# Investigating Zhishi's Therapeutic Mechanism in Stroke Management via Network Pharmacology

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**Abstract:** Purpose: To investigate the mediation process of Zhishi in stroke using network pharmacology methodologies. Methods: To pinpoint the key components and potential focus areas of Zhishi, the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and analysis tools were put to work. Targets relevant to strokes were identified across several gene-related databases, including GeneCards, OMIM, DrugBank, and TTD. By utilizing an online Venny analysis tool, common targets between Zhishi and stroke conditions were uncovered. A network depicting these shared Zhishi-stroke targets was then mapped out with Cytoscape software. A protein-protein interaction (PPI) network was crafted using the STRING database, which was followed by a detailed topological analysis of the shared targets to pinpoint crucial ones. With RStudio, the most relevant top 10 GO terms and the top 30 KEGG pathways for these shared targets were meticulously selected and visualized for a comprehensive enrichment analysis. Result: An analysis of the Zhishi database revealed 17 active components and 111 associated targets. Notably, 78 of these targets have been linked to stroke. The herb's primary active components are luteolin, naringenin, and nobiletin. The study underscored key targets like TNF, AKT1, and BCL2. The gene ontology enrichment indicates involvement in biological processes such as response to UV, response to xenobiotic stimulus, and response to light stimulus. Furthermore, KEGG pathway enrichment hints at how Zhishi might intervene in strokes, potentially through roles in Lipid and atherosclerosis, Prostate cancer signaling pathway, Toxoplasmosis, and Hepatitis B. Conclusion: Zhishi demonstrates therapeutic potential for stroke via its various components impacting multiple targets.

**Keywords:** Network Pharmacology; Stroke, Mechanism; Zhishi

## 1. Introduction

Stroke is a series of brain damage diseases caused by the rupture or obstruction of cerebral blood vessels, among which ischemic stroke accounts for 80%, characterized by high morbidity, high mortality, high recurrence rate, and high disability rate, causing great harm to human health and survival, and bringing a heavy burden to the patients' families and society[1, 2]. Due to its complex pathogenesis, treatment from a single factor and single perspective often cannot achieve good therapeutic effects. In recent years, the effect of traditional Chinese medicine in the prevention and treatment of cerebral ischemia has received increasing attention, mainly from the perspective of replenishing qi to activate blood circulation and dredging meridians to activate collaterals, acting through multiple levels, multiple targets, and multiple links, with relatively small toxic and side effects[3].

Network pharmacology is a method based on systems biology theory to study bioactive components and their action targets[4]. Because the components of traditional Chinese medicine are complex and their mechanisms of action are not yet clear, and network pharmacology integrates multiple databases, uses bioinformatics methods to bridge drugs and diseases, and is suitable for the analysis of traditional Chinese medicine components, this study adopts network pharmacology methods to comprehensively analyze the mechanism of Zhishi in treating stroke.

In traditional Chinese medicine theory, illness is mainly due to the imbalance of organ system in the body, which will affect the normal flow of qi and blood. Coupled with the wind, heat, phlegm, blood stasis, these bad things will make the brain stupid[5]. Clinical research data show that syndromes related to phlegm and congestion are very common in stroke patients. Studies have shown that traditional Chinese medicine can treat stroke in many ways, such as reducing oxidative stress, inhibiting cell death and regulating vascular function.

Pomelo peel is the dried young fruit of the Rutaceae family's sour orange and its cultivated varieties or sweet oranges, possessing the effects of breaking qi, eliminating stagnation, resolving phlegm, and dissipating fullness. There is a saying, "No pomelo peel can dissipate fullness," and clinically, pomelo peel is used to treat many diseases[6]. By tapping into the power of network pharmacology, this investigation dissects Zhishi's bioactive components, molecular targets, and signaling cascades to shed light on its protective mechanisms against stroke. The findings not only paint a clearer picture of how this traditional remedy operates but also open up fresh perspectives on how ancient Chinese medicine might contribute to modern approaches in preventing and treating cerebrovascular disorders.

## 2. Materials and Methods

### *2.1 Identifying Active Components in Zhishi and Forecasting Corresponding Targets*

By leveraging the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP), researchers pinpointed the active constituents in Zhishi that met stringent pharmacokinetic benchmarks—specifically, those with oral bioavailability (OB) of at least 30% and drug-likeness (DL) scores of 0.18 or higher. Subsequently, the UniProt database was utilized to extract and annotate the protein targets linked to these bioactive elements, facilitating precise gene identification.

### *2.2 Zhishi—Shared Targets in Stroke and Network Construction*

Conducting a search with "stroke" as the focal point, dive into GeneCards, OMIM, DrugBank, and TTD databases. Once there, synthesize and eliminate duplicates to pinpoint disease-specific targets. Utilize the Venn 2.1.0 online utility to scrutinize and merge the disease targets with those of Chinese herbal elements, zeroing in on any overlaps. With Cytoscape, craft a network map that depicts the connections between Zhishi's targets and stroke. This will enable the construction of a comprehensive Protein-Protein Interaction (PPI) network for Zhishi and stroke.

### *2.3 Establishing the PPI Network for Zhishi—Identifying Stroke-Linked Targets*

Plugging the Zhishi stroke-related target genes into the STRING database and selecting Homo sapiens as the species allowed us to generate a protein-protein interaction network. Subsequently, we turned to Cytoscape to conduct a topological analysis, where we applied the connectivity degree filter on two separate occasions to pinpoint and identify the key core targets that really made the network tick.

### *2.4 Zhishi—Stroke Collective Objective GO Evaluation and KEGG Route Enrichment Assessment*

To conduct GO and KEGG pathway enrichment analyses in RStudio, integrate the Zhishi—stroke shared targets. The GO analysis will encompass Biological Processes (BP), Molecular Functions (MF), and Cellular Components (CC). With a threshold of P≤0.05, sort the findings from lowest to highest and cherry-pick the top 10 GO analyses and the top 30 KEGG pathways for a comprehensive visualization of the analysis outcomes.

## 3. Result

### *3.1 Active Components of Zhishi and Related Targets*

The research pinpointed 17 bioactive components tied to 111 possible targets. After sifting through the inactive elements, 78 crucial targets linked to Zhishi were narrowed down. A network diagram was crafted (Figure 1) that illustrated these connections, where the bioactive components were displayed in the outer circle and their corresponding targets in the inner circle. The connections were then ranked according to their strength, pinpointing there key active ingredients based on their centrality in the network: luteolin, naringenin, and nobiletin. These components stood out as the main bioactive players in the analysis.

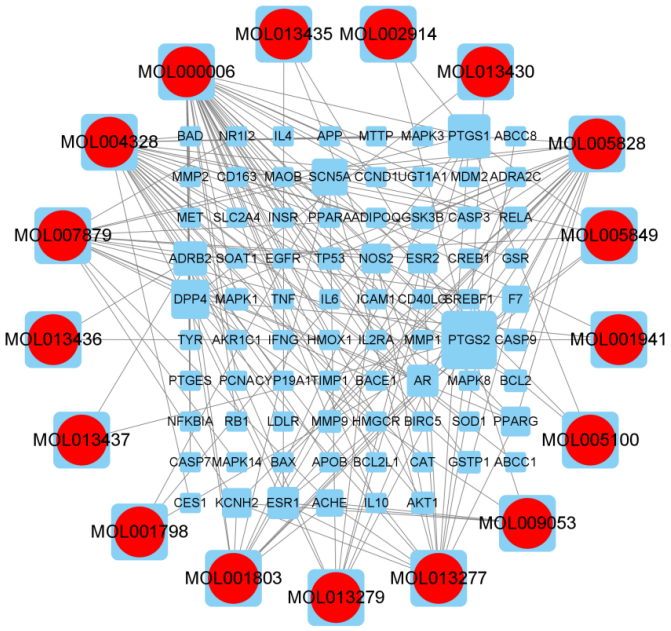


**Figure 1:** Components-Gene Interaction Network Visualization.

### *3.2 Pinpointing Common Zhishi Targets in Stroke Therapy and Charting Their Network Relationships*

A thorough investigation spanning numerous databases uncovered stroke-related targets, with GeneCards contributing 5097 entries, OMIM adding 5, TTD providing 37, and DrugBank chipping in with 2. After weeding out the duplicates, researchers were left with 2915 distinctive stroke-linked targets (Figure 2A). When these targets were put side by side with the 186 known therapeutic targets of Zhishi, a Venn diagram analysis revealed 78 common ground targets (Figure 2B). This intersection suggests these overlapping targets could be the heavy hitters when it comes to the herb's

neuroprotective punch against stroke.

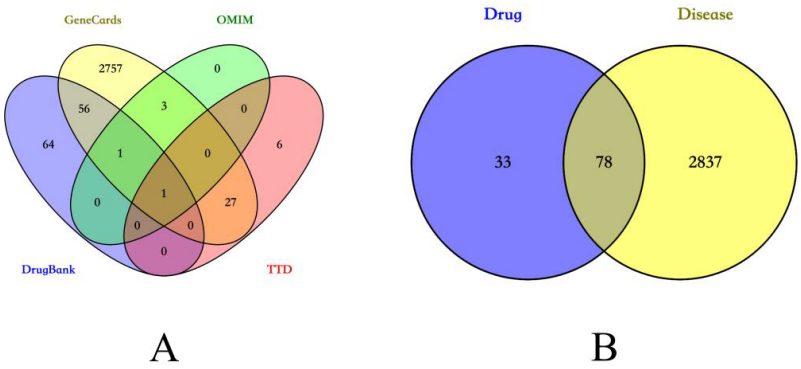


**Figure 2:** Knowledge Network in Stroke Therapy Interventions.

### *3.3 Zhishi Protein Interactions and Central Stroke Therapeutic Targets*

To delve deeper into the therapeutic prowess of Zhishi in treating strokes, an extensive examination of the 78 potential targets pinpointed earlier was meticulously carried out. This exploration was facilitated by the String database, and the findings are vividly depicted in Figure 3.

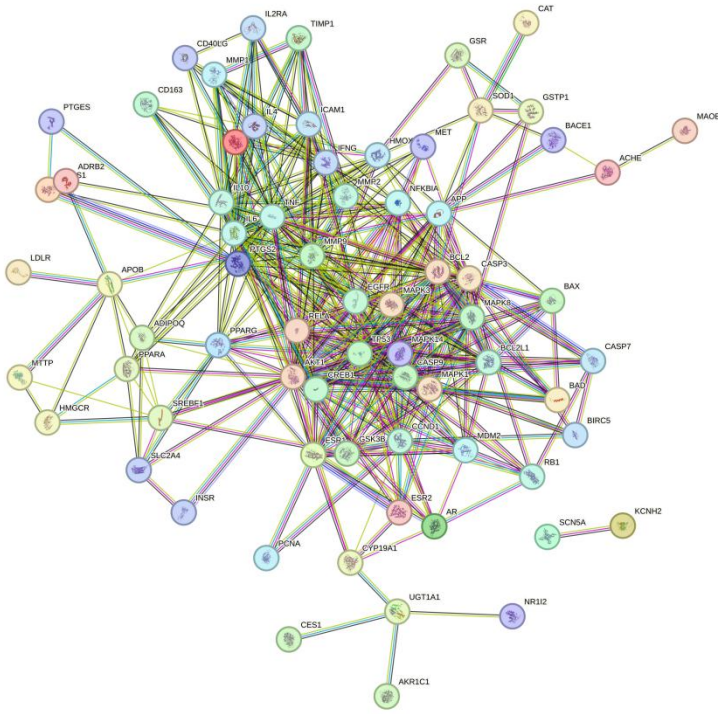


**Figure 3:** PPI network.

By making the most of the CytoNCA plug-in, we conducted a thorough topological examination of the protein interaction network. Our investigation zeroed in on crucial hub targets that cleared a high bar, with BC scores topping 4.49, CC scores going over 0.72, DD scores above 15, EC scores beyond 0.19, LAC scores exceeding 11, and NC scores surpassing 12.56. From this pool, we singled out the core targets and identified the top three heavy hitters for Zhishi stroke treatment as TNF, AKT1, BCL2, and several others. These results are laid out in Figure 4.

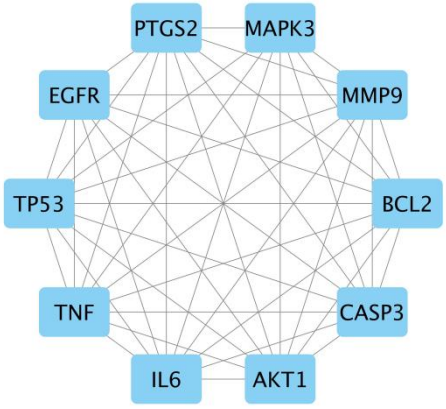


**Figure 4:** PPI Core Network Screening.

### *3.4 Enrichment Outcomes from Go and KEGG Pathways Analysis for Zhishi in Stroke Therapy*

Using the R language software, a total of 2191 GO terms were enriched, including 1980 BP terms, 148 CC terms, and 63 MF terms. These mainly involved biological processes such as rresponse to UV, response to xenobiotic stimulus, and rresponse to light stimulus. Cellular components such as membrane raft, membrane microdomain, and endoplasmic reticulum lumen. Molecular functions such as phosphatase binding, DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, and nuclear receptor activity (Figure 5). Additionally, a total of 182 KEGG pathways were enriched. These mainly included Lipid and atherosclerosis, Prostate cancer, and Toxoplasmosis (Figure 6).

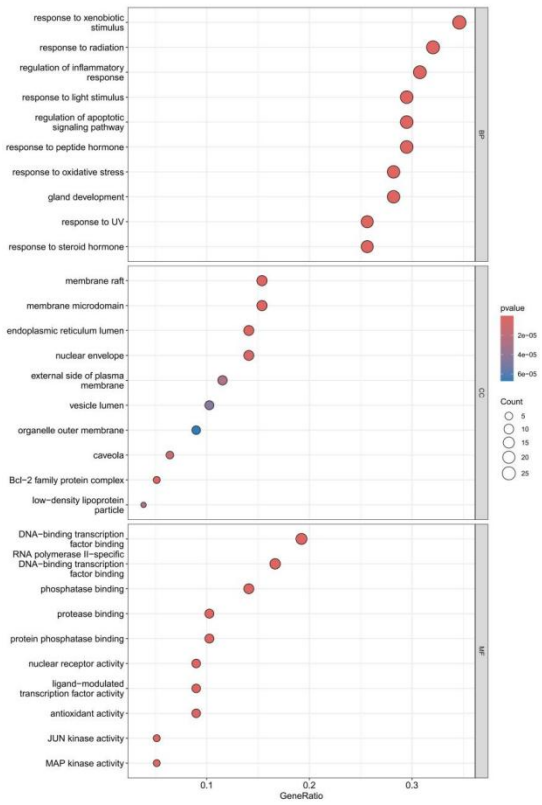


**Figure 5:** Gene Ontology (GO) Analysis for Therapeutic Targets Identified in Zhishi.

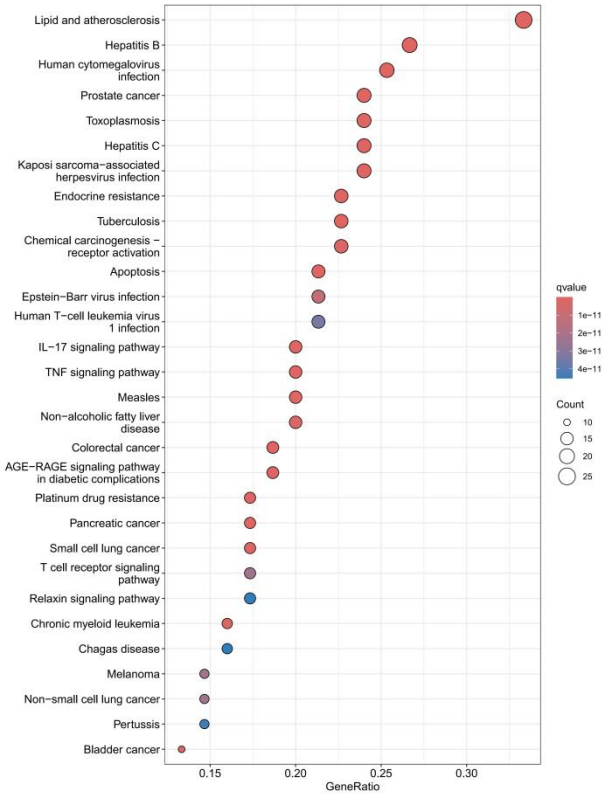


**Figure 6:** KEGG Pathway Enrichment Study for Therapeutic Candidate Targets of Zhishi.

## 3. Discussion

Stroke is an acute cerebrovascular disease caused by focal cerebral ischemia or hemorrhage, being one of the leading global causes of death and disability, imposing a heavy medical burden[7]. Global Burden of Disease (GBD) 2021 data indicates that its annual mortality rate accounts for approximately 10.7% of global total deaths, with around 11.9 million new cases annually, over 70% of which are concentrated in low- and middle-income countries; the situation in our country is even more severe, with stroke being the primary cause of death from disease domestically, the age-standardized incidence rate in adults reaching 246.8 per 100,000 person-years, and showing a trend of becoming more youthful[8, 9]. Stroke is mainly classified as ischemic (70%–80%) and hemorrhagic, with complex pathological mechanisms: the former originates from energy metabolic disorders following cerebral blood flow interruption, subsequently inducing multi-linkage damage; the latter is characterized by core features such as hematoma compression, both leading to irreversible brain tissue damage and neurological sequelae[10, 11]. Clinical treatment is highly time-sensitive and varies by subtype; existing reperfusion therapy, symptomatic interventions, and secondary preventive drugs all have limitations in application or adverse effects, making it difficult to cover multi-linkage pathological mechanisms, thus there is an urgent need for new drug development[12]. Traditional Chinese Medicine (TCM) possesses multi-component, multi-target characteristics, capable of synchronously regulating key pathological nodes of stroke, showing significant therapeutic potential[13]. This study investigates the mechanism of Zhishi in treating stroke through network pharmacology, which can provide a new basis for developing alternative or complementary therapies.

The findings of this study indicate that the primary active components in Zhishi responsible for

treating stroke are likely luteolin, naringenin, and nobiletin. The pathological process of stroke involves a cascade reaction of multiple links, including ischemia-reperfusion injury, inflammatory storm, oxidative stress overload, blood-brain barrier disruption, and neuronal apoptosis. It is difficult for single-target drugs to effectively block the complex pathological network[12, 14]. However, luteolin, naringenin, and nobiletin, as natural flavonoid components, exhibit synergistic neuroprotective potential due to their pharmacological characteristics of multiple components and multiple targets. Luteolin focuses on early-stage inflammation and oxidative stress in stroke, by inhibiting NF-κB nuclear translocation, upregulating the Nrf2/HO-1 pathway, reducing the release of pro-inflammatory factors and the accumulation of reactive oxygen species, while simultaneously inhibiting the caspase-3/9 apoptosis pathway to reduce cerebral infarct volume[15]. Naringenin centers on blood-brain barrier protection and vascular repair, by activating the PI3K/Akt/eNOS pathway to stabilize tight junction proteins and inhibit MMP-9 activity, while simultaneously promoting VEGF expression to improve cerebral microcirculation and alleviate cerebral edema[16, 17]. Nobiletin targets mitochondrial function protection and long-term neuronal survival, by inhibiting mPTP opening and upregulating the SIRT1 and BDNF/TrkB pathways to restore energy metabolism and promote synaptic remodeling, thereby improving long-term neurological function prognosis[18, 19]. The three form a temporal complementarity of "early-stage damage blockade, mid-stage reduction of secondary injury, and long-term promotion of repair," and at the signal pathway level, they form a synergistic regulatory network through cross-pathways such as NF-κB, PI3K/Akt, and SIRT1, which can both enhance neuroprotective effects and reduce the dosage of individual components to decrease adverse reactions[20, 21]. Nevertheless, the three still face challenges such as low bioavailability and limited blood-brain barrier penetration, and there is a lack of systematic research and clinical trial verification for their combined application. Future efforts are needed to improve delivery efficiency through nanocarrier modification, optimize the combination ratio, and clarify the molecular mechanisms to provide theoretical and experimental support for the development of novel therapeutic strategies for stroke[22].

According to the core target analysis of the PPI network in this study, Zhishi may treat stroke by acting on TNF, AKT1, BCL2. After ischemic stroke, microglia in the ischemic brain region rapidly activate and secrete large amounts of TNF-$\alpha$, which promotes the progression of secondary brain injury by disrupting the integrity of the blood-brain barrier, exacerbating inflammatory damage to neurons, and inducing neuronal apoptosis in the ischemic penumbra[23]. The human BCL2 gene is located on chromosome 18 and encodes the BCL2 protein, which is a core member of the anti-apoptotic subfamily of the BCL2 family[24]. After stroke, BCL2 expression in the ischemic region of neurons is downregulated, and the balance between pro-apoptotic and anti-apoptotic proteins is disrupted, leading to massive apoptosis of neurons[25]. Exogenous upregulation of BCL2 expression or activation of its anti-apoptotic function through drugs can significantly reduce infarct volume and improve neurological functional prognosis[26]. The human AKT1 gene is located on chromosome 14 and encodes protein kinase B$\alpha$ (PKB$\alpha$), which is a core node molecule in the PI3K/Akt signaling pathway[27]. Activation of the PI3K/Akt pathway can stabilize the tight junction proteins of the blood-brain barrier (Occludin, ZO-1), inhibit neuronal apoptosis, promote angiogenesis in the ischemic area, and alleviate cerebral ischemia-reperfusion injury[28].

## 4. Conclusions

To sum up, this investigation demonstrates that Zhishi combats stroke through a multifaceted

approach, engaging various constituents, targeting multiple sites, and affecting numerous biological routes. The research suggests that its proposed mode of action likely centers on active components ingredients such as luteolin, naringenin, and nobiletin regulating key genes including TNF, AKT1, BCL2, achieving treatment via pathways like Lipid and atherosclerosis, HepatitisB, and Humancytomegalovirus infection signaling pathway. This study offers a theoretical foundation for investigating Zhishi's therapeutic mechanisms and clinical use in stroke treatment.

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