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Network Pharmacological Analysis of Juhua's Therapeutic Action in Stroke Management

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Abstract: The purpose of this study was to investigate the effective active substances in Juhua for treating stroke and their specific mechanisms. Traditional Chinese Medicine systems pharmacology platform (TCMSP) screened effective Juhua components and their target proteins. Stroke-related targets were obtained from the GeneCards, OMIM, DrugBank, and TTD databases. Intersecting elements were pinpointed through Venn diagrams. A PPI network was assembled by leveraging the STRING database. An active ingredient-target-pathway network of Juhua was established using Cytoscape 3.8.2 software. The analysis of Gene Ontology, encompassing the biological processes (BP), molecular functions (MF), and cellular components (CC) was executed through the DAVID database. Moreover, to delineate the underlying mechanisms responsible for Juhu's efficacy, we conducted Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Juhua contains 15 active components effective against stroke and 11 key target proteins. GO analysis showed that Juhua's treatment of stroke is associated with 2322 biological processes, 71 cellular components, and 215 molecular functions. KEGG pathway enrichment analysis indicated that Juhua exerts its therapeutic effects on stroke through pathways including Lipid and Atherosclerosis, AGE-RAGE signaling pathway, Hepatitis B, and others. The active components of Juhua, targeting multiple targets and pathways, play a role in the prevention and treatment of stroke. The potential mechanisms of Juhua in preventing and treating stroke predicted in this study can provide a basis for conducting experimental research and clinical applications.

1. Introduction

Stroke, as an acute cerebrovascular disease, poses major global public health challenges due to its high incidence, mortality, and disability rates. It seriously threatens human health and imposes a significant socioeconomic burden[1]. The WHO reports that stroke is the world's second-highest mortality cause, third in disability rates, and the primary cause of death in China[2]. The primary clinical treatment for ischemic stroke remains intravenous thrombolysis, particularly with recombinant tissue plasminogen activator (rt-PA). However, this treatment is limited by a strict 4.5-hour therapeutic window and carries risks of serious complications like cerebral hemorrhage

and reperfusion injury[3]. Moreover, patients with large vessel occlusions have a low recanalization rate after thrombolysis. Factors such as patient age, comorbidities, and coagulation status further limit the number of patients eligible-less than 10% actually receive thrombolytic therapy. These challenges have spurred ongoing research into innovative treatments including mechanical thrombectomy, neuroprotection, and stem cell therapies[4]. At the same time, efforts are underway to strengthen stroke centers, streamline emergency protocols to reduce time from symptom onset to treatment, and implement comprehensive measures such as secondary prevention and rehabilitation. These initiatives aim to overcome current therapeutic limitations, increase stroke treatment success, and improve patients' long-term quality of life[5].

Juhua is a perennial herbaceous plant belonging to the Asteraceae family. As a traditional Chinese medicine with a long history, it has been widely used in Chinese medicinal prescriptions for treating various diseases. Its rich active components and unique pharmacological effects have also made it a research focus in the field of traditional Chinese medicine. Juhua is rich in various active components (polysaccharides, flavonoids, volatile oils, etc.), some of which have been widely studied and reported[6]. For example, the volatile oils in Juhua are among its most representative active components, with main constituents including α-pinene, jasmonyl alcohol, and eugenic acid. In addition, plants of the Juhua genus also contain abundant terpenoids, phenylpropanoids, and flavonoids. Furthermore, Juhua has multiple pharmacological effects, which is one of the important reasons for its extensive use in clinical traditional Chinese medicine. It has been confirmed that Juhua possesses antioxidant, anti-inflammatory, antibacterial, antitumor, and anti-aging effects[7, 8].

However, currently there is no research indicating whether Juhua has a therapeutic effect on stroke. Therefore, this study will use a network pharmacology approach to investigate its mechanism of action from aspects such as the effective active components of Juhua, anti-stroke targets, and pathways, providing a reference for further clarifying the pharmacological effects of Juhua.

2. Materials and Methods

2.1 Collection of active pharmaceutical components and their corresponding targets

This study is based on the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) to systematically retrieve the active components of Juhua. Considering the complexity of traditional Chinese medicine components and human absorption characteristics, strict screening criteria were set: only effective components with oral bioavailability not less than 30% and drug-likeness index above 0.18 were retained. Subsequently, potential therapeutic targets of the active components meeting these criteria were obtained through the TCMSP platform.

2.2 Screening stroke-related disease targets and pharmacological targets of Juhua in stroke treatment

Using "stroke" as a keyword, stroke-related disease targets were obtained through four databases: GeneCards, OMIM, TTD, and DrugBank. By creating a Venn diagram, the intersection of the targets of action corresponding to each active components in Juhua and stroke target genes was taken, and the common targets were screened out as the pharmacodynamic targets of Juhua active components for treating stroke.

2.3 Developing PPI Networks and Identifying Key Targets

Drug action target data obtained from Venn diagram screening were imported into the String database platform, limiting the species to “Homo sapiens”, setting the interaction confidence threshold to the highest level (0.900), keeping other parameters at default, and constructing the target protein interaction network after removing isolated nodes. Later, key targets for Juhua’s stroke intervention were identified via network topology analysis executed with the CytoNCA plugin within Cytoscape 3.8.2.

2.4 Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

This study investigated how Juhua influences the biological pathways involved in cerebral stroke by analyzing predicted pharmacodynamic targets. Using R software's ClusterProfiler, we performed comprehensive Gene Ontology (GO) annotation and KEGG pathway enrichment analyses. The findings were then visualized to clearly illustrate the underlying mechanisms and functional relationships.

3. Result

3.1 Extraction of key bioactive substances from Juhua and their action sites

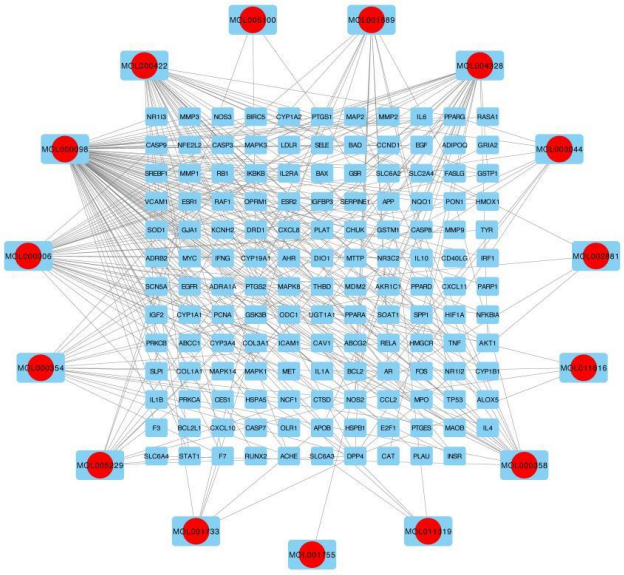

Figure 1: Illustration of Compound-Target Gene Interactions Network

This study conducted a systematic retrieval of the chemical constituents of Juhua based on the TCMSP database, obtaining a total of 210 known compounds. According to the screening criteria of Oral Bioavailability (OB) ≥30% and Drug-likeness (DL)≥18%, 17 components with potential pharmacological activities were ultimately determined, involving a total of 225 action targets. The Cytoscape software was used to perform a network topology analysis on the screening results, constructing a “Juhua-Target” interaction network model (Figure 1). In this visualization network, red nodes represent the active components of Juhua, and light cyan nodes represent the corresponding protein targets. The network analysis results indicate that components such as quercetin, luteolin, and kaempferol exhibit a high degree of network connectivity.

3.2 Screening of stroke-related disease targets and pharmacological targets of Juhua for treating stroke

This study, by integrating the resources of four major databases—GeneCards, OMIM, TTD, and DrugBank, acquired a total of 2915 genes related to the pathological mechanism of stroke (see Figure 2A for details). Furthermore, by employing a Venn diagram analysis method, the intersection operation was performed between the action targets of 17 active components of Chrysanthemum and the aforementioned stroke-related genes, ultimately identifying 142 common action targets. These targets are considered the potential pharmacodynamic basis for Juhua's active components to exert their anti-stroke effects (the results are presented in Figure 2B).

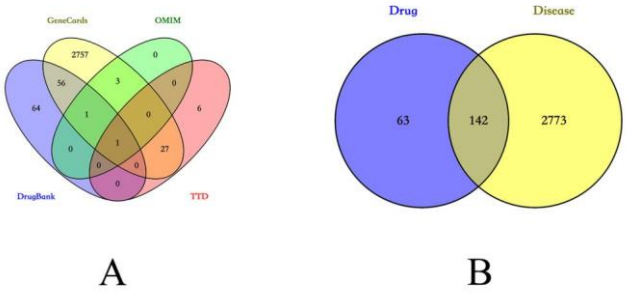

Figure 2: Juhua-Stroke target map

3.3 Construct Protein-Protein Interaction Network Diagram and Screen Core Targets

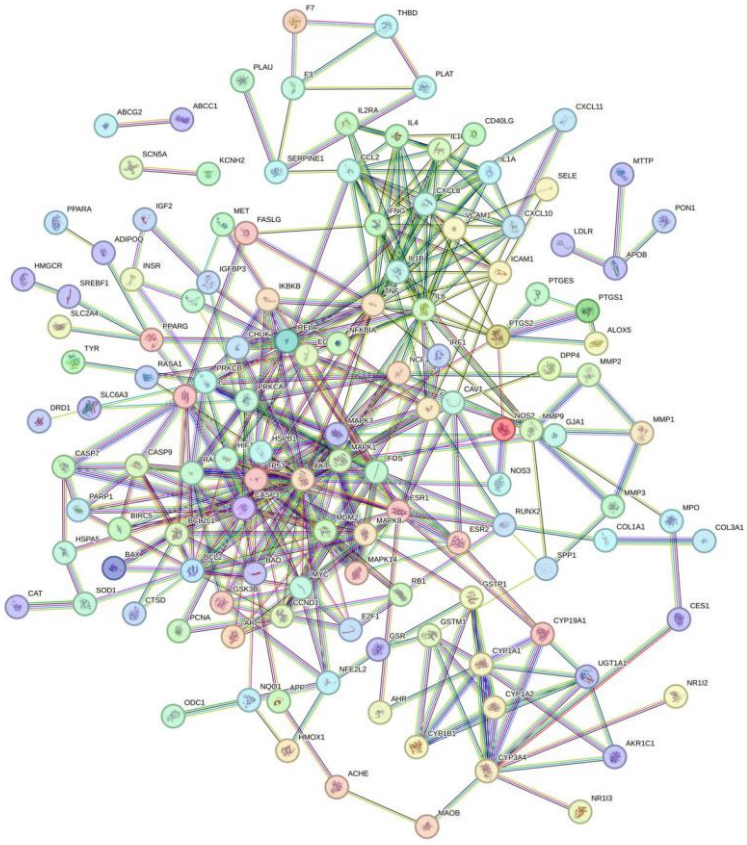

Figure 3: PPI network

To thoroughly investigate the pharmacodynamic mechanism of Juhua in the treatment of stroke, this study imported 142 potential action targets, previously identified through screening, into the String database to construct a protein-protein interaction network (Figure 3). The CytoNCA plugin was instrumental in carrying out a comprehensive topological examination of the network at hand. Through the application of a variety of stringent filters—such as a BC threshold greater than 10.3, a CC threshold above 0.5, a DC threshold of over 8, an EC threshold surpassing 0.13, a LAC threshold above 4.5, and an NC threshold greater than 5.7—an intersectional analysis was executed, pinpointing 11 crucial core nodes, including MAPK3, TNF, MAPK1, IL6, and AKT1 (refer to Figure 4). These targets may constitute the key molecular basis for the anti-stroke effects of Juhua.

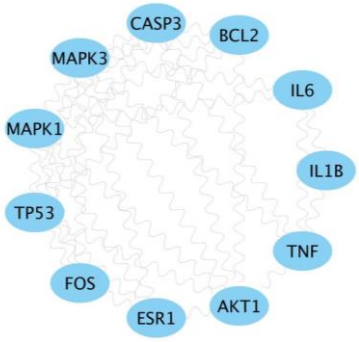

Figure 4: Key core targets construction

3.4 Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

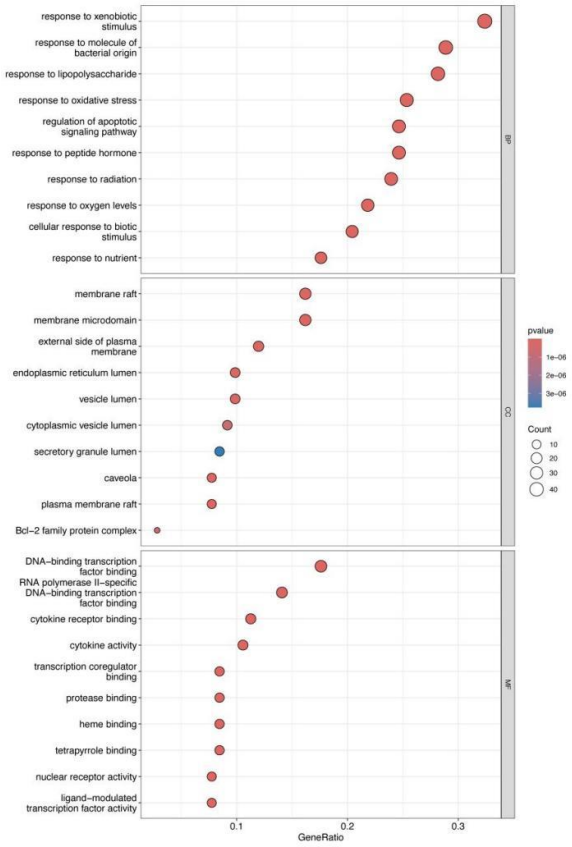

Figure 5: Gene Ontology Assessment of Stroke Intervention Targets

To investigate the biological mechanisms behind chrysanthemum's effects on cerebral stroke, we uploaded 142 pharmacodynamic targets of its active components into the DAVID online platform. We conducted both Gene Ontology (GO) functional analysis and KEGG pathway enrichment analysis, maintaining a statistical significance threshold of $P<0.05$ to ensure our findings were robust and reliable. The results of the GO functional enrichment analysis showed 2608 entries, of which 2322 were biological processes (BP), mainly involving response to xenobiotic stimulus, response to molecule of bacterial origin, response to lipopolysaccharide, response to oxidative stress, regulation of apoptotic signaling pathway, and so on. 71 were cellular components (CC), mainly involving membrane raft, membrane microdomain, caveola, plasma membrane raft, external side of plasma membrane, endoplasmic reticulum lumen, etcand so on. And 136 were molecular functions (MF), involving DNA-binding transcription factor binding, nuclear receptor activity, ligand-modulated transcription factor activity, RNA polymerase II-specific DNA-binding transcription factor binding, transcription coregulator binding, and so on. In this study, the top 10 entries for each category were selected to draw bubble plots, as shown in Figure 5. The results of the KEGG pathway enrichment analysis showed a total of 189 pathways involved, mainly involving Lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, Hepatitis B, Fluid shear stress and atherosclerosis, Prostate cancer, etc. In this study, the top 30 signaling pathways were selected to draw bubble plots, as shown in Figure 6.

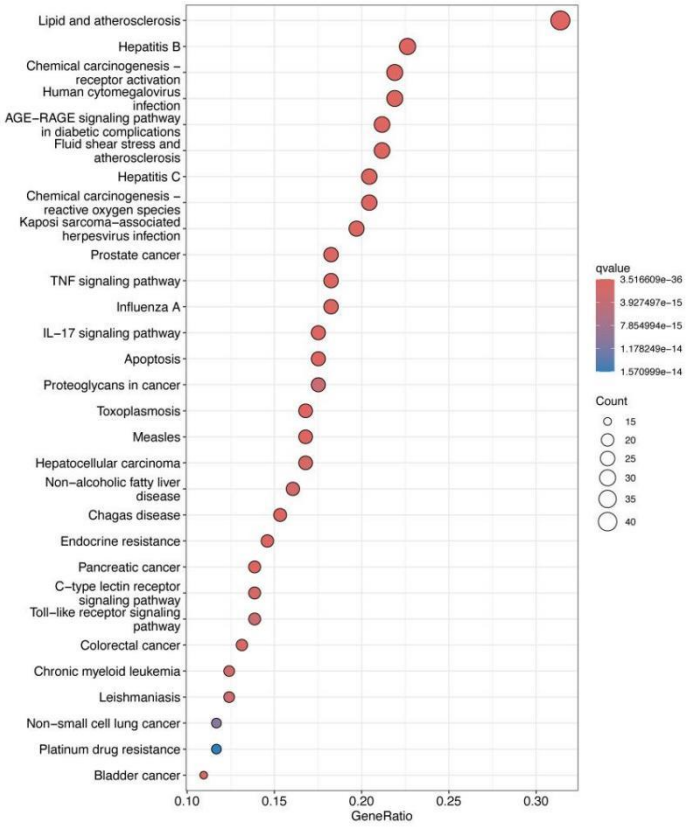

Figure 6: KEGG Pathway Analysis of Stroke Treatment Targets (Top 30 Pathways)

4. Discussion

Stroke is considered a leading cause of death and disability worldwide, accompanied by various complications, which in turn lead to prolonged hospital stays and an increased medical burden[9]. Amidst societal aging and urbanization trends, coupled with rising unhealthy living habits,

cerebrovascular disease cases are progressively mounting[10]. Stroke has emerged as the leading cause of mortality and long-term impairment among adults nationwide. The prevalence of stroke-related health challenges in our country is skyrocketing, with cases increasingly affecting younger populations[11]. Traditional Chinese medicine treatment is a commonly used clinical method for treating stroke patients. At present, there are more than 140 traditional Chinese medicine prescriptions that can be used for the treatment of post-stroke cognitive impairment[2].

Juhua, as a traditional Chinese medicinal herb, has been extensively documented to contain numerous active compounds that exert significant effects in areas such as antioxidant, anti-inflammatory, antibacterial, antitumor, and anti-aging activities. While research on the active constituents of Juhua has advanced, their investigation is complicated by the multi-component, multi-target nature of their synergistic effects[12]. This complexity is further compounded by the intricate structures of these active components, which often feature multiple chiral centers and exist as various conformational and configurational isomers, rendering their separation and identification particularly challenging[13]. Furthermore, pharmacological studies have predominantly concentrated on the in vitro activity screening of purified compounds, with a notable lack of research concerning in vivo pharmacodynamic evaluation and structure-activity relationships.

This study employs network pharmacology to investigate the therapeutic mechanisms of chrysanthemum in the treatment of stroke. The screening identified eleven core targets: MAPK3, TNF, MAPK1, IL6, and others, which are the key targets through which chrysanthemum exerts its therapeutic effects in stroke treatment. MAPK3 is an important component of the mitogen-activated protein kinase (MAPK) pathway, playing a crucial connecting role in the process of cell signal transduction, and is involved in processes such as neuronal remodeling and cell migration[14], and plays a key role in the process of apoptosis induced by oxidative stress[15]. After activation in the process of cerebral ischemia-reperfusion, it can reduce apoptosis and inflammatory responses, exhibiting a significant neuroprotective effect[16]. Tumor necrosis factor (TNF) is a key factor in many classical pathways, playing a very important role in regulating cell survival and death, apoptosis, and necrosis. Studies have shown that the regulatory network centered on NR3C1 may be the key regulatory network for necrosis and apoptosis of cardiac microvascular endothelial cells[17]. MAPK1, also known as ERK2, is a member of the mitogen-activated protein kinase (MAPK) family and a key regulator of proliferation, differentiation, and survival signals[18]. IL-6 is an inflammatory cytokine, associated with unstable carotid plaques, and is significantly elevated during the acute phase of ischemic stroke. In the subacute and chronic phases, it can also act as a neurotrophic mediator, participating in the injury repair process after cerebral ischemia[19], and its neuroprotective effect is achieved by upregulating the expression and function of neuronal adenosine A1 receptors[20].

This study systematically reveals the multi-mechanistic effects of Juhua in treating stroke. Its therapeutic effects may be achieved through multiple active components (such as quercetin, luteolin, and kaempferol) acting on multiple key targets (including the genes MAPK3, TNF, MAPK1, IL6, and AKT1), and through synergistic action across multiple biological pathways, including Lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, Hepatitis B, Fluid shear stress and atherosclerosis. This discovery lays a crucial theoretical foundation for further elucidating the molecular mechanisms of Juhua in treating stroke and its clinical translation and application.

Data Availability

Data underlying the study’s conclusions are available from the corresponding author upon request.

Conflicts of Interest

The authors confirm no competing interests related to this publication.

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