Secondary targets." Then, the files were imported into Cytoscape v3.9.0 to build a "ingredients-disease-gene symbols" network.

3.9. Computational Validation of I-T Interactions. The computational software was used to simulate the interaction between active compounds and core targets and to explore the binding ability and binding mode between compounds and targets. Therefore, 12 drug components were selected for the molecular docking with 14 core targets. The PDB format of the target protein was downloaded from the structural bioinformatics protein database (PDB, https://www.rcsb.org/). The protein structure was used to simulate dehydration, hydrogenation, and removal of proteins using DiscoveryStudio2016 (DS) software, and then molecular docking was performed.

4. Results

4.1. Identification of the Chemical Constituents in FA by UPLC-QTOF-MS/MS. UPLC-QTOF-MS/MS is an analytical technique widely used in drug research, which can perform chromatography and identify fragment ions and cleavage patterns. Figure 2 represents a (+) ESI-MS mass total ion chromatogram of the FA extract (TIC). A total of 46 compounds were identified using PeakView®1.2 software and literature comparison, including 33 flavonoids, 10 coumarins, 3 limonoids, and other compounds. Twelve

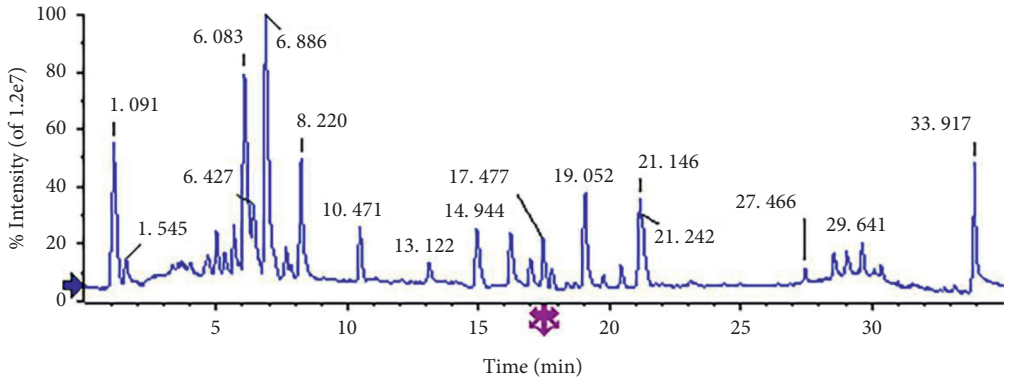


Figure 2: The (+) ESI-MS mass total ion chromatogram (TICs) of the FA by UPLC/Q-TOF-MS/MS.

ingredients (eriodictyol; 5-demethylnobiletin; naringenin; nobiletin; 3′, 4′, 3, 5, 6, 7, 8-heptamethoxyflavone; auraptene; ferulic acid; umbelliferone, hesperidin; naringin; neohesperidin; limonin) were confirmed by the comparison with the reference substance (Table 1).

4.2. Screening of the Active Components in FA. The blood-brain barrier (BBB) is an important structure in the human body to maintain a stable brain environment; thus, lipophilic and water-soluble drugs do not cross easily the BBB, limiting the effect of 95% of drugs on the central nervous system [25]. Depression is a neurological disorder occurring in the central nervous system; thus, the relationship between the ability of antidepressants and the permeability of the BBB is of utmost importance to obtain an effective result.

Forty-six compounds were screened by DS software, and the screening standard used was "ADMET_BBB ≥ −0.3" [26]. Seven active compounds of FA were obtained, such as isomerancin, meranzin, prangenin, bergapten, 5, 6, 7, 4′-tetramethoxyflavone, 3′, 4′, 7, 8-tetramethoxyflavone, and Scutellarein. Scutellarein has a good permeability in the BBB model in vitro, has the ability to improve BBB dysfunction, and treats stroke, Alzheimer's disease, and other central nervous system diseases [27, 28]. Bergapten has extremely high bioavailability, can cross the BBB, protect nerves, and treat brain diseases, and it is a potential candidate as antidepressant drug [29]. However, some compounds not meeting the screening criteria were also chosen in order to comprehensively evaluate MDD treatment. For example, naringin and neohesperidin are the main components of FA according to the 2020 "*China Pharmacopoeia*." In addition, according to the literature, meranzin hydrate and nobiletin can reduce ROS, MDA, IL-6, and TNF-α levels in MDD rats; these compounds can cause anti-inflammatory and antioxidative stress [30]. Naringin can improve the hippocampus mTOR and P70S6K phosphorylation level [31]. Hesperidin can improve CUMS rat behavior by regulating the function of the hypothalamic-pituitary-adrenal (HPA) axis [32]. Although these compounds do not satisfy the screening criteria of crossing the BBB, some studies showed their biological activity; thus, these 5 compounds were also used as candidate active components. Finally, 12 compounds were chosen as active ingredients (Table 2).

4.3. Acquisition of the Related Targets of FA Components. All the targets of the compounds in FA were collected from the SwissTargetPrediction software and Pharmmapper software. A total of 827 FA-related targets were obtained after the removal of the repeated targets.

4.4. Identification of the MDD-Associated Targets. The screening standard used was "Relevance score>10" [33], which allowed obtaining 71 and 189 MDD-related targets by Genecard database and OMIM database, respectively. A total of 238 known MDD-related targets were collected after the removal of the redundant information.

4.5. PPI Network Analysis. The disease targets and composition targets were combined and imported into Venny2.1 software, and 41 overlapping targets were obtained by the Venny diagram (Figure 3(a)) and PPI network (Figure 3(c)). The PPI network diagram was visualized by the Cytoscape software for a further analysis (Figure 3(b)). The results showed that NCS1 did not intersect with other targets. "Degree" could allow the evaluation of the effect to each target to a certain extent; thus, the median value of "degree" was used as a screening condition [34], and degree was ranked as a core target (Figure 3(d)). For example, DRD2, FASP3, and PIK3P1 could be important research targets for the treatment of MDD with FA.

4.6. Analysis of the Go and KEGG Pathway Enrichment. WebGestalt and Metascape were used to perform Go function and KEGG pathway enrichment analysis on 20 core targets. The results showed the involvement of 12 Biological Processes (BP), 16 Cellular Components (CC), and 13 Molecular Functions (MF). BP was related to cell communication, response to stimulus, and biological regulation, CC was related to membrane, cell projection, and endomembrane system, and MF was related to protein binding, molecular transducer activity, and ion binding (Figure 4(a)).

There is a close relationship between the core target and the signaling pathway. The screening results with "value of enrichment ratio" resulted in 10 pathways, including EGFR tyrosine kinase inhibitor resistance (hsa04080), ErbB signaling pathway (hsa04072), thyroid hormone signaling pathway

Table 1: Identification of chemical constituents of FA immaturus ethanol extract.

No	Molecular formula	Extraction mass (Da)	Error (ppm)	tR/ (min)	Identity	Classification	Ref.
1	$C_{27}H_{30}O_{15}$	511.1658	2.8	3.73	Lonicerin	Flavonoid	[20]
2	$C_{15}H_{12}O_6$	289.0707	0.2	4.98	Eriodictyol	Coumarin	[21]
3	$C_{22}H_{24}O_9$	433.1493	1.6	20.45	3′,4′,3,5,6,7,8-Heptamethoxyflavone	Flavonoid	[22]
4	$C_{27}H_{32}O_{15}$	597.1814	2.3	4.99	Neoeriocitrin	Flavonoid	[20]
5	$C_{27}H_{32}O_{15}$	597.1814	2.3	4.7	Eriocitrin	Flavonoid	[20]
6	$C_{10}H_{10}O_4$	195.0652	−2.8	5.37	Ferulic acid	Total phenolic acids	[20]
7	$C_{16}H_{14}0_6$	303.0863	0.6	6.88	Hesperetin	Flavonoid	[21]
8	$C_{27}H_{32}O_{14}$	581.1865	2.4	5.67	Narirutin	Flavonoid	[21]
9	$C_{15}H_{12}O_5$	273.0758	−1	12.04	Naringenin	Flavonoid	[21]
10	$C_{21}H_{22}O_{10}$	435.1286	1.2	6.07	Naringenin-7-O-glucoside	Flavonoid	[20]
11	$C_{22}H_{24}O_{11}$	465.1391	0.8	6.87	Hesperetin-7-O-glucoside	Flavonoid	[21]
12	$C_{28}H_{34}O_{15}$	611.1971	1.6	6.42	Hesperidin	Flavonoid	[20]
13	$C_{27}H_{32}O_{14}$	581.1865	2.4	6.06	Naringin	Flavonoid	[23]
14	$C_{28}H_{34}O_{15}$	611.1971	1.6	6.87	Neohesperidin	Flavonoid	[23]
15	$C_{22}H_{24}O_{11}$	465.1391	0.8	7.24	Eriodictiol-7-O-glucoside	Flavonoid	[21]
16	$C_{15}H_{16}O_4$	261.1121	−0.9	16.22	Isomerancin	Coumarin	[20]
17	$C_{15}H_{16}O_4$	261.1121	−0.9	8.22	Meranzin	Coumarin	[20]
18	$C_{15}H_{18}O_5$	279.1227	−0.1	8.23	Meranzin hydrate	Coumarin	[20]
19	$C_{11}H_6O_4$	203.0339	−2.7	9.36	Xanthotoxol	Coumarin	[20]
20	$C_{26}H_{27}O_{14}$	564.1474	2.6	9.86	Isonaringin	Flavonoid	[20]
21	$C_{16}H_{14}O_5$	287.0914	−0.8	10.45	Prangenin	Coumarin	[21]
22	$C_{16}H_{14}O_5$	287.0914	−0.8	10.47	Isosakuranetin	Flavonoid	[21]
23	$C_{28}H_{34}O_{14}$	595.2021	1.7	6.88	Poncirin (isosakuranetin-7-O-neohesperidoside)	Flavonoid	[21]
24	$C_{28}H_{34}O_{14}$	595.2021	1.7	8.22	Neoponcirin	Flavonoid	[21]
25	$C_{28}H_{34}O_{14}$	595.2021	1.7	9.85	Isosakuranetin-7-O-rutinoside	Flavonoid	[21]
26	$C_{28}H_{34}O_{14}$	595.2021	1.7	5.89	Poncirin	Flavonoid	[20]
27	$C_{18}H_{16}O_6$	329.102	0.3	11.53	4′-Hydroxy-5,6,7-trimethoxyflavone	Flavonoid	[22]
28	$C_{12}H_8O_4$	217.0495	−2.3	14.77	Bergapten	Coumarin	[20]
29	$C_{19}H_{24}O_5$	333.1697	0.5	14.93	Marmin	Coumarin	[20]
30	$C_{19}H_{18}O_6$	343.1176	−0.5	16.98	5,7,8,4′-Tetramethoxyflavone	Flavonoid	[22]
31	$C_{19}H_{18}O_6$	343.1176	−0.5	16.99	5,6,7,4′-Tetramethoxyflavone	Flavonoid	[22]
32	$C_{19}H_{18}O_6$	343.1176	−0.5	19.08	3′, 4′,7,8-Tetramethoxyflavone	Flavonoid	[22]
33	$C_{19}H_{18}O_6$	343.1176	−0.5	11.08	Scutellarein	Flavonoid	[21]
34	$C_{21}H_{22}O_8$	403.1387	0.6	19.04	3,5,7,8,3′, 4′-Hexamethoxyflavone	Flavonoid	[22]
35	$C_{21}H_{22}O_8$	403.1387	0.6	19.05	Nobiletin	Flavonoid	[20]
36	$C_{28}H_{34}O_9$	515.2276	1	19.75	Nomilin	Limonoid	[22]
37	$C_{20}H_{20}O_7$	373.1282	0.7	15.04	5,7,8,3′,4′-Pentamethoxyflavone	Flavonoid	[22]
38	$C_{20}H_{20}O_7$	373.1282	0.7	16.72	Auranetin	Flavonoid	[21]
39	$C_{20}H_{20}O_7$	373.1282	0.7	21.15	Tangeretin	Flavonoid	[23]
40	$C_{20}H_{20}O_7$	373.1282	0.7	16.97	Sinensetin	Flavonoid	[20]
41	$C_{20}H_{20}O_7$	373.1282	0.7	16.72	Isosinensetin	Flavonoid	[21]
42	$C_{21}H_{22}O_9$	419.1337	1.1	21.27	Natsudaidain	Flavonoid	[20]
43	$C_{20}H_{20}O_8$	389.1231	0.2	[23].07	5-Demethylnobiletin	Flavonoid	[22]
44	$C_9H_6O_3$	163.039	−4.1	5.33	Umbelliferone	Coumarin	[20]
45	$C_{19}H_{22}O_3$	299.1642	−0.3	29.63	Auraptene	Coumarin	[24]
46	$C_{26}H_{30}O_8$	471.2013	−4	17.47	Limonin	Limonoid	[22]

(hsa04919), PI3K-Akt signaling pathway (hsa04151), and neuroactive ligand-receptor interaction (hsa04722). Some antidepressants exert a therapeutic effect through the ErbB signaling pathway and often induce the downregulation of NRG1/ErbB4; thus, our hypothesis was that antidepressants could play a role through this channel [35]. Therefore, the above pathways were obtained by screening, providing a direction in the research of a new mechanism of action of FA.

4.7. I-D-G Network Analysis. Cytoscape v3.9.0 software was used to build an "ingredients-disease-gene symbols" network, as shown in Figure 5, in which the pink node represented the common targets, the twelve magenta nodes represented the core ingredients of FA, and the 20 green nodes represented the core gene symbols between FA and MDD. This result revealed that FA could play a role in the treatment of MDD through multiple targets.

Table 2: Absorption parameters of 12 FA components.

No	Component	Molecular formula	BBB
C1	Hesperidin	$C_{28}H_{34}O_{15}$	ND
C2	Naringin	$C_{27}H_{32}O_{14}$	ND
C3	Neohesperidin	$C_{28}H_{34}O_{15}$	ND
C4	Isomerancin	$C_{15}H_{16}O_4$	−0.126
C5	Meranzin	$C_{15}H_{16}O_4$	−0.098
C6	Meranzin hydrate	$C_{15}H_{18}O_5$	−0.852
C7	Prangenin	$C_{16}H_{14}O_5$	−0.282
C8	Bergapten	$C_{12}H_8O_4$	−0.233
C9	5,6,7,4′-Tetramethoxyflavone	$C_{19}H_{18}O_6$	−0.185
C10	3′,4′,7,8-Tetramethoxyflavone	$C_{19}H_{18}O_6$	−0.185
C11	Scutellarein	$C_{19}H_{18}O_6$	−0.185
C12	Nobiletin	$C_{21}H_{22}O_8$	−0.478

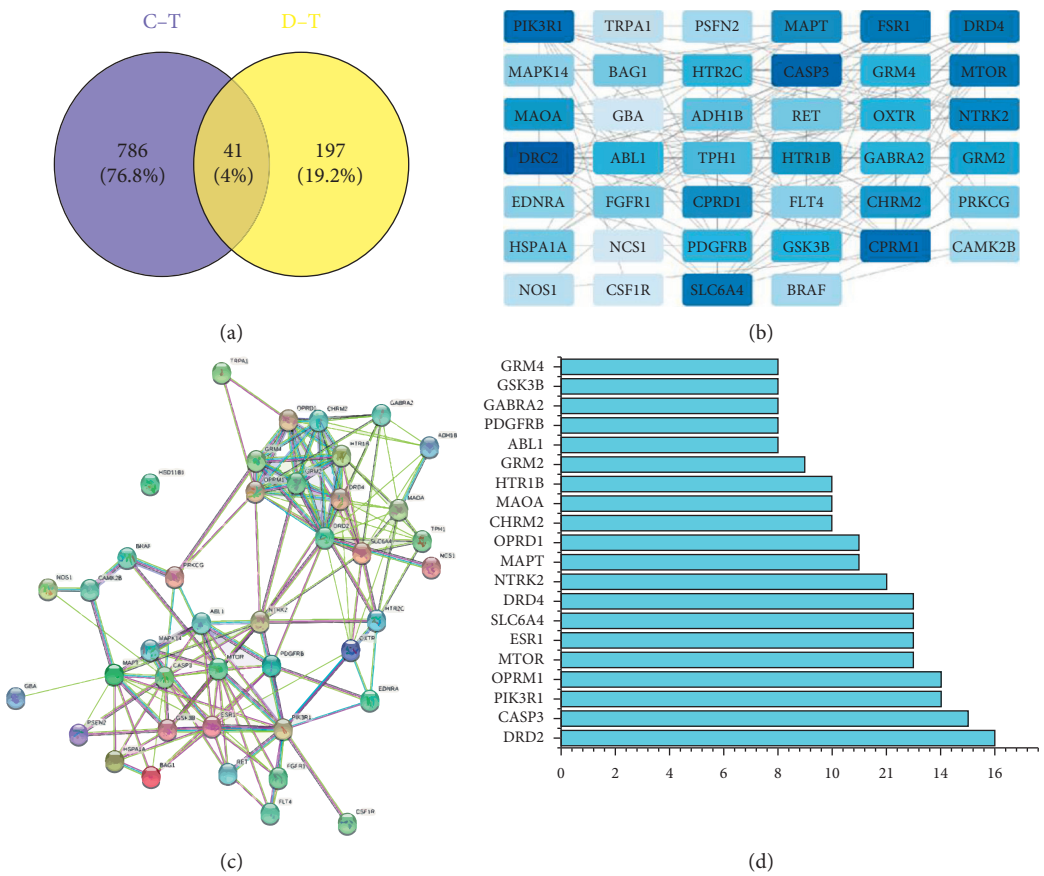


Figure 3: (a) Venn diagram of related targets of Aurantii Fructus (FA) and Major Depressive Disorder (MDD). (b) PPI network of overlapping targets between drug and disease. The more the color, the higher the value. (c) PPI network. (d) Bar plot of the 20 core targets. The x-axis represents the degree. The y-axis represents the target protein.

Cytoscape software analysis revealed that the values of C4 (isomerancin, degree = 7), C1 (hesperidin, degree = 6), C5 (meranzin, degree = 6), C2 (naringin, degree = 5), and C7 (prangenin, degree = 5) were at the top of the list, meaning that these compounds could be the core components of FA effective in the treatment of MDD.

4.8. Computational Validation of I-T Interactions. The binding ability of the receptor to the ligand is closely related to the molecular docking score. The higher the score, the stronger the binding ability of the receptor. The docking scoring results showed that most of the receptors had good binding ability to the ligands except ABL1 and FASP3 genes, which had low docking scores (Table 3). Therefore, the two gene symbols ABL1 and FASP3 were removed during the building of the heat map. Figure 6(a) shows that the better the color, the higher the docking score. Our results revealed that neohesperidin, naringin, and hesperidin have a high binding ability with DRD4 and mTOR.

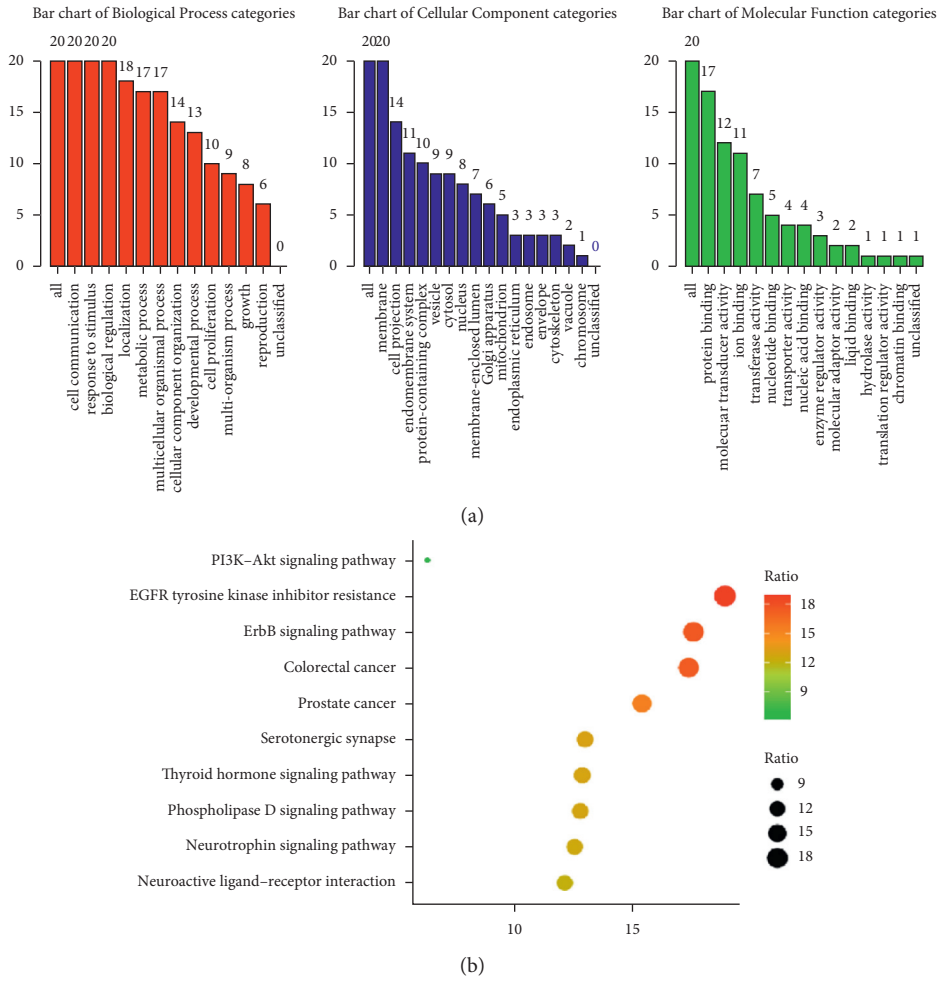


Figure 4: (a) Bar plot of the GO function enrichment of core targets. Red represents the Biological Process (BP). Blue represents the Cellular Component (CC). Green represents the Molecular Function (MF). (b) Bubble chart of KEGG enrichment of core targets. The *x*-axis represents the ratio enrichment. The *y*-axis represents the pathway.

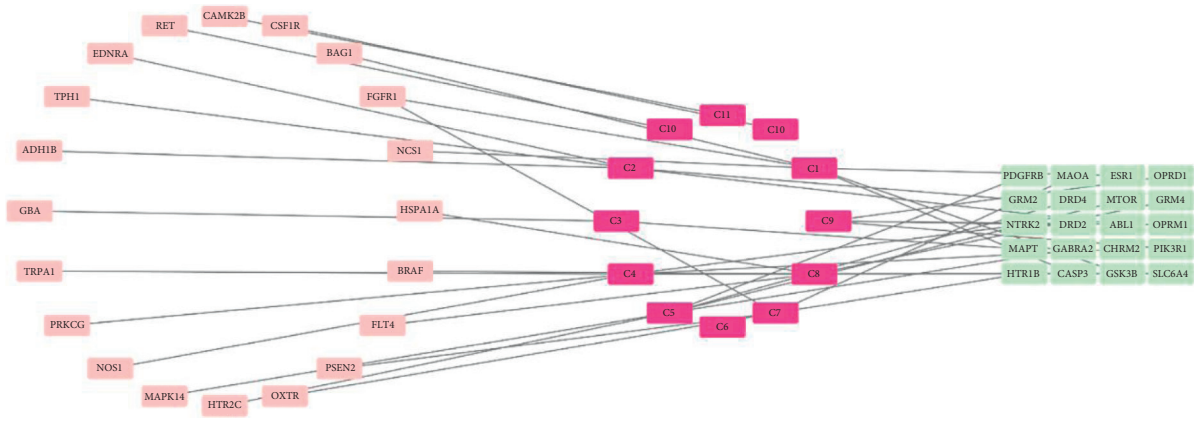


Figure 5: The I-D-G network.

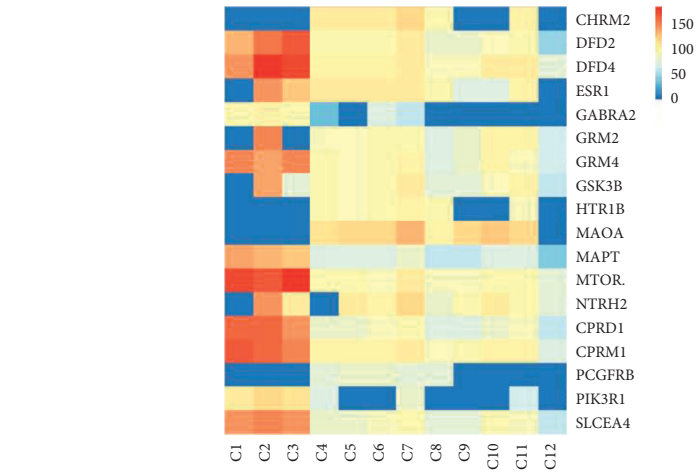
Based on these data, we found that the interaction between each component and the core target is the basis of biological activity. Therefore, FA in the treatment of MDD is a complex process among a series of multitargets and multicomponents.

5. Discussion

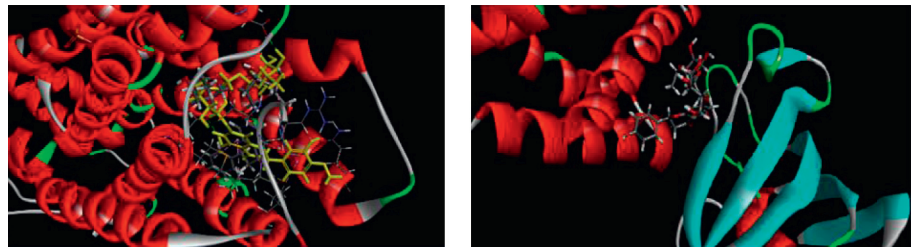
MDD is a common mental disease that has become one of the world's major diseases, resulting in the change of the quality of life and work ability. The number of patients

Table 3: DRD docking with the component score.

No	Component	Score
C2	Naringin	186.427
C3	Neohesperidin	178.642
C1	Hesperidin	144.394
C11	Scutellarein	111.055
C10	3′, 4′, 7, 8-Tetramethoxyflavone	109.533
C7	Prangenin	107.563
C4	Isomerancin	103.292
C5	Meranzin	100.242
C6	Meranzin hydrate	100.109
C9	5, 6, 7, 4′-Tetramethoxyflavone	91.912
C8	Bergapten	87.6184
C12	Nobiletin	77.2802



(a)



(b)

Figure 6: (a) Heatmap of I-G docking scores. (b) 3D diagram of naringin-DRD4 and naringin-mTOR.

increases year by year. At present, most of the patients suffering from clinical depression are treated by the western medicine, which commonly use selective serotonin reuptake inhibitors (fluoxetine and paroxetine) and serotonin and norepinephrine reuptake inhibitors (venlafaxine and duloxetine). However, many problems are associated with the use of the western medicine, such as high recurrence rate, poor tolerance, and adverse reactions. The treatment using traditional Chinese medicine is gradual, and the existing research results show that the curative effect of the Chinese medicine does not differ significantly from that of the western medicine. Nevertheless, the Chinese medicine has a faster effect, less adverse reactions, and more benefits. Therefore, the research on MDD of traditional Chinese medicine has been more intense and fruitful in recent years.

FA is one of the commonly used medicinal compounds, which is widely used to cure gastrointestinal diseases. Previous studies showed that MDD often leads to gastrointestinal dysfunction, and the FA extract can regulate the HPA axis [36] and neuroprotection [37], regulate gastrointestinal hormones [38], and can participate in regulating monoamine-based syndrome systems [39]. However, the potential mechanism used by FA to cure depression is unknown. This work used network pharmacological

methods, detected the active ingredients, targets, and passage, and combined them to obtain the potential mechanism of action of FA on MDD.

UPLC-Q-TOF-MS/MS technology was used to identify FA chemical components. Then, the combination of the literature with the related software resulted in a selection of 12 main active ingredients. Finally, the overlapping target was used to perform the PPI network analysis. DRD2, DRD4, OPRM1, and mTOR were the most relevant targets for the treatment of MDD by FA. DRD2 is divided into two subtypes, D2S and D2L [40], it is a target of many antipsychotic drugs, and its down- or upregulation is closely associate with MDD [41]. mTOR is a receptor serine/threonine kinase, and in its activated, phosphorylated form can regulate the nerves and promote topical protein synthesis, thereby further promoting the synaptic formation [42, 43]. A study found that hesperidin exerted an antidepressant effect by activating the hippocampus mTOR pathway [44]. Interestingly, neohesperidin, naringin, and hesperidin were the key components of FA involved in the treatment of MDD, as shown by the hot map. For example, DRD2 and mTOR had a good molecular binding ability than forneohesperidin and hesperidin. Therefore, our hypothesis was that the above ingredients and targets might represent the key elements on the role of FA as antidepressant.

Metascap was used to perform the pathway analysis on 20 core targets. The results showed that FA mainly activates and regulates PI3K-Akt signaling pathway (hsa04151). EGFR tyrosine kinase inhibitor resistance (hsa04080) and ErbB signaling pathway (hsa04072) exert pharmacological effects. The feature to pay attention to is PI3K-Akt signaling pathway, Akt is upstream of the mTOR signaling pathway, and the phosphorylated form can activate the mTOR signaling pathway. Key targets for PI3K/Akt signaling pathway influence the glutamic acid system function, hippocampal neuron apoptosis, and mitochondrial function involved in MDD. Many studies show the main role of TCM as an antidepressant by acting on the PI3K/Akt/mTOR signaling pathway. Caihujialong Tang increases the expression and activity of PI3K in the rat hippocampus to protect neurons, thus exerting an antidepressant effect [45]. Jiaotai Wan regulates PI3K/AKT/mTOR signaling pathway related proteins to improve depression and reverse behavioral changes in rats [46]. The above evidence generated the hypothesis that FA is effective by acting on AKT, PI3K, and mTOR protein expression.

FA in China is often used as a hydraulic medicine, commonly used to cure gastrointestinal diseases, but the literature does not give any explanation regarding its therapeutic effects on MDD, such as the mitigation of diarrhea caused by MDD. Therefore, our results by UPLC-Q-TOF-MS/MS technology and system pharmacology revealed a potential mechanism of FA to treat MDD, providing a reference for the development and use of antidepressants.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

The funder has no role/influence in this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yating Xie and Ying Liu have contributed equally to this work and share first authorship. XYT integrated the data and wrote the manuscript. ZJL completed the ingredient identification. YXW and ZT accomplished the pharmacological study. ZP and LY executed the literature search. LMM directed the data processing. HM and WQ implemented corrections in the manuscript.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (nos. 82060724 and 81560651), Science and Technology Project of Jiangxi Health Committee (20210015), and Doctoral Research Startup Fund (no. 2018WBZR009).

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2021, Article ID 7604537, 13 pages
https://doi.org/10.1155/2021/7604537

Review Article

Efficacy and Safety of Chaihu Jia Longgu Muli Decoction in the Treatment of Poststroke Depression: A Systematic Review and Meta-Analysis

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Received 24 June 2021; Accepted 9 August 2021; Published 20 August 2021

Academic Editor: Junqing Huang

Objective. Chaihu Jia Longgu Muli decoction (CLMD) is widely used in the treatment of poststroke depression (PSD) in China. Some evidences show that it has advantages, but there lacks reliable evidence. This study aims to systematically evaluate the efficacy and safety of CLMD in the treatment of PSD. *Methods*. All randomized controlled trials (RCTs) of CLMD in the treatment of PSD were searched from the following databases: PubMed, Cochrane Library, Embase, Web of Science, China National Knowledge Infrastructure (CNKI), Wanfang Database, VIP Database, and Chinese Biomedical Literature Service System (CBM), from their inception to May 2021. Two researchers independently screened the literature, extracted the data, and evaluated the risk of bias in the included studies. Meta-analysis was performed using RevMan5.3 software. *Results*. A total of 13 RCTs involving 1665 patients were finally included in this study, among which 5 RCTs were oral CLMD alone versus antidepressants, and 8 RCTs were oral CLMD with antidepressants versus antidepressants. Meta-analysis results showed that oral administration of CLMD could improve Hamilton's Depression Scale (HAMD) and the Modified Edinburgh-Scandinavian Stroke Scale (MESSS) scores, improve the Barthel index, and have a low rate of adverse reactions, but there was no significant difference in the total effective rate ($p = 0.21 > 0.05$) and the National Institute of Health Stroke Scale (NIHSS) score ($p = 0.47 > 0.05$) between the antidepressants group and the oral administration of the CLMD group. Oral CLMD combined with antidepressants could improve the total effective rate, HAMD, and MESSS score, but there was no significant difference in Barthel index ($p = 0.06 > 0.05$) and the adverse reaction rate ($p = 0.14 > 0.05$) between the two groups. *Conclusion*. Current evidence suggests that oral CLMD alone or with antidepressants is more effective and safer in the treatment of PSD than oral antidepressants. Due to the limitation of the quality and quantity of the included studies, more high-quality studies are needed to confirm the above conclusion.

1. Introduction

Poststroke depression (PSD) refers to a series of psychological and physical syndromes featured with depression, slow response, loss of interest, and other symptoms after stroke [1]. The incidence of PSD ranges from 29% to 31% [2], and it usually occurs within 1 year after stroke [3]. PSD is closely related to the poor prognosis of stroke, which leads to prolonged hospitalization, neurological recovery disorder, more loss of independent living ability, and even increased mortality [1, 4, 5]. Studies have shown that the mortality of patients with PSD is significantly higher than that of patients with stroke alone, which is 1.28–1.75 times higher, and the severity of depression is highly correlated with the mortality [6]. Antidepressants are the first choice for the treatment of PSD, including the selective serotonin reuptake inhibitor (SSRI), serotonin norepinephrine

reuptake inhibitor (SNRI), noradrenergic and specific serotonergic antidepressant (NaSSA), and tricyclic antidepressants (TCAs) [1]. Although clinical studies have confirmed that antidepressants are effective for PSD [7, 8] and recommended by guidelines [9], these drugs require long-term use and are prone to dependence and many adverse reactions [10]. These negative factors may force PSD patients or clinicians to explore other treatment options. Therefore, it is crucial to provide better treatment strategies for PSD patients.

Traditional Chinese medicine (TCM) has many advantages, such as multitarget, multipathway, and strong safety, which plays an important role in the complementary and alternative therapies, and has accumulated rich experience in the practice of treating PSD [11]. According to TCM theory, the pathogenesis of PSD is mainly liver qi stagnation, accompanied by the damage to brain collaterals and imbalance of qi, blood, and Yin and Yang after stroke, and the pathological characteristic is intermingled deficiency and excess [12]. Chaihu Jia Longgu Muli decoction (CLMD), a representative prescription for the treatment of mental diseases, is composed of Chaihu (Radix Bupleuri), Longgu (Os Draconis), Muli (Concha Ostreae), Huang Qin (Radix Scutellariae), Shengjiang (Rhizoma Zingiberis Recens), Da Zao (Fructus Jujubae), Qian Dan (Miniumite), Ren Shen (Radix Ginseng), Gui Zhi (Ramulus Cinnamomi), Fuling (*Poria*), Ban Xia (Rhizoma Pinelliae), and Da Huang (Radix et Rhizoma Rhei), which has the effect of soothing liver qi stagnation, regulating qi and blood, calming the mind, and relieving fright. It is widely used in dementia, insomnia, anxiety, depression, and other mental diseases, and its effect is reliable [13]. Meanwhile, there are more and more clinical studies on the application of CLMD in PSD. Liu et al. [14] showed that CLMD can significantly improve the depression and quality of life of the patients through a randomized controlled trial, which has similar efficacy with fluoxetine, and has less side effects. Zhao et al. [15] observed that CLMD combined with antidepressant has a synergistic effect in ameliorating depression, improving the ability of daily living, and reducing inflammatory cytokines, without increasing adverse reactions. There are more and more similar reports, but it is difficult to draw a reliable conclusion due to the differences in the clinical efficacy of CLMD, research design, and course of treatment. Therefore, the purpose of this systematic review is to evaluate the efficacy and safety of CLMD in the treatment of PSD and to provide a reliable treatment option and evidence-based basis for clinical work and scientific research.

2. Methods

The protocol and registration information are available at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021255407 (registration number: CRD42021255407). We performed this meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Table S1).

2.1. Search Strategy. PubMed, the Cochrane Library, Embase, Web of Science, China National Knowledge Infrastructure, Wanfang Database, VIP Database, and China Biomedical Literature Service System (CBM) were searched by computer from the establishment of the databases to May 2021. The retrieval method adopted the combination of medical subject headings (MeSH) terms and free terms, and the English retrieval words mainly included stroke, cerebrovascular accident, depression, Chaihu Jia Longgu Muli decoction , and Chaihu Jia Longgu Muli granules (Table S2). All literature were reviewed by two investigators (Renhong Wan and Yihua Fan) independently. Any disagreement was resolved by the consultation with a third researcher (Ruiwen Song).

2.2. Inclusion and Exclusion Criteria

2.2.1. Inclusion Criteria. Inclusion criteria were as follows:

(1) Study type: randomized controlled trials (RCTs) of CLMD in the treatment of PSD

(2) Diagnostic criteria: the diagnostic criteria for stroke refer to the Diagnostic Points of Various Cerebrovascular Diseases [16], for depression refer to the ones for PSD in the Classification and Diagnostic Criteria of Mental Disorders in China [17], and for TCM refer to the Diagnostic Efficacy Criteria for Diseases of TCM

(3) Interventions: the treatment group was given CLMD or combined with the antidepressant, while the control group received the same antidepressant as the treatment group. (Oral preparations of Chaihu Jia Longgu Muli (CLM) included different forms such as CLMD and CLM granules. Modified CLMD referred to the addition or subtraction of no more than 3 herbs in PSD patients with different symptoms [18]).

(4) Outcome indicators: the main outcome indicators are (i) total effective rate: efficacy was assessed by the reduction rate of Hamilton's Depression Scale (HAMD) score. The criteria for the efficacy of depression were recovery, score reduction rate >75%; significant effect, the reduction rate >50%; effectiveness, score reduction rate ≥25%; ineffectiveness, subtraction rate <25%; and total effective rate = (recovery number + significant effect number + effectiveness number)/total number ∗ 100%; and (ii) HAMD score: secondary outcome indicators: (i) Barthel index for activities of daily living; (ii) National Institute of Health Stroke Scale (NIHSS); (iii) the Modified Edinburgh-Scandinavian Stroke Scale (MESSS); and (iv) adverse reactions rate.

2.2.2. Exclusion Criteria. Exclusion criteria were as follows:

(1) For repetitive studies, only the studies with the highest quality and best data were included

(2) Studies with incomplete data or significant errors that cannot be resolved after contact with the author

(3) Studies in which random methods or allocation concealment were evaluated as high risk

(4) Studies without outcome indicators

2.3. Study Selection and Data Extraction. Two independent reviewers screened the literature strictly according to the inclusion and exclusion criteria, extracted the data, and cross-checked the data after completion. For literature and data with objections, two reviewers would discuss, and if there was no agreement, the third reviewer would be invited to evaluate. The extracted data included (i). basic information of the included studies: title, first author, year of publication, number of cases in each group, and baseline characteristics of patients; (ii) intervention measures and treatment course of treatment group and control group; (iii) outcome indicators; and (iv) each risk bias assessment elements in RCTs.

2.4. Risk of Bias Assessment. The risk of bias in the included studies was independently evaluated by two investigators, and the results were cross-checked. If there was any disagreement, it would be discussed and resolved with the third researcher. Bias risk assessment adopted the RCT bias risk assessment tool recommended in the Cochrane manual 5.1.0 [19].

2.5. Statistical Analysis and Data Synthesis for Meta-Analysis. RevMan5.3 software recommended by the Cochrane Collaboration was used for meta-analysis. For continuous variables, if the measurement tools and units were the same, the weighted mean difference (WMD) was used for analysis. If the measurement tools or units were inconsistent, the standard mean difference (SMD) was used. The dichotomous variables were analyzed by relative risk (RR), and 95% confidence interval (95% CI) was used for each effect size. The heterogeneity among the included results was analyzed by the χ^2 test, and the magnitude of the heterogeneity was determined quantitatively by combining with I^2. If $p > 0.10$ and $I^2 < 50\%$, there was no significant heterogeneity between studies, and a fixed-effect model was used for meta-analysis. If $p > 0.10$ and $I^2 \geq 50\%$, the heterogeneity between studies was considered significant, and then, subgroup analysis or sensitivity analysis was used to explore the source of heterogeneity. After the exclusion of obvious clinical heterogeneity and methodological heterogeneity, a random-effect model was used for meta-analysis. Sensitivity analysis was used to observe the influence of single study on the combined effect size and to analyze the stability of the meta-analysis results. For main outcome indicators, if the included studies were ≥10, the funnel plot was used to qualitatively detect publication bias. Egger's and Begg's tests were used to quantitatively assess the potential publication bias.

3. Results

3.1. Characteristics of the Studies. Of 277 related articles obtained by the initial search, 102 were obtained after removing the duplicates. After reading the titles and abstracts, 55 articles were excluded, and 34 were excluded after reviewing the full text in the remaining 47, so 13 studies were eligible for inclusion. The literature screening process is shown in Figure 1.

The basic characteristics of the included studies are given in Table 1. The treatment group was treated with CLMD alone or combined with antidepressants, while the control group was treated with the antidepressants, and the characteristics of the intervention measures are given in Table 2.

3.2. Risk of Bias Assessment. The RCT risk of bias assessment tool recommended in the Cochrane Manual 5.1.0 was used to evaluate the quality of the 13 included studies, and the random sequence generation method was correctly used in 6 studies [15, 20, 22, 25, 26, 29]. None of the studies [14, 15, 20–30] mentioned the use of the blind method. All studies [14, 15, 20–30] were assessed as low risk of bias in terms of allocation concealment, incomplete outcome indicators, selective reporting, and other biases. The results are shown in Figure 2.

3.3. Meta-Analysis Results. Among the 13 studies included, 5 studies [14, 23, 27, 28, 30] compared oral CLMD alone with antidepressants and 8 studies [15, 20–22, 24–26, 29] compared oral CLMD combined with antidepressants with antidepressants.

3.3.1. Oral CLMD Alone vs. Antidepressant

(1) Total Effective Rate. 4 RCTs reported the total effective rate. The heterogeneity test showed no statistical heterogeneity ($p = 0.97$; $I^2 = 0\%$). Meta-analysis of the data using a fixed-effect model showed no statistically significant difference between the treatment group and the control group ((RR = 1.05, 95% CI: 0.97, 1.15, $p = 0.21 > 0.05$), Figure 3). Due to the large span of treatment courses in each study, we performed a subgroup analysis based on the course. According to the course of treatment, they were divided into two subgroups: <30 days and ≥60 days. The heterogeneity test of total effective rate showed no statistical heterogeneity in <30 days ($p = 0.88$; $I^2 = 0\%$) and ≥60 days ($p = 0.88$; $I^2 = 0\%$) (Figure 4). Meta-analysis of data using a fixed-effect model indicated that there was no statistical difference in the two groups (<30 days (RR = 1.04, 95% CI: 0.95, 1.14, $p = 0.40 > 0.05$) and ≥60 days (RR = 1.08, 95% CI: 0.92, 1.27, $p = 0.35 > 0.05$)).

(2) HAMD Score. 4 RCTs reported the HAMD score. The heterogeneity test showed no statistical heterogeneity ($p = 0.64$; $I^2 = 0\%$). Meta-analysis of the data using a fixed-effect model showed that the score of the treatment group was lower than that of the control group, and the difference was statistically significant ((MD = −1.30, 95% CI: −1.99, −0.61, $p = 0.002 < 0.05$), Figure 5). At the same time, we performed subgroup analysis according to the course of treatment. The heterogeneity test of the HAMD score showed no statistical heterogeneity in <30 days ($p = 0.38$; $I^2 = 0\%$) and ≥60 days ($p = 0.87$; $I^2 = 0\%$) (Figure 6). Meta-

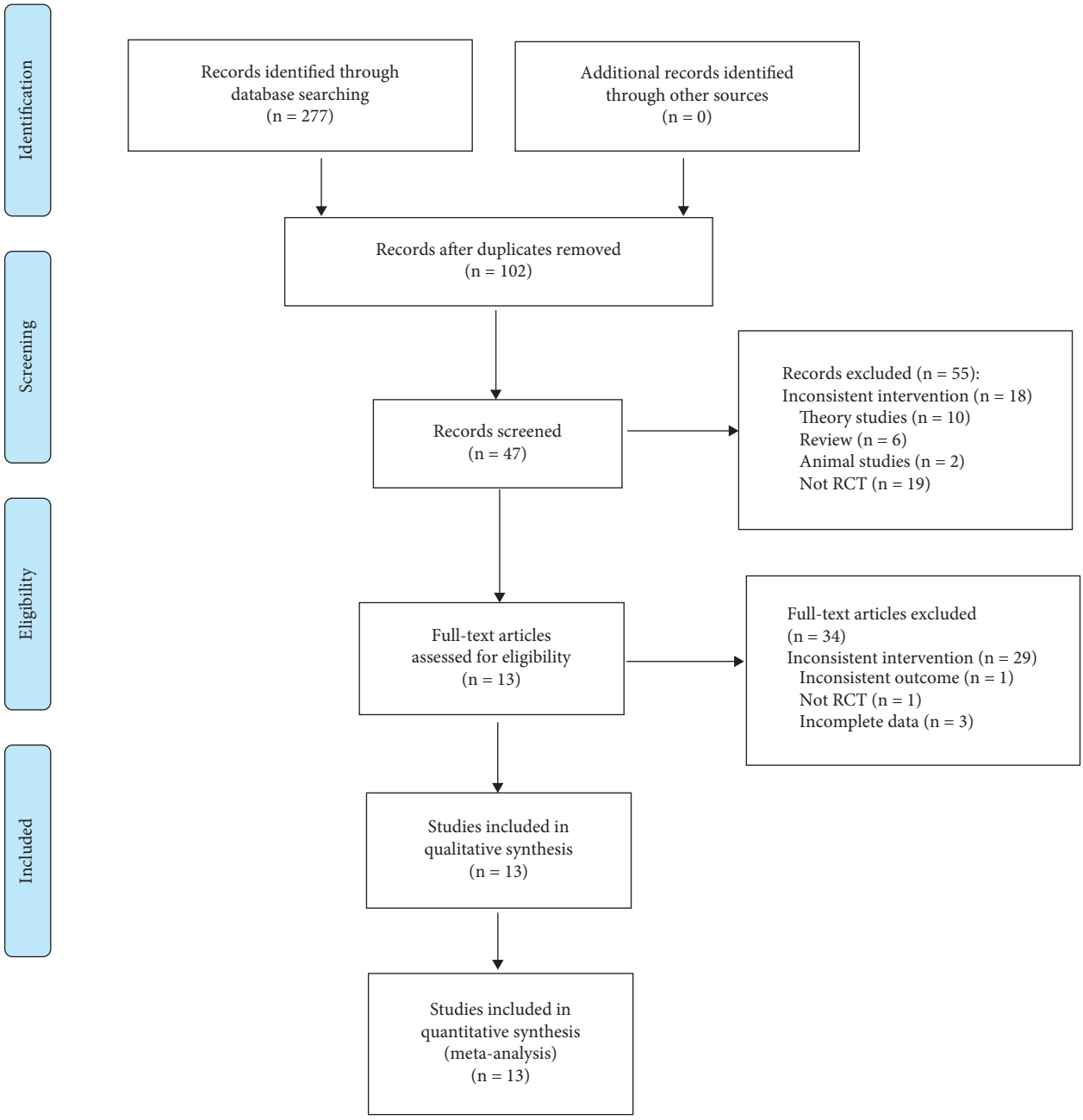

Figure 1: Flow diagram.

analysis of data was performed using a fixed-effect model, and the results indicated that the HAMD score of the treatment group was better than that of the control group after treatment in the <30 days subgroup (MD = −1.57, 95% CI: −2.36, −0.78, $p < 0.0007$), but there was no statistically significant difference between the two groups in the ≥60 days subgroup (MD = −0.66, 95% CI: −2.07, 0.76, $p = 0.36 > 0.05$).

(3) MESSS Score. 1 RCT reported the MESSS score, which could not be used for meta-analysis. Descriptive analysis showed that the treatment group was superior to the control group, and the difference between the two groups was statistically significant ((MD = −5.72, 95% CI: −8.05, −3.39), $p < 0.00001$).

(4) NIHSS Score. 1 RCT reported the NIHSS score, and descriptive analysis showed no statistically significant difference between the two groups ((MD = −0.37, 95% CI: −1.37, −0.63), $p = 0.47 > 0.05$).

(5) Barthel Index. 3 RCTs reported Barthel index. The heterogeneity test suggested significant heterogeneity ($p = 0.004$; $I^2 = 82\%$), so the elimination method was adopted one by one. When Liu's study [14] was excluded, heterogeneity disappeared ($p = 1.00$; $I^2 = 0\%$, Figure 7), suggesting that this study was the source of heterogeneity, and a fixed-effect model was adopted after the exclusion of heterogeneity. Results showed that the score of the treatment group was better than that of the control group, and the

Table 1: Basic characteristics of the included studies.

Study cohort	No. (T/C)	Gender		Age		Course (day)	Outcome
		T	C	T	C		
An [20]	38/38	22/16	20/18	57.03 ± 7.33	56.23 ± 7.26	30	①④
Chen et al. [21]	52/52	30/22	29/23	49.6 ± 3.3	49.1 ± 3.6	21	①②④
Gao and Zhang [22]	75/75	30/45	33/42	66.5 ± 11.3	69.5 ± 12.0	30	①②④
Huang [23]	20/20	13/7	13/7	64.80 ± 7.08	65.30 ± 6.89	60	①②⑤⑥⑦
Lai et al. [24]	34/34	18/16	23/11	58.2 ± 5.8	62.1 ± 6.9	56	①②⑤
Li [25]	36/34	17/19	15/19	—	—	56	①②⑦
Liu and Yang [26]	48/47	26/22	26/21	48.35 ± 6.24	48.35 ± 6.24	63	①②④⑦
Liu et al. [14]	28/32	13/15	15/17	65.4 ± 8.7	63.7 ± 9.3	28	②④⑤
Wang and Li [27]	49/49	36/13	35/14	59.63 ± 5.27	60.05 ± 5.69	28	①②③⑦
Wu [28]	42/42	23/19	21/21	59.78 ± 7.82	60.54 ± 8.04	90	①②⑥
Zhao et al. [15]	35/35	20/15	21/14	60.4 ± 4.5	60.5 ± 4.3	28	①②④⑤⑦
Zhu [29]	38/38	24/14	26/12	46.34 ± 10.97	45.28 ± 11.26	42	①②③
Zhang et al. [30]	68/66	29/39	19/47	65.91 ± 10.442	68.12 ± 9.731	14	①②④⑤

Note: ①, efficiency; ②, HAMD; ③, MESSS; ④, NIHSSS; ⑤, Barthel; ⑥, CSS; ⑦, adverse reactions rate; —, unclear.

Table 2: Characteristics of the interventions.

Study	Interventions of the treatment group		Interventions of the control group	Days
	CLMD	Antidepressants	Antidepressants	
An [20]	Chaihu Jia Longgu Muli decoction, 100 ml bid	Fluoxetine hydrochloride capsules 20 mg qd	Fluoxetine hydrochloride capsules 20 mg qd	30
Chen et al. [21]	Chaihu Jia Longgu Muli decoction, 100 ml bid	Paroxetine 10 mg qd, 10 mg was added after 1 week	Paroxetine 10 mg qd, 10 mg was added after 1 week	21
Gao and Zhang [22]	Chaihu Jia Longgu Muli decoction, 100 ml bid	Flupentixol 0.5 mg bid and melitracen 10 mg bid	Flupentixol 0.5 mg bid and melitracen 10 mg bid	30
Huang [23]	Chaihu Jia Longgu Muli decoction, 100 ml bid	None	Fluoxetine hydrochloride 20 mg qd	60
Lai et al. [24]	Chaihu Jia Longgu Muli decoction, 125 ml bid	Flupentixol and melitracen tablets (flupentixol 0.5 mg and melitracen 10 mg) 2#qd	Flupentixol and melitracen tablets (flupentixol 0.5 mg and melitracen 10 mg) 2#qd	56
Li [25]	Chaihu Jia Longgu Muli decoction, 100 ml bid	Fluoxetine hydrochloride 20 mg qd	Fluoxetine hydrochloride 20 mg qd	56
Liu and Yang [26]	Chaihu Jia Longgu Muli decoction, 100 ml bid	Paroxetine 10 mg qd, 10 mg was added after 1 week	Paroxetine 10 mg qd, 10 mg was added after 1 week	63
Liu et al. [14]	Chaihu Jia Longgu Muli decoction, 100 ml bid	None	Fluoxetine hydrochloride tablets 20 mg qd	28
Wang and Li [27]	Chaihu Jia Longgu Muli decoction, 100 ml bid	None	Fluoxetine hydrochloride tablets 20 mg qd	28
Wu [28]	Chaihu Jia Longgu Muli decoction, bid	None	Fluoxetine hydrochloride 20 mg qd	90
Zhao et al. [15]	Chaihu Jia Longgu Muli decoction, bid	Fluoxetine hydrochloride capsules 20 mg bid	Fluoxetine hydrochloride capsules 20 mg bid	28
Zhu [29]	Chaihu Jia Longgu Muli decoction	Conventional Western medicine	Conventional Western medicine	42
Zhang et al. [30]	Chaihu and Longgu Muli granules, 100 ml bid	None	Citalopram 20 mg qd	14

difference was statistically significant (MD = 9.00, 95% CI: 6.45, 11.55, $p < 0.00001$).

(6) Adverse Reactions Rate. 2 RCTs reported adverse reactions rate. The heterogeneity test showed no statistical heterogeneity ($p = 0.59$; $I^2 = 0\%$, Figure 8). Meta-analysis of the data using a fixed-effect model showed that the adverse reactions rate in the treatment group was lower than that in the control group, and the difference was statistically significant (RR = 0.10,95% CI: 0.01, 0.75, $p = 0.03 < 0.05$).

3.3.2. Oral CLMD + Antidepressant vs. Antidepressant

(1) Total Effective Rate. 8 RCTs reported the total effective rate. The heterogeneity test showed no statistical heterogeneity ($p = 0.84$; $I^2 = 0\%$). Meta-analysis of the data using a fixed-effect model showed that the total effective rate of the treatment group was better than that of the control group, and the difference between the two groups was statistically significant ((RR = 1.28, 95% CI: 1.19, 1.39, $p < 0.00001$), Figure 9). We also conducted subgroup analysis according to the course of treatment, and the

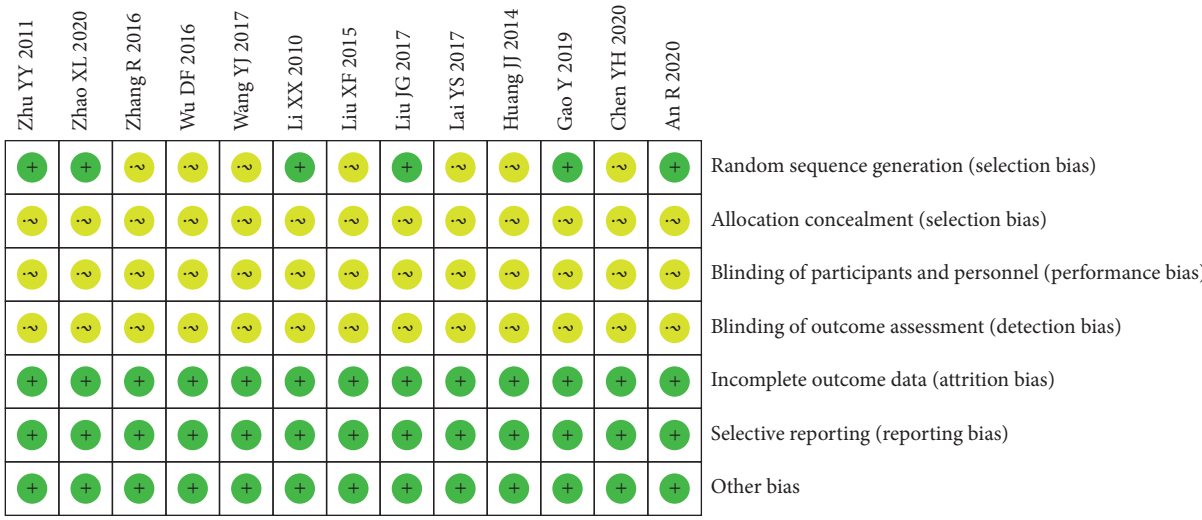

Figure 2: Summary of risk of bias.

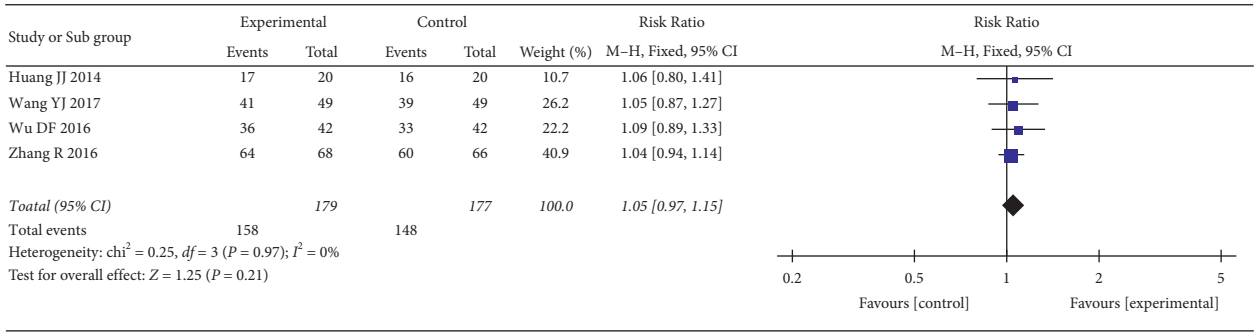

Figure 3: Meta-analysis of oral CLMD alone vs. antidepressant in total effective rate.

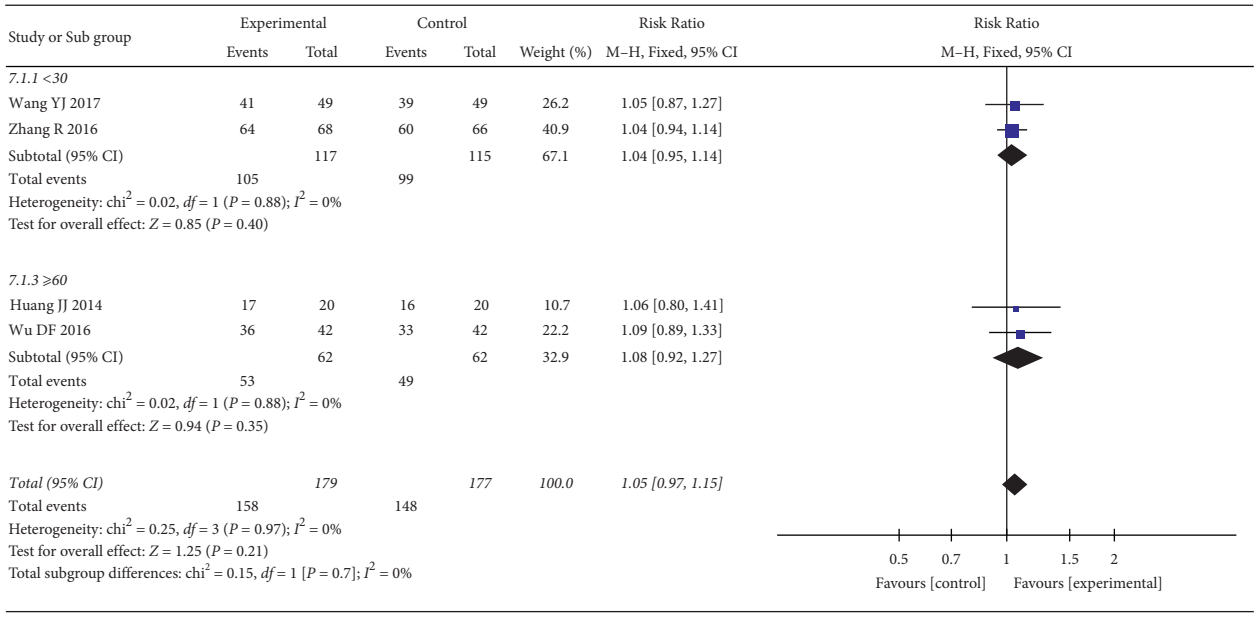

Figure 4: Subgroup analysis of oral CLMD alone vs. antidepressant in total effective rate.

studies were divided into three subgroups: <30 days, ≥30 days and <60 days, and ≥60 days. The heterogeneity test showed no statistical heterogeneity in the <30 days subgroup ($p = 0.83$; $I^2 = 0\%$) and the ≥30 days and <60 days subgroup ($p = 0.59$; $I^2 = 0\%$) (Figure 10). Meta-analysis was performed on the data using a fixed-effect model, and the results showed that oral

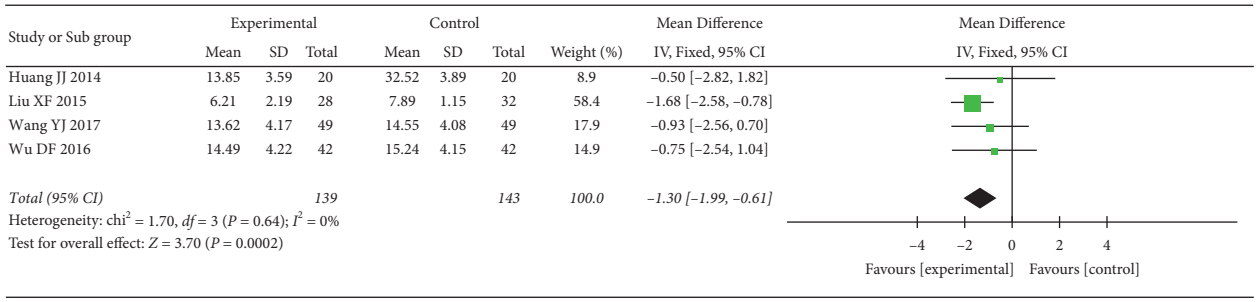

Figure 5: Meta-analysis of oral CLMD alone vs. antidepressant in the HAMD score.

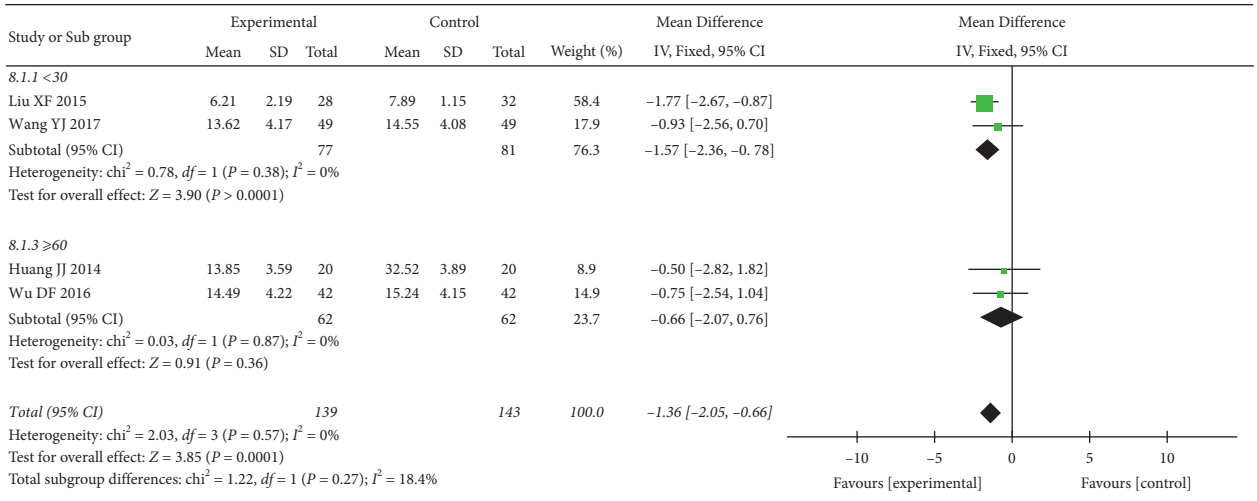

Figure 6: Subgroup analysis of oral CLMD alone vs. antidepressant in the HAMD score.

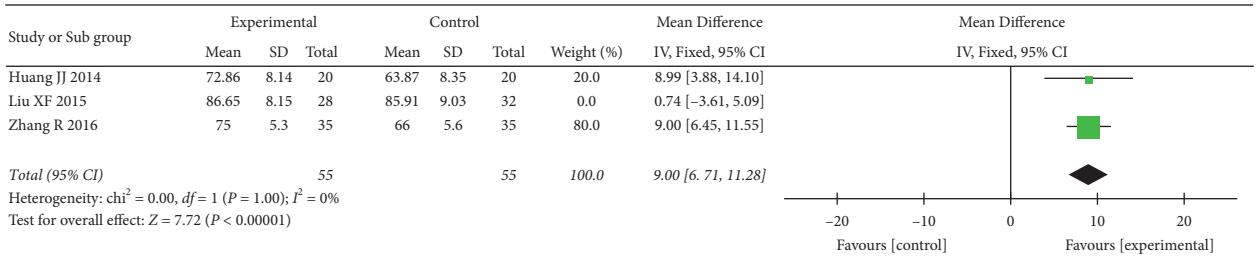

Figure 7: Meta-analysis of oral CLMD alone vs. antidepressant in Barthel index.

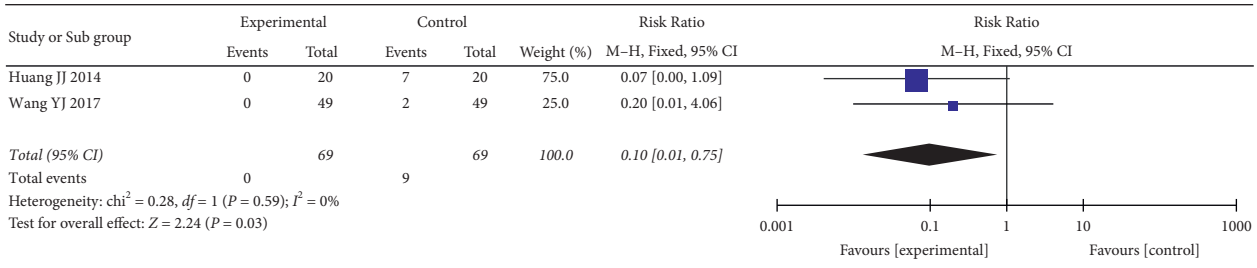

Figure 8: Meta-analysis of oral CLMD alone vs. antidepressant in adverse reactions rate.

CLMD combined with antidepressants was better than oral antidepressants alone within 60 days of treatment (<30 days, (RR = 1.25, 95% CI: 1.09, 1.43, $p = 0.001$) and ≥30 and <60 days (RR = 1.32, 95% CI: 1.19, 1.47, $p < 0.00001$)). There was no significant difference between the two groups after >60 days of treatment ($p = 0.05$).

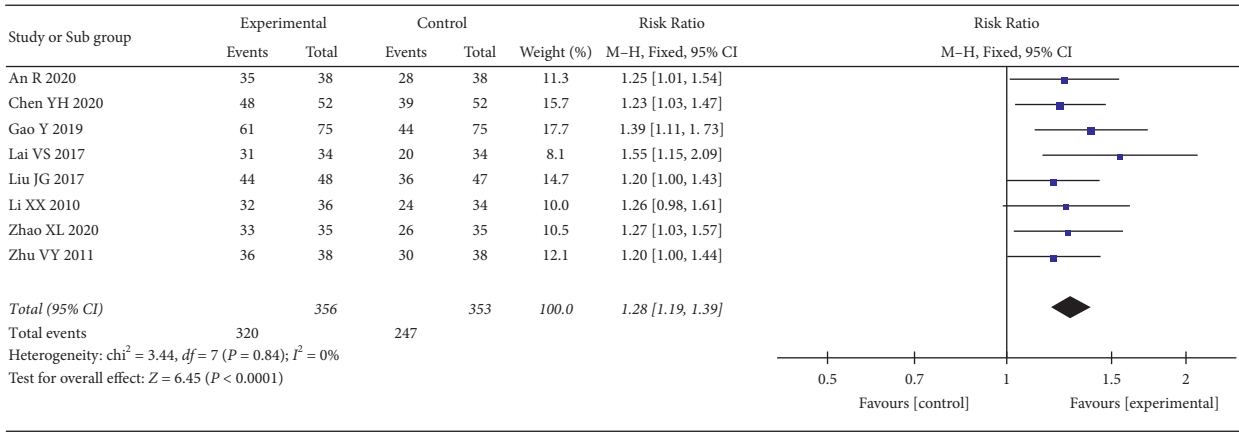

Figure 9: Meta-analysis of oral CLMD + antidepressant vs. antidepressant in total effective rate.

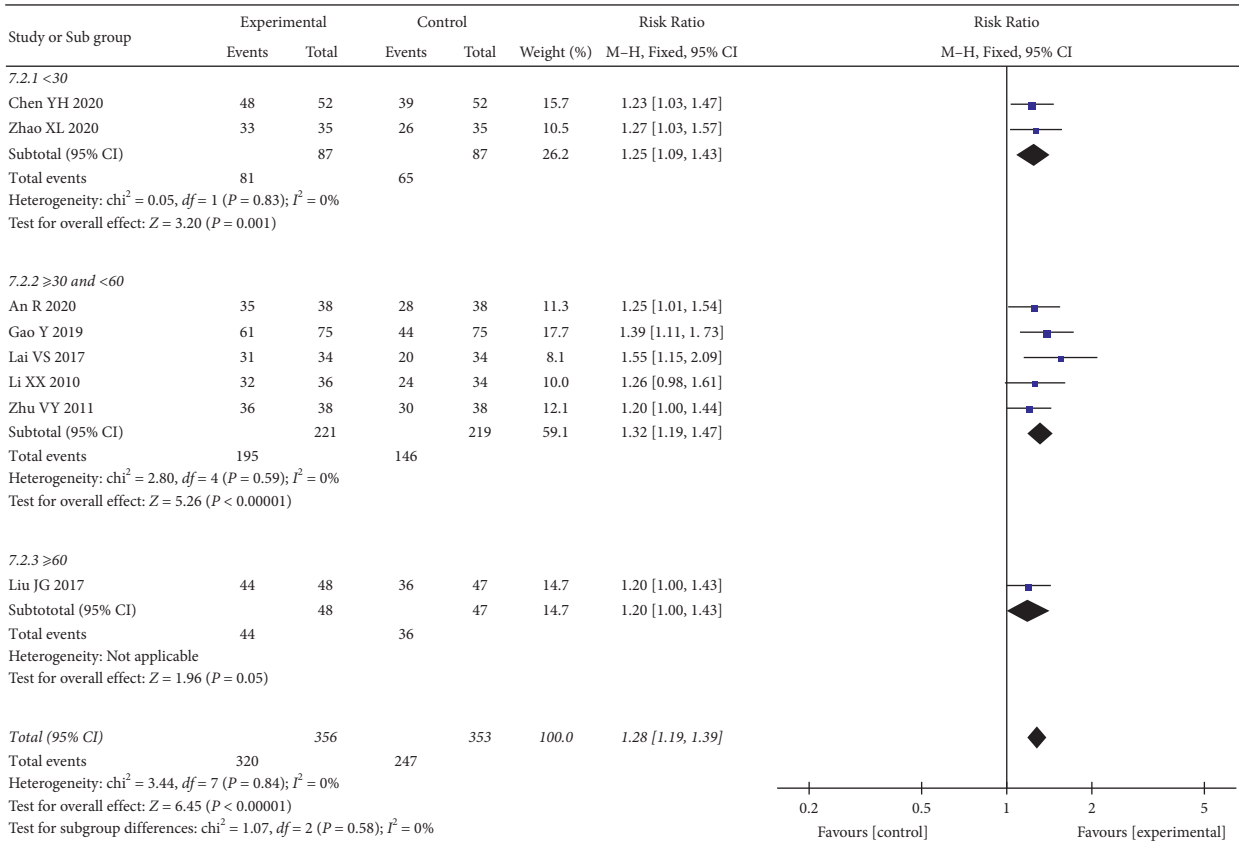

Figure 10: Subgroup analysis of oral CLMD + antidepressant vs. antidepressant in total effective rate.

(2) HAMD Score. 7 RCTs reported the HAMD score. The heterogeneity test indicated significant heterogeneity ($p < 0.00001$; $I^2 = 99\%$). However, the confidence intervals in the forest plot were all on the left side of the invalid line, indicating that the heterogeneity among studies did not affect the results. Therefore, a random-effect model was adopted for the combination. The results showed that the HAMD score of the treatment group was better than that of the control group, and the difference was statistically significant ((MD = −5.64, 95% CI: −10.11, −1.16, $p = 0.01$ <0.05), Figure 11). To explore the source of heterogeneity, we performed subgroup analysis according to the course of treatment. The heterogeneity test result of the subgroup (≥30 and <60 days) was $p < 0.00001$ and $I^2 = 99\%$, indicating significant heterogeneity. When Lai's study was excluded [24], the heterogeneity disappeared ($p = 0.41$; $I^2 = 0\%$), suggesting that this study was the source of heterogeneity. A fixed-effect model was used for meta-analysis of the data,

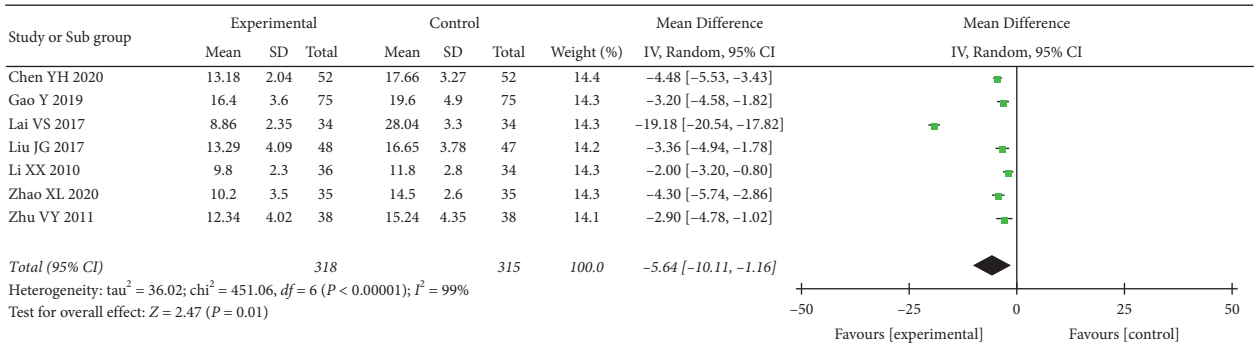

Figure 11: Meta-analysis of oral CLMD + antidepressant vs. antidepressant in the HAMD score.

and the results showed that HAMD scores in the treatment groups were all better than those in the control groups, with statistically significant differences in the <30 days subgroup (MD = −4.42, 95% CI: −5.27, −3.57, $p < 0.00001$), the ≥30 and <60 days subgroup (MD = −2.59, 95% CI: −3.41, −1.78, $p < 0.00001$), and the ≥60 days subgroup (MD = −3.36, 95% CI: −4.94, −1.78, $p < 0.00001$) (Figure 12).

(3) MESSS Score. 1 RCT reported the MESSS score, which could not be used for meta-analysis. Descriptive analysis showed that the treatment group was superior to the control group, and the difference between the two groups was statistically significant (MD = −5.26, 95% CI: −7.55, −2.97, $p < 0.00001$).

(4) NIHSS Score. 4 RCTs reported the NIHSS score. The heterogeneity test indicated significant heterogeneity ($p < 0.00001$; $I^2 = 99\%$). A one-by-one elimination method was used to analyze the source of heterogeneity. When Liu and Zhang's study [26] was excluded, the heterogeneity was significantly reduced ($p = 0.19$; $I^2 = 37\%$, Figure 13), suggesting that the study was the source of heterogeneity, and a fixed-effect model was adopted after the exclusion of heterogeneity. Results showed that the NIHSS score of the treatment group was better than that of the control group, and the difference was statistically significant (MD = −2.93, 95% CI: −3.39, −2.47, $p < 0.00001$).

(5) Barthel Index. 2 RCTs reported Barthel index, and the heterogeneity test suggested significant heterogeneity ($p = 0.03$; $I^2 = 80\%$). Due to the small number of the included studies that could not be further analyzed and the study results were all on the side of the invalid line, a random-effect model was adopted. The result showed that there was no statistically significant difference between the two groups ((MD = 8.23, 95% CI: −0.41, −16.87, $p = 0.06$ >0.05), Figure 14).

(6) Adverse Reactions Rate. 3 RCTs reported the rate of adverse reactions. The heterogeneity test showed no statistical heterogeneity ($p = 0.98$; $I^2 = 0\%$, Figure 15). Meta-analysis of the data using a fixed-effect model showed that there was no significant difference between the two groups (RR = 0.65, 95% CI: 0.37, 1.16, $p = 0.15$ >0.05).

3.4. Sensitivity Analysis. Sensitivity analysis of the above indicators was conducted by the one-by-one elimination method, and changes of the effect size and p value were observed after the one-by-one exclusion of the included studies. The results showed that the effect size of outcome indicators did not change significantly, suggesting that the results of the meta-analysis were reliable and stable.

3.5. Publication Bias. Egger's test and Begg's test were used to evaluate whether there was publication bias in the main outcome indicators. For oral CLMD alone vs. antidepressant, no evidence of publication bias was found in the effective rate (Egger's test $p = 0.7165$ >0.05, Begg's test $p = 0.382$ >0.05) as well as the HAMD score (Egger's test $p = 0.6926$ >0.05, Begg's test $p = 1.6918$ >0.05). As for oral CLMD + antidepressant vs. antidepressant, there was publication bias in the effective rate (Egger's test $p = 0.002$ <0.05, Begg's test $p = 0.0354$ <0.05) and the HAMD score (Egger's test $p < 0.0001$, Begg's test $p = 1.9285$ >0.05).

4. Discussion

In this study, a meta-analysis of 13 RCTs of CLMD in the treatment of PSD showed that the following. (1) In terms of total effective rate, we found that CLMD combined with antidepressants was more effective than antidepressants alone, while there was no difference between CLMD and antidepressants alone; (2) HAMD is the most commonly used in the assessment of depressive symptoms, and both CLMD alone and CLMD with antidepressants were better than antidepressants alone in reducing HAMD scores. Depending on the course of treatment, we found different conclusions. When the course of treatment was <30 days, oral CLMD was more effective than antidepressants alone. When the course of treatment was ≥60 days, the efficacy of oral CLMD was comparable to that of antidepressants alone. Whether the treatment course was short or long, the efficacy of CLMD combined with antidepressants was better than that of the antidepressants group, indicating that the treatment of CLMD combined with antidepressants was more conducive to improving the clinical efficacy; (3) MESSS and NIHSS are the international major indicators for the evaluation of neurological function recovery after stroke, which are of great significance for the judgment of

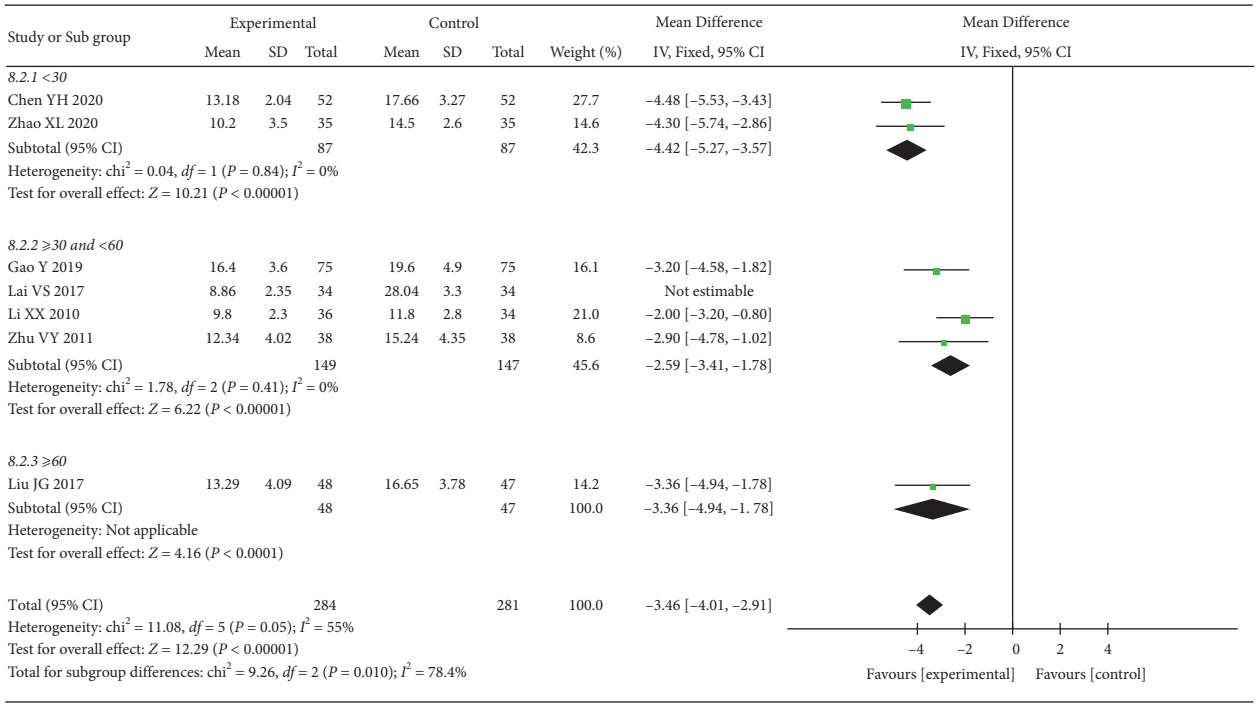

Figure 12: Subgroup analysis of oral CLMD + antidepressant vs. antidepressant in the HAMD score.

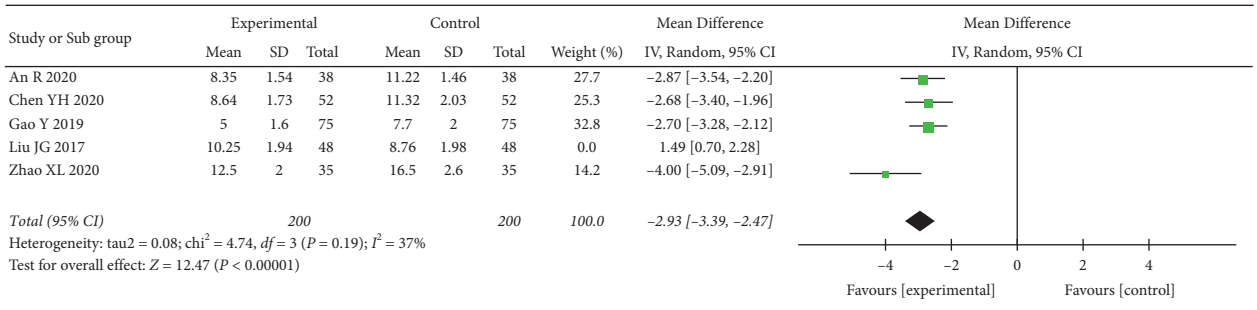

Figure 13: Meta-analysis results of oral CLMD + antidepressant vs. antidepressant in the NIHSS score.

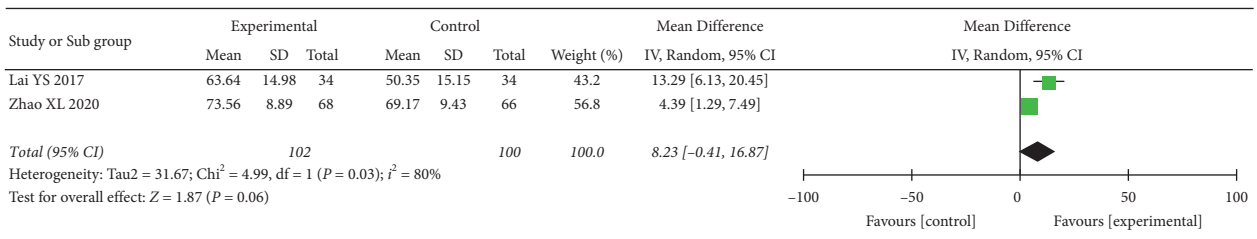

Figure 14: Meta-analysis results of oral CLMD + antidepressant vs. antidepressant in Barthel index.

postoperative recovery after stroke. Clinical observation shows that MESSS and NIHSS have good predictive validity for the prognosis of stroke and are significantly correlated with Barthel index [31]. The higher the score is, the lower the BI value is [32]. Due to the limited number of the included studies, only the NIHSS scores of oral CLMD combined with antidepressants were meta-analyzed, and the results showed that the combined treatment group was superior to the antidepressant group; (4) Barthel index is an indicator to test the independent living ability of patients, which can reflect the degree of nursing need of the patients. Barthel index also can be used to evaluate the functional recovery of PSD patients. As the score of specific items of Barthel index was not reported in the included studies, it was impossible to objectively evaluate the specific impact of CLMD alone or CLMD + antidepressant on the

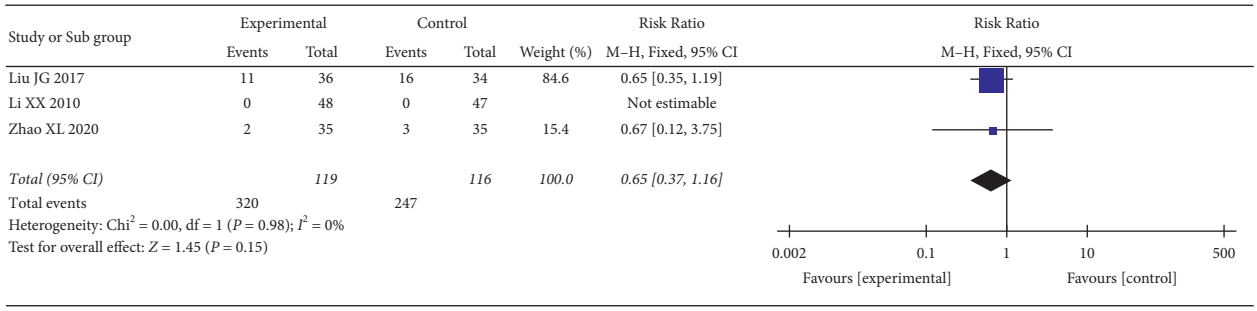

Figure 15: Meta-analysis results of oral CLMD + antidepressant vs. antidepressant in adverse reactions rate.

independent living activities of PSD patients. The results showed that the BI score of oral CLMD alone was better than that of the antidepressant group, indicating that CLMD was positive and effective in improving the independent living ability of PSD patients, but there was no significant difference between oral CLMD combined with antidepressants and the antidepressant group, which may be related to the course of treatment; (5) in terms of adverse reactions, there were a total of 5 RCTs in our study that described adverse reactions, among which two RCTs were about CLMD alone vs. antidepressant, which reported that no adverse reactions occurred in the treatment group, while the adverse reactions in the control group included insomnia, gastrointestinal discomfort, dizziness, and headache. The remaining three RCTs were related to CLMD + antidepressant vs. antidepressant, among which one RCT reported no adverse reactions in the treatment group and the control group, while two RCTs reported no significant differences in adverse reactions between the treatment group and the control group, including abnormal blood and urine routine, abnormal liver function, insomnia, and digestive tract discomfort. It is seen that CLMD does not increase the risk of adverse reactions, but it does not reduce the side effects of depression; and (6) we conducted sensitivity analysis on all outcome indicators through the one-by-one exclusion method, and the results showed that our meta-analysis was robust.

Stroke is an important social psychological factor leading to depression. Neurological dysfunction and long-term disability caused by stroke lead to the psychological stress response, which brings about psychological imbalance [1]. Depression hinders the recovery of the neurological function after stroke. Antidepressant treatment can not only relieve the symptoms of depression but also promote the physical recovery of stroke patients, which is far more vital than the treatment of depression itself [33].

After thousands of years of exploration, Chinese medicine has advantages in the treatment of mental disorders [34–36]. CLMD is one of the most common prescriptions used in the treatment of mental diseases in TCM. Clinical studies have found that CLMD has the effect of psychotropic drugs and have shown a significant antidepressant effect on animal models [37, 38]. It can regulate the hypothalamopituitary-adrenal system dysfunction by preventing the dopaminergic and serotonergic transmission in the prefrontal cortex [39] and upregulate the expression of the brain-derived neurotrophic factor (BDNF) to alleviate the depression-like state induced by chronic stress [37]. It also has immediate and long-lasting antidepressant effects by enhancing BDNF expression in the hippocampus [38]. Chaihu (Radix Bupleuri) and Huang Qin (Radix Scutellariae) are the key drugs in many prescriptions for mental disorders. Modern pharmacological studies have found that they can reduce neuroinflammation [40] and neuronal apoptosis [41] and increase the concentration of the nerve growth factor and BDNF [42]. Baicalin in Huang Qin (Radix Scutellariae) can inhibit inflammation [43] and promote nerve regeneration [44, 45]. Baicalin also has an antidepressant-like effect [46], which is associated with the increase of BDNF in the hippocampal region [44].

Limitations of this study are as follows: some of the included studies seldom describe the specific operation of the allocation concealment and blind method, and there may be selectivity bias and measurement bias; all the studies are from China, and there may be regional restrictions; due to the particularity of TCM decoction, the composition and dosage of CLMD in the study were different, which may have a certain influence on the results of the study.

5. Conclusion

Current evidence supports the efficacy of CLMD in PSD patients, which can not only improve depressive symptoms but also promote the recovery of neurological and limbs functions in stroke patients. The efficacy of CLMD alone is no less than that of antidepressants, and there are fewer adverse reactions. In addition, CLMD alone was more effective when treatment was less than 30 days, while oral CLMD combined with antidepressants was more effective than antidepressants alone in both short- and long-term treatment.

Abbreviations

PSD: Poststroke depression
SSRI: Selective serotonin reuptake inhibitor
SNRI: Serotonin norepinephrine reuptake inhibitor
NaSSA: Noradrenergic and specific serotonergic antidepressant
TCAs: Tricyclic antidepressants
TCM: Traditional Chinese medicine
CLMD: Chaihu Jia Longgu Muli decoction

CBM: China biomedical literature service system
RCTs: Randomized controlled trials
CLM: Chaihu Jia Longgu Muli
HAMD: Hamilton's Depression Scale
NIHSS: National Institute of Health Stroke Scale
MESSS: Modified Edinburgh-Scandinavian Stroke Scale
WMD: Weighted mean difference
SMD: Standard mean difference
RR: Relative risk
BDNF: Brain-derived neurotrophic factor.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Renhong Wan and Ruiwen Song are the co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Renhong Wan and Ruiwen Song contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81503637 and 81973939).

Supplementary Materials

Table S1. The PRISMA checklist. Table S2. Search strategy in PubMed database. (*Supplementary Materials*)

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