

Exploring the mechanism of action of Buyang Huanwu Tang in treating post-stroke sequelae based on network pharmacology

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Abstract: Objective: To explore the mechanism of action of Buyang Huanwu Tang in treating post-stroke sequelae through network pharmacology methods. Method: Based on the TCMSP database, screen the active ingredients in Buyang Huanwu Tang. Screening relevant targets for post-stroke sequelae through Gene Card database and OMIM database, and drawing Venn diagrams using Venn package in R language, and using Cytoscape 3.10.3 Software is used to construct a network of "prescription drugs active ingredients disease targets", followed by enrichment analysis of GO and KEGG pathways. Finally, the analysis results are molecular docked to identify potential targets for the treatment of post-stroke sequelae with Buyang Huanwu Tang and exert therapeutic effects. Result: A total of 63 potential active ingredients were screened out, and 67 potential targets were predicted. GO and KEGG analysis showed that Buyang Huanwu Tang can improve post-stroke sequelae symptoms by regulating the apoptotic signaling pathway, membrane raft, DNA binding transcription factor binding, and so on. Conclusion: This study elucidates that Buyang Huanwu Tang can exert therapeutic effects through a multi-component synergistic mode of action. These findings provide new ideas and scientific basis for the mechanism of action of Buyang Huanwu Tang in treating post-stroke sequelae.

Keywords: Network pharmacology; Buyang Huanwu Tang; Sequelae of stroke; Mechanism of Action

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Stroke is a common clinical disease in neurosurgery^[1]. With the intensification of aging, the incidence rate of the disease is increasing year by year. According to the results of 2023 China Health Statistics Summary, the number of stroke patients in China is as high as 13 million^[2]. The clinical manifestations of ischemic stroke include neurological dysfunction, communication disorders, swallowing disorders, changes in consciousness, loss of independent living ability, etc. In severe cases, it can lead to hemiplegia and limb dysfunction. The commonly used Western medicine in clinical practice for treatment and intervention is not ideal, and there may be many adverse reactions^[3]. It has a high disability rate and high mortality rate^[4]. Bu Yang Huan Wu Tang originated from the book "Yi Lin Gai Cuo" by the Qing Dynasty physician Wang Qingren, and is a representative formula for treating ischemic stroke^[5]. The main medicines are Astragalus, Angelica sinensis tail, Red Peony Root, Earthworm, and Chuanxiong Rhizome. In the formula, qi tonifying drugs and a small amount of blood activating drugs are commonly used. Qi tonifying and blood activating drugs are used to treat the root cause, while blood stasis removing and meridian unblocking drugs are used to treat the symptoms. Together, they play a role in tonifying qi, promoting blood circulation, and meridian unblocking^[6]. However, due to its multiple components and targets, the mechanism of action of Buyang Huanwu Tang has not been fully elucidated. In recent years, network pharmacology, as an emerging research method, has provided new ideas and tools for revealing the complex mechanisms of action of traditional Chinese medicine formulas.

I. Materials and Methods

1.1 Active ingredient screening and target prediction of Buyang Huanwu Tang

Utilize the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP)^[7]. Using oral bioavailability (OB) $\geq 30\%$ and drug likeness (DL) ≥ 0.18 as standards, screen the active ingredients of each drug in Buyang Huanwu Tang^[8]. Due to the lack of relevant information on Earthworm in the TCMSP database, the active protein components in Earthworm were obtained by reading relevant literature^[9]. Then, the corresponding component structures were drawn using the computer-aided drug design platform (Swiss ADME), and the CAS number was obtained to obtain the corresponding protein names. Convert into gene names through the UniProt database to obtain corresponding targets^[10].

1.2 Target and intersection acquisition of post-stroke sequelae

Using the keyword 'Post stroke sequelae of qi deficiency blood stasis type', search for relevant information on disease targets in GeneCard and OMIM databases, and then draw Venn diagrams of disease targets and drug targets through Venn packages in R language.

1.3 Drawing of the "Formula Drug Active Ingredient Disease Target" Network

The action network of "compound drug active ingredient target" was constructed using Cytoscape 3.10.3 software, which includes the target of the compound drug components and active ingredients in Buyang Huanwu Tang, as well as the target of post-stroke sequelae.

1.4 Construction of protein-protein interaction network (PPI)

Load the common targets of diseases and drugs into the STRING database, set the species as "Homo Sapiens", and obtain the network relationship diagram between component targets and disease targets. Then, use the bioinformatics analysis platform Cytoscape (v3.10.3) to construct the protein-protein interaction network diagram, hide independent nodes, set the interaction threshold as "highest confidence (0.400)", save the downloaded file in TSV file format, and perform interaction network topology analysis^[11]. Afterwards, the results will be imported into the bioinformatics analysis platform Cytoscape (v3.10.3) to draw an interaction network of intersecting targets. At the same time, CytoNCA plugin will be used to participate in data analysis and select the core targets for the treatment of post-stroke sequelae with Buyang Huanwu Tang.

1.5 GO Function and KEGG Pathway Enrichment Analysis

The intersection of the active ingredients in Buyang Huanwu Tang and potential targets in post-stroke sequelae was analyzed using the cluster profiler package in R language for GO biological process analysis, including cellular component analysis (CC), biological processes analysis (BP), molecular function analysis (MF)^[12], and KEGG signaling pathway enrichment analysis. Analyze the results through the visualization technology of ggplot2 package.

1.6 Molecular docking

Molecular docking is the characteristic and affinity of the interaction between small molecule ligands and protein receptors used to predict drug activity. The protein receptor retrieves the corresponding gene of the target protein from the uniprot database, and then downloads the PDB format file of the three-dimensional structure of the target protein from the PDB database^[13] as the receptor file for molecular docking. Obtain the two-dimensional structure of drug molecules from the PubChem database using small molecule ligands. Import the PDB format file of the target protein into Py MOL software for operations such as removing water molecules and ligands, and then use Auto Dock Tools software for visual analysis^[14]. Docking small molecule ligands into the active pocket of the target protein for molecular docking.

II. Results

2.1 Active ingredients and targets of Buyang Huanwu Tang

Search and analysis were conducted in the TCMSP database, with screening criteria of $OB \geq 30\%$ and $DL \geq 0.18$. Through systematic search and verification, active ingredients from Astragalus, Red Peony Root, Angelica sinensis, Earthworm, and Chuanxiong Rhizome were identified as 20, 29, 2, 7. A total of 63 active ingredients were obtained from Buyang Huanwu Tang, as shown in Tables 2-1, 2-2, and 2-3 Tables 2-4 and 2-5. The information on the common active ingredients of the six traditional Chinese medicines in Buyang Huanwu Tang is shown in Table 2-6.

Table 2-1 Active Ingredient Data of Astragalus in Buyang Huanwu Decoction

Mol ID	Molecule Name	DL	OB (%)
MOL000433	FA	0.71	68.96
MOL000371	3,9-di-O-methylnissolin	0.48	53.74
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl) chroman-7-ol	0.26	67.67
MOL000387	Bifendate	0.67	31.10
MOL000033	(3S,8S,9S, 10R, 13R, 14S, 17R)-10, 13-dimethyl-17-[(2R,5S)-5-propan-2-yl-octan-2-yl]-2,3,4,7,8,9, 11, 12, 14, 15, 16, 17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	0.78	36.23
MOL000417	Calycosin	0.24	47.75

MOL000098	quercetin	0.28	46.43
MOL000378	7 - O - methylisomucronulatol	0.30	74.69
MOL000239	Jaranol	0.29	50.83
MOL000380	(6aR, 11aR)-9, 10-dimethoxy-6 a, 11a-dihydro-6H-benzofuran o[3,2-c]chromen-3-ol	0.42	64.26
MOL000296	hederagenin	0.75	36.91
MOL000439	isomucronulatol-7,2'-di-O-glu cosiole	0.62	49.28
MOL000379	9, 10-dimethoxypterocarpan-3- O-β-D-glucoside	0.92	36.74
MOL000354	isorhamnetin	0.31	49.60
MOL000422	kaempferol	0.24	41.88
MOL000211	Mairin	0.78	55.38
MOL000374	5'-hydroxyiso-muronulatol-2', 5'-di-O-glucoside	0.69	41.72
MOL000392	formononetin	0.21	69.67
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	0.48	39.05
MOL000398	isoflavanone	0.30	109.99

Table 2-2 Active ingredient data of Red Peony Root in Buyang Huanwu Tang

Mol ID	Molecule Name	DL	OB (%)
MOL000359	Sitosterol	0.75	36.91
MOL002714	Baicalein	0.21	33.52
MOL000492	(+)-catechin	0.24	54.83
MOL007016	Paeoniflorigenone	0.37	65.33
MOL001925	Paeoniflorin qt	0.4	68.18
MOL000449	Stigmasterol	0.76	43.83
MOL007012	4-o-methyl-paeoniflorin qt	0.43	56.7
MOL002883	Ethyl oleate (NF)	0.19	32.4
MOL002883	Ethyl oleate (NF)	0.19	32.4
MOL001924	paeoniflorin	0.79	53.87
MOL007005	Albiflorin qt	0.33	48.7
MOL007022	evofolinB	0.22	64.74
MOL005043	campest-5-en-3beta-ol	0.71	37.58
MOL007008	4-ethyl-paeoniflorin qt	0.44	56.87
MOL006994	1-o-beta-d-glucopyranosyl-8-o- benzoylpaeonisuffrone qt	0.3	36.01
MOL002776	Baicalin	0.75	40.12
MOL006996	1-o-beta-d-glucopyranosylpaeonisuffrone qt	0.35	65.08
MOL007004	Albiflorin	0.77	30.25
MOL001921	Lactiflorin	0.8	49.12
MOL007018	9-ethyl-neo-paeoniaflorin A qt	0.3	64.42
MOL001918	paeoniflorigenone	0.37	87.59
MOL007003	benzoyl paeoniflorin	0.54	31.14
MOL000358	beta-sitosterol	0.75	36.91
MOL007014	8-debenzoylpaeonidanin	0.45	31.74

MOL006992	(2R,3R)-4-methoxyl-distylin	0.3	59.98
MOL001002	ellagic acid	0.43	43.06
MOL006990	(1S,2S,4R)-trans-2-hydroxy-1,8-cineole-B-D-glucopyranoside	0.27	30.25
MOL007025	Isobenzoylpaeoniflorin	0.54	31.14
MOL004355	Spinasterol	0.76	42.98
MOL006999	stigmast-7-en-3-ol	0.75	37.42

Table 2-3 Active ingredient data of Chuanxiong Rhizome in Buyang Huanwu Tang

Mol ID	Molecule Name	DL	OB (%)
MOL001494	Mandenol	0.19	42
MOL002157	wallichilide	0.71	42.31
MOL002140	Perlolyrine	0.27	65.95
MOL000359	Sitosterol	0.75	36.91
MOL002135	Myricanone	0.51	40.6
MOL002151	senkyunone	0.24	47.66
MOL000433	FA	0.71	68.96

Table 2-4 Active ingredient data of Earthwormin in Buyang Huanwu Tang

Mol ID	Molecule Name	DL	OB (%)
CID11326977	14-acetyl-12-photoacyl-8-diloltriol	0.31	60.31
CID7004037	2-Amino-2-methylpentanoic acid	0.31	72.92
CID123293	Dipropyl ketone	0.28	73.58
CID6114187	Ethyl oleate	0.19	32.40
CID474588	β -D-glucopyranoside	0.62	20.63
CID3391864	Amylvinyl methanol	0.24	32.79
CID80626	Methyl 2-methyl-2-acrylate	0.21	34.20

Table 2-5 Active ingredient data of Angelica sinensis in Buyang Huanwu Tang

Mol ID	Molecule Name	DL	OB (%)
MOL000449	Stigmasterol	0.76	43.83
MOL000358	Beta-sitosterol	0.75	36.91

Table 2-6 Common components of drugs in Buyang Huanwu Tang

MOL ID	Component Name	Source Herbs
MOL000359	Sitosterol	Chuanxiong Rhizome、Red Peony Root
MOL000449	Stigmasterol	Red Peony Root、Angelica Sinensis
MOL000358	Beta-sitosterol	Angelica Sinensis、Red Peony Root
MOL000433	Folic Acid	Astragalus、Chuanxiong Rhizome

2.2 Potential targets of Buyang Huanwu Tang in treating post-stroke sequelae

Using the keyword 'Post stroke sequelae of qi deficiency blood stasis type', a search was conducted through online human Mendelian genetics (OMIM) and human genome databases (Gene Card) [15], resulting in a total of 317 and 322 disease-related targets, respectively. The genes obtained from the Gene Cards database were screened with a relevance score ≥ 1 , and then integrated with the targets retrieved from the OMIM database to obtain 489 disease targets. Using the Venn algorithm to analyze and plot the effective targets of drug active

ingredients and disease-related targets (Figure 2-1), a total of 67 intersecting targets were obtained.

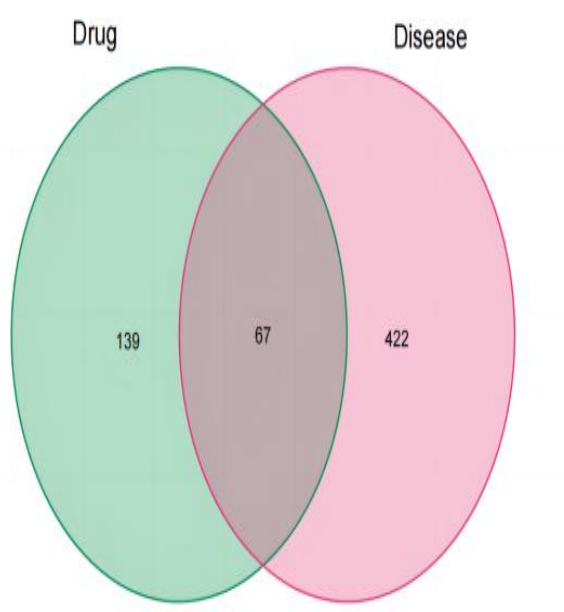


Figure 2-1 Venn diagram of disease and drug component related targets

2.3 Construction and Analysis of the "Formula Drug Active Ingredient Disease Target" Network

Using the bioinformatics analysis platform Cytoscape (v3.10.3), the action network of the "prescription drug active ingredient target" of Buyang Huanwu Tang was obtained by analyzing the 614 drug components, active ingredients, and their effects, as well as the 489 disease gene targets in Buyang Huanwu Tang. There are a total of 97 nodes and 215 edges (Figure 2-2).

The connection between node connections and drug/disease target genes is positively correlated. Separate the drug component nodes from the disease nodes, and sort the nodes in descending order according to their degree values. The core components include quercetin, kaempferol, baicalein, 7-O-methylisomucronulatol, Myricanone, formononetin, etc. These components are significantly associated with the treatment of post-stroke sequelae with Buyang Huanwu Tang and play a key role in the recovery of post-stroke sequelae.

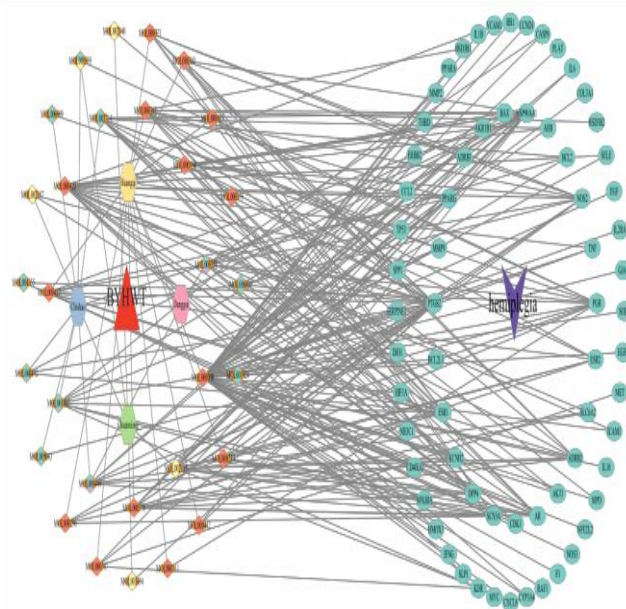


Figure 2-2 Network diagram of the action of "prescription drugs active ingredients targets" in Buyang Huanwu Tang

2.4 PPI network and core components, core targets

Import the intersection targets into the STRING database, set the species as "Homo Sapiens", hide unrelated nodes [16], and set the minimum interaction threshold to "highest confidence (0.400)" to obtain a network relationship graph of drug component targets and disease targets (Figure 2-3), with 67 nodes and 1048 edges. The imported nodes represent related proteins, and each edge represents the interaction between proteins. Save the file in TSV format for PPI analysis in Cytoscape 3.10.3 software [17]. Import the TSV file into Cytoscape 3.10.3 software and use CytoNCA plugin for network analysis to obtain Figure 2-4, which includes a total of 67 nodes and 1048 edges; Then, using R language, the core gene files of Figure 2-4 were obtained. The median values of Eigenvector, Betweenness, LAC, Degree, Network, and Closeness were calculated for 67 nodes. Only nodes with values greater than the median were selected and imported into Cytoscape 3.10.3 software for PPI analysis. Obtain Figure 2-5, with a total of 27 nodes and 341 edges. The same operation resulted in Figure 2-6, with 9 nodes and 36 edges. Each node is HIF1A, TP53, TNF, IL1B, BCL2, IL6, PTGS2, MMP9, AKT1. The above targets may be the core targets of Buyang Huanwu Tang in treating post-stroke sequelae.

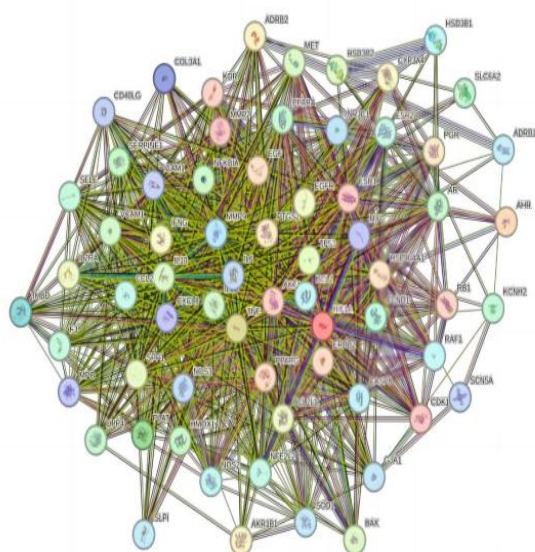


Figure 2-3

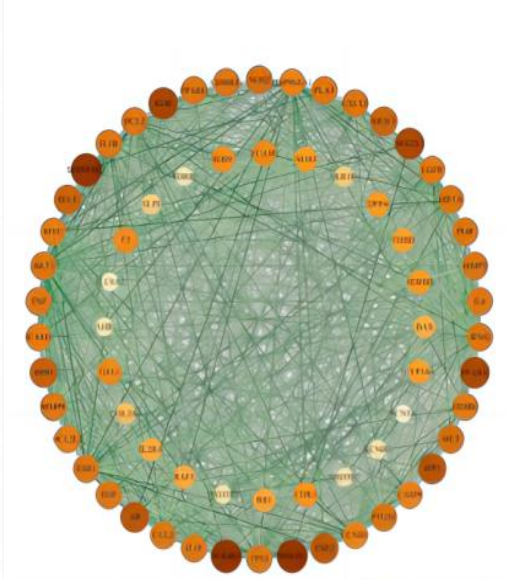


Figure 2-4

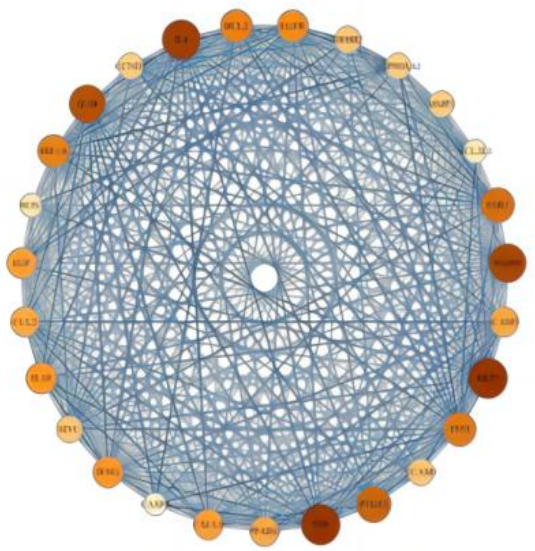


Figure 2-5

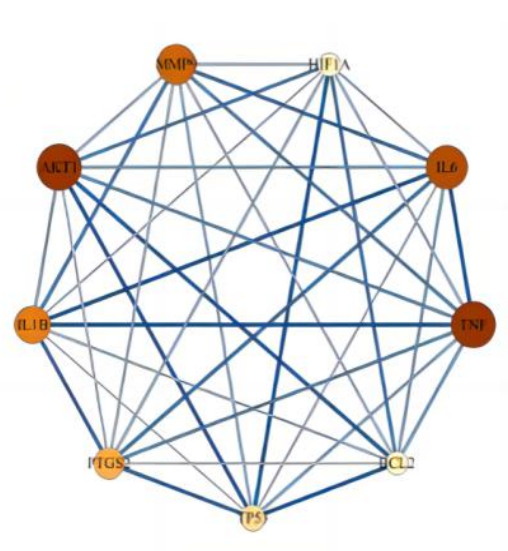


Figure 2-6

2.5 Analysis of GO Function and KEGG Pathway Enrichment Results

In order to elucidate the biological functions of the key targets of Buyang Huanwu Tang in treating post-stroke sequelae and the relationships between various signaling pathways, GO enrichment analysis and KEGG enrichment pathway analysis were performed on the obtained information, and the top 10 gene biological processes and signaling pathways related to the disease were selected for visualization analysis through charts.

Firstly, the Bioc Manager package in R language is used to analyze the gene numbers (Entrez IDs) of the intersection targets of diseases and drug components. Then, the exported gene number files are placed in R language. The results of GO enrichment analysis are visualized using three packages: cluster Profiler package, enrich plot package, and ggplot2 package. The screening criteria are $P < 0.05$ [18], and the smaller the P value, the higher the enrichment significance. The GO enrichment analysis results are shown in Figures 2-7 and 2-8. The X-axis corresponds to the ratio of the number of genes for each function to the total number of genes; The Y-axis corresponds to the name of each function and the number of enriched genes on each function. The larger the diameter of the bubble, the more targets it participates in [19]. The GO enrichment analysis results showed that the potential targets of Buyang Huanwu Tang in treating post-stroke sequelae were mainly enriched in the regulation of apoptotic signaling pathway, epithelial cell proliferation, and other aspects related to biological processes analysis (BP); Cellular Component (CC) analysis is mainly enriched in membrane rafts (membrane raft), Membrane microdomain, etc; Molecular Function (MF) analysis mainly involves enrichment in DNA binding transcription factor binding, ubiquitin protein ligase binding, and so on.

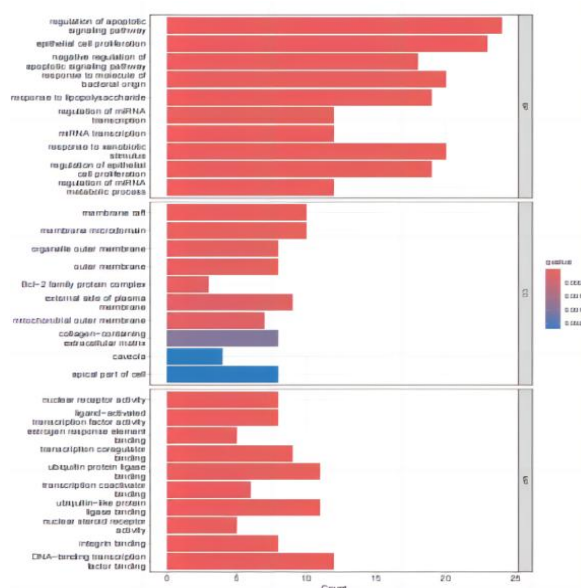


Figure 2-7 GO Enrichment Analysis Bar Chart

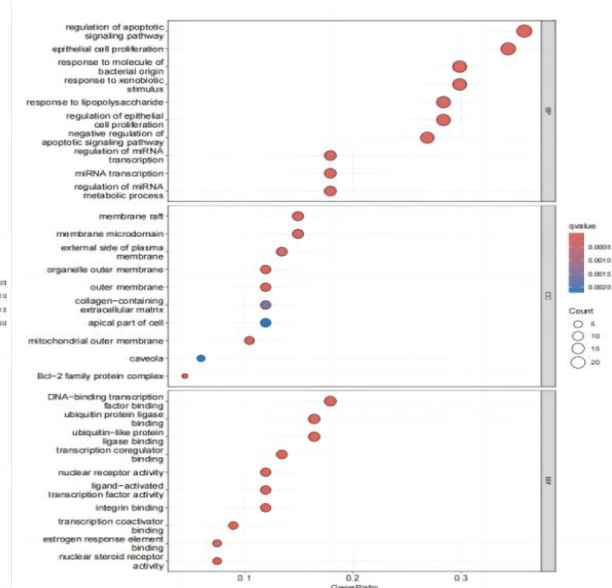


Figure 2-8 GO Enrichment Analysis Bubble Chart

KEGG enrichment analysis results show that the signal pathways involved in the target mainly include: lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetes complications, fluid shear stress and atherosclerosis, chemical carcinogenesis receptor activation and other pathways.

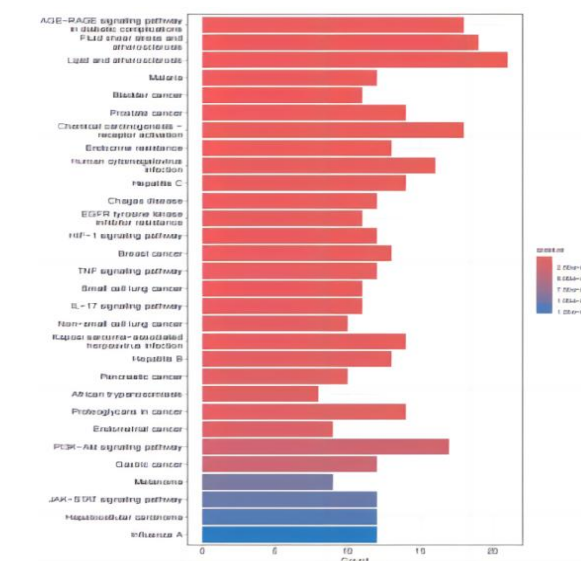


Figure 2-9 KEGG Enrichment Analysis Bar Chart

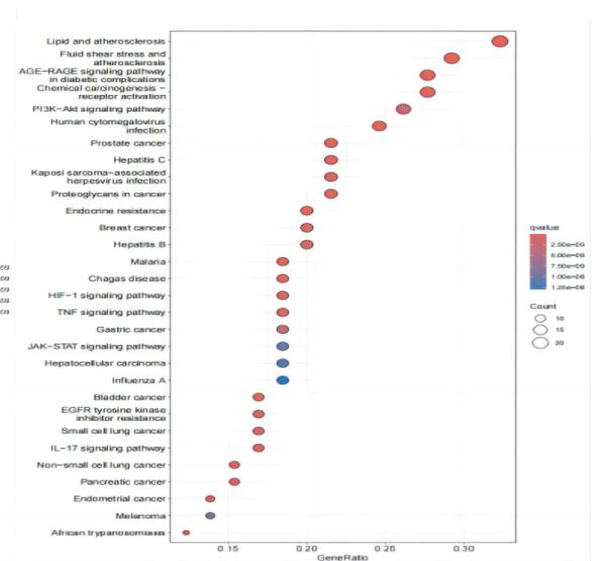


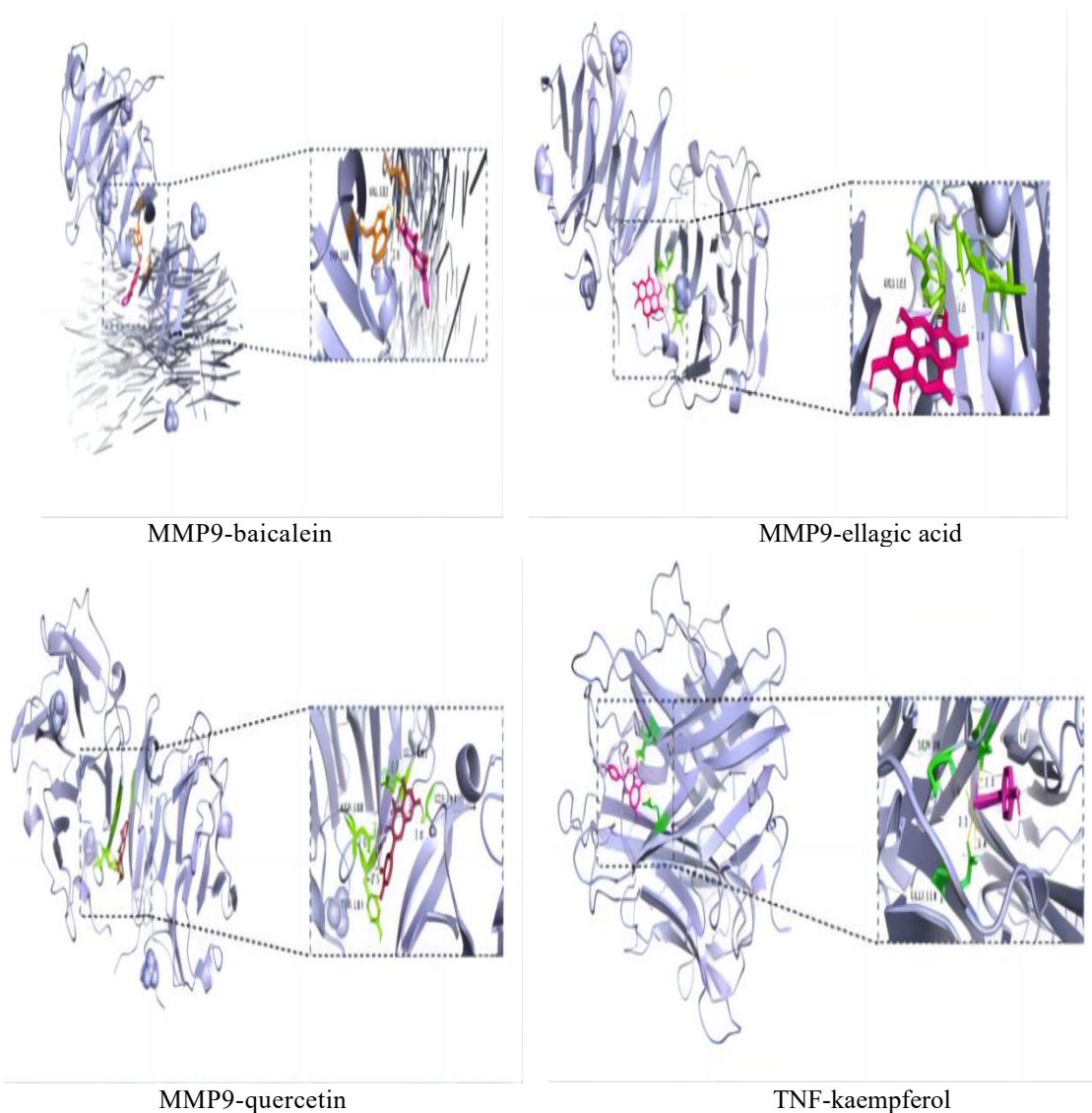
Figure 2-10 KEGG Enrichment Analysis Bubble Chart

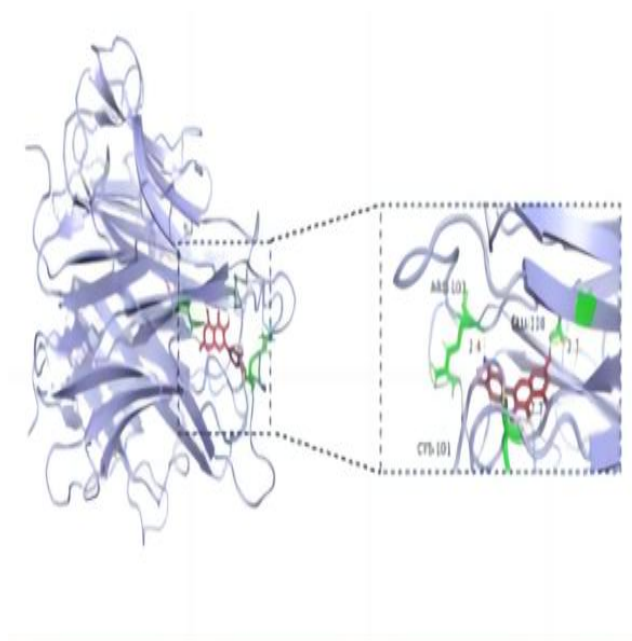
2.6 Molecular docking results

After docking the small molecule ligand with the protein receptor, the top 20 docking sites were compared to select the top 5 pairs for subsequent molecular docking analysis. When the binding energy is less than -5 kcal/mol, it indicates that the formed complex has relatively high binding activity [20]. Based on the above conditions, the 5 pairs with the lowest energy were selected for molecular docking, and the results are shown in Table 2-7. The docking results were plotted using Py Mol software, as shown in Figure 2-11. The effective ingredients of Buyang Huanwu Tang have good docking with the core targets, among which the most stable in order are MMP9 docking with tannic acid (-8.70 kcal · mol⁻¹), TNF docking with quercetin (-8.70 kcal · mol⁻¹), TNF docking with kaempferol (-8.60 kcal · mol⁻¹), MMP9 docking with quercetin (-7.90 kcal · mol⁻¹), and MMP9 docking with baicalein (-7.10 kcal · mol⁻¹).

Table 2-7 Binding Energy of Buyang Huanwu Decoction to Core Target Genes (kcal/mol)

Target	Binding Energy
MMP9-baicalein	-7.1
MMP9-ellagic acid	-8.7
MMP9-quercetin	-7.9
TNF-kaempferol	-8.6
TNF-quercetin	-8.7





TNF-quercetin
Figure 2-11 Molecular Docking Results

III. Discussions

Modern medicine divides stroke into two categories: ischemic and hemorrhagic, which have significant differences in pathological and physiological mechanisms, clinical treatment, and other aspects. Among them, ischemic stroke accounts for about 85% [21], and leaves a large number of sequelae that greatly affect the quality of life of the population. Buyang Huanwu Tang is a commonly used formula for treating ischemic stroke. The mechanism has not yet been clearly elucidated. The experiment analyzed the 9 core nodes of HIF1A, TP53, TNF, IL1B, BCL2, IL6, PTGS2, MMP9, and AKT1 in the PPI network results of Buyang Huanwu Tang treatment for post-stroke sequelae. It was found that HIF-1 α protein is an important transcriptional regulator of cell survival under hypoxic conditions and plays a crucial role in angiogenesis [22]. The TP53 gene is responsible for regulating key biological processes such as cell cycle, DNA damage repair, cell apoptosis, and anti-aging [23]. TNF can inhibit virus replication and kill virus-infected cells [24]. IL-1 β is involved in various autoimmune inflammatory responses and cellular activities. Bcl-2 can alter the redox state of mitochondrial thiol groups to control their membrane potential and regulate cell apoptosis. IL-6 plays an important role in inflammatory response, inducing the synthesis of acute phase proteins and exerting anti-inflammatory effects by inhibiting the activity of tumor necrosis factor alpha (TNF - α) and interleukin-1 (IL-1), while activating the activity of IL-1ra and IL-10 [25]. IL-6 is involved in regulating the generation of blood cells and affecting the differentiation and survival of lymphocytes [26]. PTGS2 and AKT1 proteins regulate various processes such as cell growth, survival, metabolism, and differentiation [27,28]. The 9 nodes in the PPI action network all have anti-inflammatory effects and exert their therapeutic effects through the synergistic effects between these targets.

Further screening the active ingredients of Buyang Huanwu Tang in the TCMSP database, including sitosterol, stigmasterol, beta sitosterol, folic acid (FA), etc. Key targets of post-stroke sequelae were identified through Gene Cards, OMIM and other databases, and a "component target pathway" network was constructed. The top ranked active ingredients include quercetin, kaempferol, baicalein, etc. Then, the core targets of drug therapy for diseases were identified through PPI analysis, such as HIF1A, TP53, TNF, IL1B, BCL2, IL6, PTGS2, MMP9, AKT1, etc. It is found that the core target of Buyang Huanwu Decoction is mainly concentrated in the regulation of apoptosis signal pathway, epithelial cell proliferation, lipid and atherosclerosis, which are closely related to anti-inflammatory, anti apoptotic, promoting angiogenesis and nerve repair. The molecular docking results showed that the main active ingredients in Buyang Huanwu Tang (such as tannic acid and quercetin) have high binding activity with core targets (such as MMP9 and TNF), verifying the reliability of network prediction.

This study systematically analyzed the synergistic mechanism of multiple components, targets, and pathways in the treatment of post-stroke sequelae with Buyang Huanwu Tang through various methods such as component screening, target prediction, network construction, and molecular docking, avoiding the limitations of single target research.

IV. Conclusion

This study explored the potential mechanism of action of Buyang Huanwu Tang in treating post-stroke sequelae through network pharmacology methods. Through active ingredient screening, target prediction, and functional pathway enrichment analysis, we found that Buyang Huanwu Tang can improve the symptoms of post-stroke sequelae through synergistic effects of multiple targets and pathways. Especially in the regulation of apoptosis signaling pathways, cell proliferation, and DNA binding transcription factor binding, the enrichment suggests that it may exert therapeutic effects by regulating cell apoptosis, improving neurological function, and promoting vascular repair. Molecular docking analysis further validated the stable binding between core targets (such as MMP9, TNF, IL6, etc.) and active ingredients (such as quercetin, baicalein, kaempferol, etc.), enhancing the potential of Buyang Huanwu Tang in the treatment of post-stroke sequelae. These research results provide new theoretical basis for the clinical application and further pharmacological mechanism research of Buyang Huanwu Tang.

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