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The efficacy and mechanism of Zhengan Xifeng Decoction in the treatment of ischemic stroke: a network pharmacology study

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Abstract: *Background: To systematically evaluate the effectiveness and mechanism of Zhengan Xifeng Decoction in the treatment of ischemic stroke based on network pharmacology research methods. Methods: The active ingredients of Zhengan Xifeng Decoction were collected by TCMSP. Uniprot database was used to obtain the list of potential targets of active ingredients of Zhengan Xifeng Decoction. GeneCards and OMIM databases were used to obtain ischemic stroke disease-related targets. String website was used to construct protein interaction network (PPI). Metascape was used for GO enrichment and KEGG pathway enrichment analysis. Results: 183 effective active components, 415 targets and 1022 disease-related targets of Zhengan Xifeng Decoction were screened by network pharmacology, and 136 intersection gene targets were obtained. PPI analysis revealed that the key targets involved TNF, AKT1, IL6, IL1B, VEGFA, etc. Enrichment analysis obtained 2076 GO items ($P < 0.01$) and 193 KEGG items ($P < 0.01$).*

Keywords: *Zhengan Xifeng Decoction; Ischemic stroke; Network pharmacology; Therapeutic mechanism*

1. Introduction

Ischemic stroke, also known as cerebral infarction, is the most common type of cerebrovascular disease, the main cause of formation is cerebral blood circulation disorders, local brain tissue hypoxia, ischemic necrosis or softening, and ultimately cause the corresponding neurological deficits [1]. Modern medical treatment of ischemic stroke mainly uses ultra-early thrombolytic therapy and interventional arterial thrombectomy [2]. Thrombolytic therapy is considered to be the most important blood flow recovery measure at present. Recombinant tissue plasminogen activator (rt-PA) and urokinase (UK) are the main thrombolytic drugs used in clinical practice at present, but they have strict "time window" and contraindications, so that most patients cannot be thrombolyzed in time or can not be thrombolyzed; endovascular mechanical thrombectomy is also an important means recommended by guidelines in recent years for the treatment of early ischemic stroke, but the requirements for operators make some primary hospitals unable to be effectively applied. For other patients who do not meet thrombolysis or mechanical thrombectomy, western medicine is mainly based on anti-platelet aggregation and lipid regulation and plaque stabilization, but the improvement of symptoms is extremely limited.Ischemic stroke belongs to the category of "stroke" in TCM. The classical Chinese medicine "Huang Di Nei Jing" believes that "Zhufeng drops dizziness and all belong to the liver". The therapeutic effect of calming the liver and extinguishing wind, resolving phlegm, removing blood stasis and dredging collaterals on stroke is significant. Modern physicians Zhang Bolong and Zhang Xichun further recognize that the occurrence of this disease is mainly liver-yang wind, qi and blood upside down, and straight offending the brain.Zhengan Xifeng Decoction is from Zhang Xichun's "Medical Xinzhong Shenxi Lu", which consists of Achyranthes bidentata, ochre, raw keel, raw oyster, raw turtle plate, Radix Paeoniae Alba, Radix Scrophulariae, Radix Asparagi, Neem, Malt, Yinchen, and Radix Glycyrrhizae, with a total of 12 herbs, which is to reuse Achyranthes bidentata to induce blood flow, break its yang hyperactivity, and Keel, oyster, turtle plate, peony to benefit yin and subside yang, Zhenggan Xifeng, a total of ministers.Ochre to reduce stomach and reduce Chong, Scrophularia, Asparagus Qing fei Qi, liver wood, Yin chen, malt, Neem Shugan Qi, to Shun liver, are

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adjuvant.Glycyrrhiza uralensis harmonizes various drugs to make.Zhuyao combined with Zhenggan Xifeng, nourishing yin and subsiding yang, for liver-yang hyperactivity, qi and blood upside down and other stroke special, the effect is quite good.In recent years, it has been widely used due to the significant advantages of multi-target and multi-link onset of action of traditional Chinese medicine.The use of traditional Chinese medicine compound treatment of cerebral infarction gradually increased, of which Zhengan Xifeng Decoction is more respected, but the efficacy is controversial [3,4]. The aim of this study was to conduct a meta-analysis and network pharmacology analysis of randomized controlled trials of Zhengan Xifeng Decoction in the treatment of ischemic stroke to assess the efficacy and mechanism of Zhengan Xifeng Decoction in the treatment of ischemic stroke and provide stronger evidence-based medical evidence for the clinical application of Zhengan Xifeng Decoction in the treatment of ischemic stroke.

2. Study method

2.1. Effective active ingredient screening and target prediction

Use the pharmacology database and analysis platform of traditional Chinese medicine system (TCMSP) [5] database to query the active ingredients of the drugs contained in Zhengan Xifeng Decoction, use oral availability (OB) ≥ 30% and drug-like activity (DL) ≥ 0.18 as screening parameters for active ingredient and target extraction, use Uniprot database [6], limit human source (Human) and certified (Reviewed) matching, and obtain the list of potential targets of active ingredients of Zhengan Xifeng Decoction.

2.2. Screening of disease-related targets

Using GeneCards [7] (Fig. www.genecards.org), and the OMIM [8] database were searched for "Cerebral Infarction", respectively, to obtain ischemic stroke-related targets after de-repetition.

2.3. Common targets of compound and disease

The network of active pharmaceutical ingredients and targets and disease gene targets was constructed using Venny 2.1.0 to obtain the common targets of compound and disease. Cytoscape 3.7.1 was used to construct the network diagram of the relationship between active pharmaceutical ingredients and targets and disease common gene targets.

2.4. PPI network construction

The protein interaction PPI network is constructed based on String database [9]. The protein interaction network (PPI) is constructed and analyzed by Cytose 3.7.1 to select the key targets.

2.5. GO enrichment and KEGG pathway enrichment analysis of key components

GO enrichment and KEGG Passways enrichment analysis of common key targets of drug diseases were performed through Metascape [10], and mapping was completed using online mapping software on the Weishengxin website.

2.6. Network construction of key pathway targets

According to the KEGG enrichment results, Cytoscape 3.7.1 was applied to construct a network map of key pathway targets.

3. Results

3.1. Screening of active ingredients and targets in traditional Chinese medicines

Screening of effective active ingredients and targets.Using the Systematic Pharmacology Database and Analysis Platform of Traditional Chinese Medicine (TCMSP), the active ingredients and targets were extracted using oral availability (OB) ≥ 30% and drug-like properties (DL) ≥ 0.18 as screening

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parameters to obtain the active ingredients of Zhengan Xifeng Decoction, 20 Achyranthes bidentata, 13 Paeonia lactiflora, 9 Scrophularia sinensis, 9 Asparagus officinalis, 9 Neem, 18 Malt, 13 Artemisia capillaris, and 92 Glycyrrhiza uralensis, 2 ochre substitutes were obtained in the Herb database, 20 turtle plates, 3 keels, and 10 oysters, using the Uniprot database, limited human source (Human) and certified (Reviewed) matching, achid5-lipogenase, Thrombin, calmodulin and other ingredients could not be obtained in the gene list, so they were excluded, and after eliminating repeated targets for each drug, 193 active ingredient targets of Zhengan Xifeng Decoction were obtained: 193 Achyranthes bidentata, 83 Radix Paeoniae Alba, 44 Scrophulariae There were 15 oysters, and a total of 415 targets were obtained after all drugs eliminated repeated targets.

3.2. Identification of disease targets

Disease target screening.GeneCards and OMIM databases were searched for "Cerebral Infarction", respectively, to have a Relevance score ≥ 4.52, and a total of 1022 targets related to ischemic stroke disease were obtained after weight removal.

3.3. Traditional Chinese Medicine - Ingredients - Diseases - Target Network Diagram Construction

Construction of Network of Active Ingredients and Targets of Compound Drugs and Disease Gene Targets.A total of 136 intersection gene targets were obtained by processing the drug targets of Zhengan Xifeng Decoction and ischemic stroke targets through Venny 2.1.0 (Figure 1) Cytoscape 3.7.1 was applied to obtain the relationship diagram of the common gene target network between Zhengan Xifeng Decoction and ischemic stroke (see Figure 2), which involved 288 nodes, 1801 edges, and the Degree value size was expressed by the shape size, and the comparison of the value results revealed that mistletoe, β-sitosterol, catharol, and isorhamnetin played an important role.

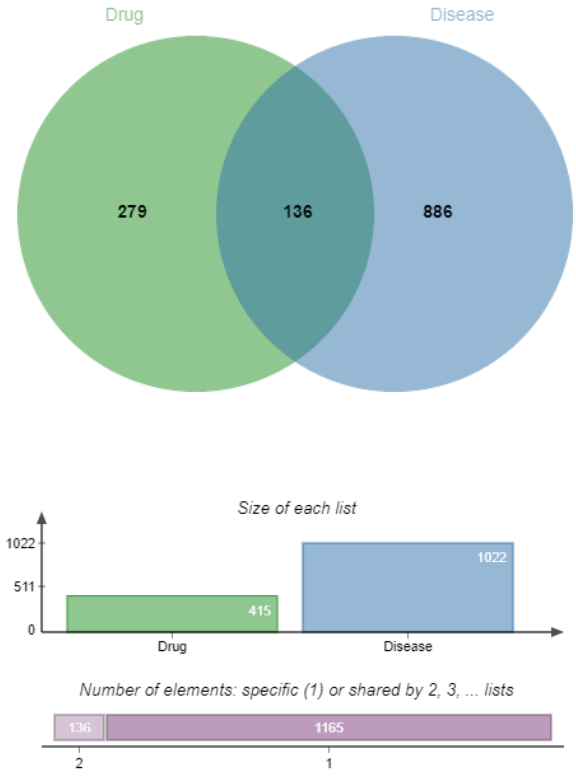

Figure 1: Venny Diagram of Ischemic Stroke Treated by Zhengan Xifeng Decoction

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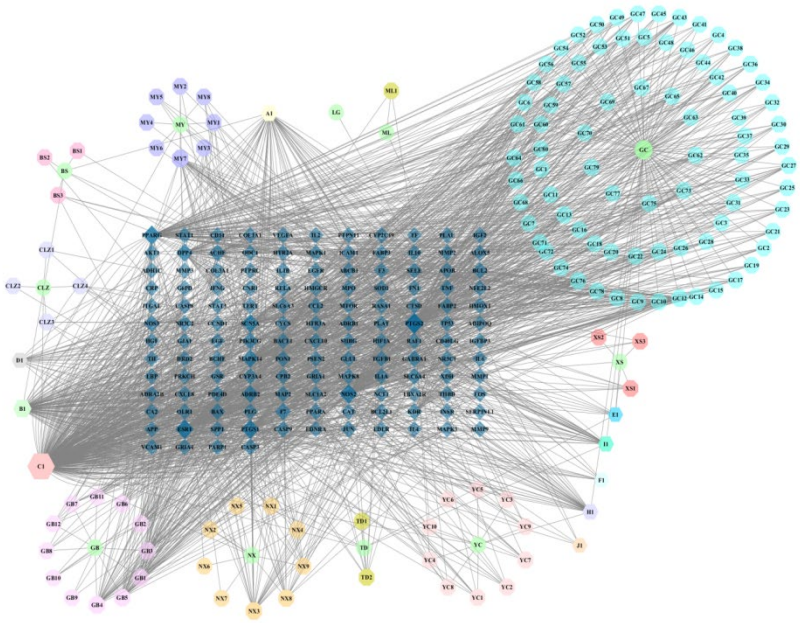

Figure 2: Relationship between Zhengan Xifeng Decoction and Common Gene Targets of Ischemic Stroke (he circle is the drug name, the octagon is the active pharmaceutical ingredient, the hexagon is the common pharmaceutical ingredient, and the diamond is the target gene)

3.4. Construction of traditional Chinese medicine-disease target PPI network

String-based PPI network construction imports the common targets into String, sets the organism as "Homo sapiens" confidence > 0.40, constructs the PPI network, and applies Cytoscape3.7.1 to analyze and screen them, as shown in Figure 3. This figure contains 136 nodes, 2702 edges, the darker the color in the figure, the higher the representing Degree value, mainly including TNF, AKT1, IL6, IL1β, VEGFA, etc.

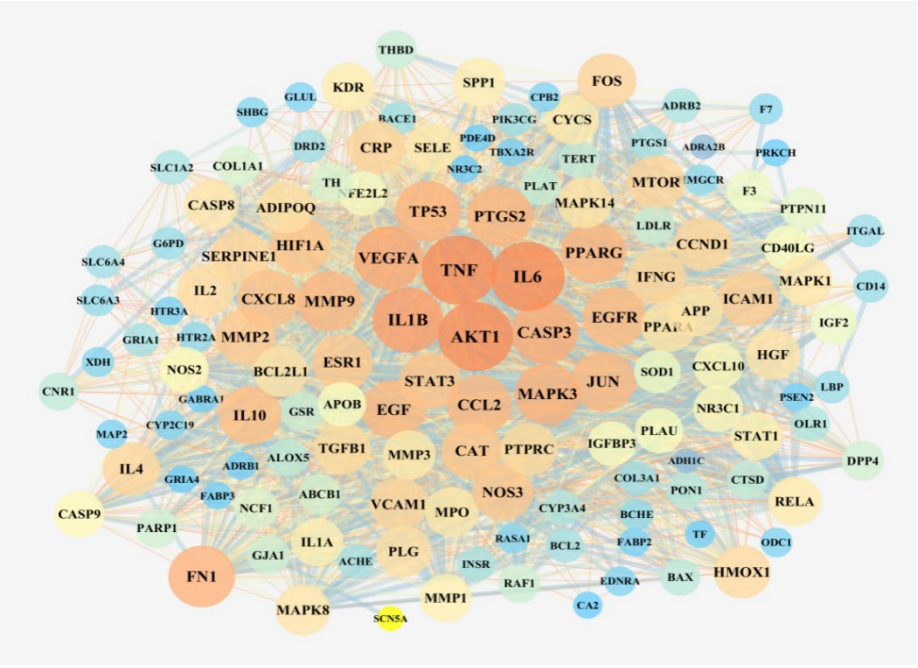

Figure 3: Network Diagram of Protein Interaction (PPI) between Zhengan Xifeng Decoction and Key Targets of Ischemic Stroke

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3.5. GO enrichment analysis results

GO enrichment analysis of key components Through Metascape database, a total of 1796 biological processes (BP) were enriched, mainly involved in response to inorganic substances, positive regulation of cellular component movement, response to extracellular stimulation, etc., and a total of 126 cellular components (CC) were enriched, indicating that the treatment process was mainly carried out through cell body medial raft, vesicle lumen, positive plasma membrane response of inorganic membrane, etc., in this part of molecular function (MF), a total of 154 were enriched, the tightest was kinase binding, transcription co-regulator binding (protease binding),For catecholamine binding activity, $P < 0.01$ was used as the screening condition. The top ten most significant items of BP, CC and MF were selected. The bar chart was drawn online using Microbiology, as shown in Figure 4.

Figure 4: Histogram of GO functional enrichment analysis of key targets of Zhengan Xifeng Decoction in the treatment of ischemic stroke (The left side represents the name and the right side represents count)

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3.6. Results of KEGG pathway enrichment analysis

KEGG pathway enrichment analysis of key components used Metascape database and enriched a total of 193 related signaling pathways. The results showed that AGE-RAGE signaling pathway in diabetic atherosclerosis, Lipid and atherosclerosis, Fluid shear stress and atherosclerosis, IL-17 signaling pathway and TNF signaling pathway were closely related to the mechanism of Zhengan Xifeng Decoction in the treatment of ischemic stroke. The top 20 pathways with the closest relationship were selected to draw a histogram and bubble diagram, as shown in Figure 5.

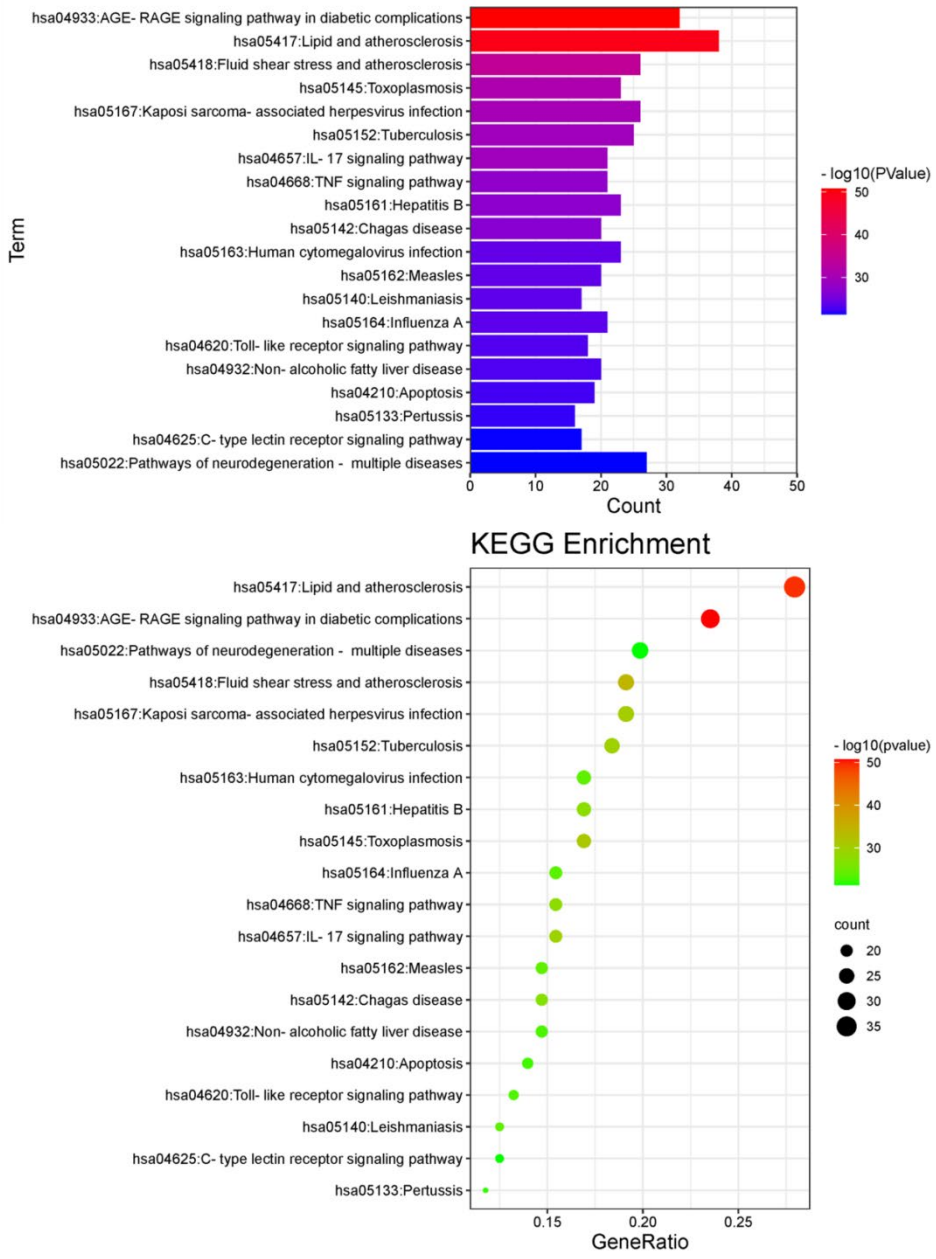

Figure 5: Histogram and Bubble Diagram of KEGG Pathway Enrichment Analysis of Key Targets of Zhengan Xifeng Decoction in the Treatment of Ischemic Stroke

3.7. Target Pathway Network Construction

Target Pathway Network Construction.According to the KEGG pathway enrichment results, the main top 20 major pathways were selected, and the network was constructed by using Cytoscape 3.7.1 to construct the 20 major pathways with target information, as shown in Figure 6.

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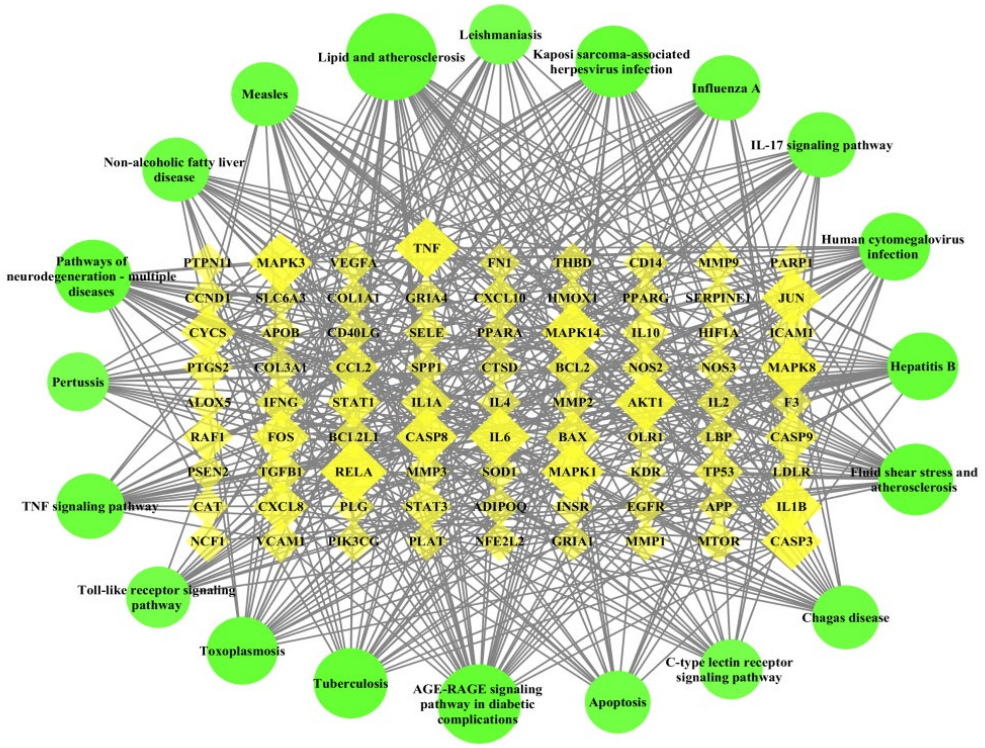

Figure 6: Network diagram of target pathway of Zhengan Xifeng Decoction in treating ischemic stroke

4. Discussion

In network pharmacology, the screening of key components of Zhengan Xifeng Decoction in the treatment of ischemic stroke revealed that mistletoe bombesin, β-sitosterol, kanamycin, and isorhamnetin play an important role.Among them, mistletoe bombesin is widely distributed in the plant kingdom. Studies such as Yang Ran [11] have found that mistletoe bombesin can improve the neurological damage caused by local cerebral ischemia-reperfusion in rats, inhibit the release of inflammatory factors, reduce the degree of brain tissue damage, and play a neuroprotective role; β-sitosterol has the effects of inhibiting apoptosis, reducing the level of density lipoprotein, and effectively preventing atherosclerosis [12]. Koo [13] and other studies have found that rich β-sitosterol can significantly improve the area of atherosclerotic lesions in mice and prevent cerebrovascular diseases. Sinopherol and quercetin belong to flavonoids, which are similar to the pharmacological effects of bombesin. The latest findings show that sinopherol can inhibit mitochondrial fission by activating Akt and maintain mitochondrial HK-II, and protect neurons from succinate-mediated ischemic stroke [14]. Isorhamnetin has a wide range of antiviral and antioxidant effects, and isorhamnetin can inhibit foam cell formation in atherosclerotic plaques, reduce lipid core area in sclerotic plaques, and stabilize plaques by up-regulating SIRT6 expression levels [15]. In the construction of PPI network of key target protein interaction, we found that TNF, AKT1, IL6, IL1β, VEGFA, etc. are the key targets of Zhengan Xifeng Decoction in the treatment of ischemic stroke. The treatment of ischemic stroke with Zhengan Xifeng Decoction may be related to the intervention of inflammatory response and abnormal proliferation of vascular endothelial cells. TNF is a pleiotropic protein, involved in the body's immune response and multiple pathological processes, and plays an important role in the central nervous system. Studies have found that TNF can be involved in almost every stage of stroke-related neuronal injury, such as inflammation and thrombotic events [16]. Interleukins IL6 and IL1β, as inflammatory factors, play an important role in the body's anti-infective immune process, and it is found that TNF-α and IL6 and IL1β have a pleiotropic synergistic effect in the brain, and IL6 can synergize with TNF to induce the VEGF pathway by "trans signaling" and increase vascular permeability. TNF-α enhances the toxic effects of IL1β in a synergistic manner [17],so these two inflammatory cytokines can somehow reconcile and induce neuroinflammation and brain injury after ischemic stroke. AKT, protein kinase, regulate cell proliferation and growth, the latest discovery of AKT signaling pathway and MAPK is a key survival pathway for cells, can regulate the

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survival and apoptosis of nerve cells, protect neurons, and become an important target for the treatment of ischemic stroke and programmed cell death [18]. VEGFA, vascular endothelial growth factor, has a strong effect of inducing angiogenesis and neuroprotection, which has attracted the attention of stroke researchers [19]. However, preclinical experiments have found that the dual regulatory effect of VEGFA in vascular endothelium, especially increasing vascular permeability may lead to brain edema and increase intracranial pressure, which is harmful to acute stroke, so there is still a lot of controversy about VEGFA [20]. GO enrichment analysis revealed that GO biological processes mainly involve pathways such as response to inorganic substances and positive regulation of cellular components, membrane rafts, vesicle lumens, and extracellular matrix may be key target positions for drug treatment of diseases, and in terms of molecular functions, they mainly converge on kinase binding, transcription factor binding, and catecholamine binding.KEGG pathway enrichment analysis revealed that Zhengan Xifeng Decoction effectively slowed down the disease process mainly through AGE-RAGE signaling pathway, lipid and atherosclerosis pathway, IL-17 signaling pathway, TNF signaling pathway, atherosclerosis is the most common cause of ischemic stroke, some studies have found that AGE-RAGE signaling pathway and lipid and atherosclerosis pathway are closely related to atherosclerosi [21], inflammatory response is an important cause of post-ischemic cranial nerve injury, a large number of studies at home and abroad focus on various inflammatory signaling channel blockers and ion function regulation, IL-17 signaling pathway and inflammatory response are closely related, effective treatment can greatly reduce the brain injury after ischemic stroke.TNF plays an important role during cerebral ischemia and can trigger the activation of NF-kβ and MAPK pathways and increase inflammatory events in the brain environment, which may become a new treatment for ischemic stroke by inhibiting TNF-α [22, 23].

5. Conclusion

Network pharmacology results showed that Zhengan Xifeng Decoction had a clear effect in the treatment of ischemic stroke, which mainly intervened the inflammatory response and abnormal proliferation of vascular endothelial cells through the active ingredients such as mistletoe, β-sitosterol, catechol, isorhamnetin, TNF, AKT1, IL6, IL1β, VEGFA and other key targets to regulate the level of ischemic stroke. However, due to the complex composition of Zhengan Xifeng Decoction, in the process of drug composition and target screening, some active ingredients were missed and the disease target was insufficient due to limited conditions. Although the results have statistical significance, but the reliability may be insufficient, so a more sample study should be carried out in the future to further clarify the key targets of Zhengan Xifeng Decoction in the treatment of ischemic stroke and provide a stronger evidence-based medicine basis for its treatment of ischemic stroke.

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