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ORIGINAL ARTICLE

Exploring the Mechanism of Action of Modified Bazhen Decoction in Modulating Chronic Cerebral Ischemic Injury through the Ferroptosis Pathway Based on Network Pharmacology

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Abstract

The modified bazhen decoction is an agreement formula used in the treatment of cerebral ischemia at the hospital affiliated with Jiangxi University of Traditional Chinese Medicine. It has been shown to effectively alleviate clinical symptoms associated with inadequate cerebral blood supply. However, its therapeutic mechanism remains unclear. Based on network pharmacology, this study investigates the mechanism by which modified bazhen decoction regulates chronic cerebral ischemic injury through the ferroptosis pathway. TCMSP database, GeneCards database, and FerrDb database were searched for the corresponding targets.A PPI network was constructed using the core targets, followed by analyses of GO functions and KEGG pathways. Finally, the therapeutic effect of modified bazhen decoction on chronic cerebral ischemic injury through the ferroptosis pathway was verified by animal experiments.PPI analysis revealed core targets, including TP53, IL6, PTGS2, JUN, STAT3, and IL1B. The top five active ingredients, ranked by degree value, were identified as quercetin, β-sitosterol, kaempferol, lignans, and stigmasterol. The results of animal experiments suggest that the modified bazhen decoction can effectively improve cognitive impairment, alleviate oxidative stress and iron death, and repair damaged cells. modified bazhen decoction effectively inhibits neuronal damage and improves neurological dysfunction in chronic cerebral ischemic injury through the ferroptosis pathway. Its neuroprotective effects are associated with modulation of the lipid and atherosclerosis pathways and its action on the PTGS2 target.

Keywords: modified bazhen decoction, chronic cerebral ischemic injury,ferroptosis, network pharmacology, lipid and atherosclerosis signaling pathways, p53/PTGS2 pathway.

Introduction

Chronic cerebral ischemia（CCI） is a condition characterized by long-term insufficient cerebral blood perfusion, which may be triggered by factors such as cerebral vascular stenosis, thrombosis, or embolism(Daulatzai, 2017). This results in a prolonged low-oxygen state in the brain tissue, significantly contributing to the development of various neurological disorders, including vascular dementia, Binswanger disease, and Alzheimer's disease(Merelli et al., 2021). The underlying mechanisms of pathological injury and neuroprotection following chronic cerebral ischemia are complex, involving factors such as glial cell changes, neuronal apoptosis, oxidative stress, alterations in neurotransmitter systems, inflammatory responses, cellular autophagy, and pyroptosis. Currently, no specific drugs exist for the treatment of chronic cerebral ischemic injury(CCII), and clinical management primarily relies on vasodilators, cerebral protective agents,

statins, calcium channel blockers, stem cell transplantation, interventional surgery, and other approaches. Therapeutic interventions such as butylphthalide, vincristine, and nimodipine are commonly used to alleviate the adverse symptoms of cerebral ischemia.

Iron ions serve as essential transmitters in the nervous system, playing a crucial role in mitochondrial oxidative reactions, oxygen transport, and the synthesis of various enzymes. However, excessive iron accumulation can trigger oxidative stress and damage cells(Kulaszynska et al., 2024). Iron overload is a known risk factor for chronic cerebral ischemic injury, with previous studies linking elevated serum iron levels to the progression of this condition. Prolonged ischemia in cerebral tissue can exacerbate neurological damage through the accumulation of excess iron, leading to iron-dependent cell death, also known as ferroptosis, via mechanisms such as oxidative damage(She et al., 2020). Ferroptosis is characterized by iron-dependent regulated cell death, primarily manifested through extensive lipid peroxidation. Morphologically, it involves structural changes in mitochondria, including a reduction in mitochondrial volume, increased density, rupture of the outer membrane, and the disappearance of mitochondrial cristae(Dixon et al., 2012). Biochemically, ferroptosis is marked by decreased synthesis or excessive depletion of glutathione (GSH), reduced activity of the enzyme glutathione peroxidase 4 (GPX4), intracellular iron accumulation, and redox imbalance, leading to the formation of toxic lipid peroxides and reactive oxygen species (ROS) (X. Jiang et al., 2021). Studies have identified various proteins involved in cellular iron homeostasis regulation, including ferritin, membrane iron transport proteins, and transferrin receptors(Zhang & Enns, 2009).

The clinical symptoms of chronic cerebral ischemic injury primarily include cognitive impairment, dizziness, headache, poor concentration, and slow reaction time. From the perspective of Traditional Chinese Medicine (TCM), this condition can be categorized under "vertigo," "dementia," and "stroke." As the disease progresses, the TCM concept of "phlegm and blood stasis" aligns with modern medical views on local brain tissue ischemia, metabolic dysfunction, and the accumulation of toxic substances due to prolonged cerebral ischemia. When analyzing ferroptosis in TCM terms, the excessive accumulation of iron ions and deposition of lipid peroxides are seen as manifestations of blood stasis and phlegm obstruction in the cerebral collaterals. The Modified Bazhen Decoction (MBZD) is a widely utilized herbal formula for treating CCII in our medical center. This formula contains 11 traditional Chinese medicinal herbs, such as Huangqi (Hedysarum Multijugum Maxim), Danshen (Radix Salviae), Gegen (Radix Puerariae), Dangshen (Codonopsis Radix), Chishao (Radix Paeoniae Rubra), Fuling (Poria cocos), Baizhu (Atractylodes Macrocephala Koidz), Chuanxiong (Chuanxiong Rhizoma), Danggui (Angelicae Sinensis Radix), Shichangpu (Acoritataninowii Rhizoma), and Tiannanxing (Arisaematis Rhizoma). In clinical settings, MBZD has been shown to significantly relieve symptoms in patients with CCCI, promoting improvements in cerebrovascular reserve and cerebral blood flow. Furthermore, it helps alleviate symptoms such as dizziness, headache, and a sensation of heaviness in the head, though its exact mechanism of action remains unclear. (Xu et al., 2019). In this study, we used network pharmacology, target screening, molecular docking, stability analysis, and animal experiments to explore the targets and mechanisms underlying the therapeutic effects of modified bazhen decoction in treating chronic cerebral ischemic injury through the ferroptosis pathway, providing a theoretical basis for the use of TCM in treating this condition. Display workflow diagram. (Fig.1)

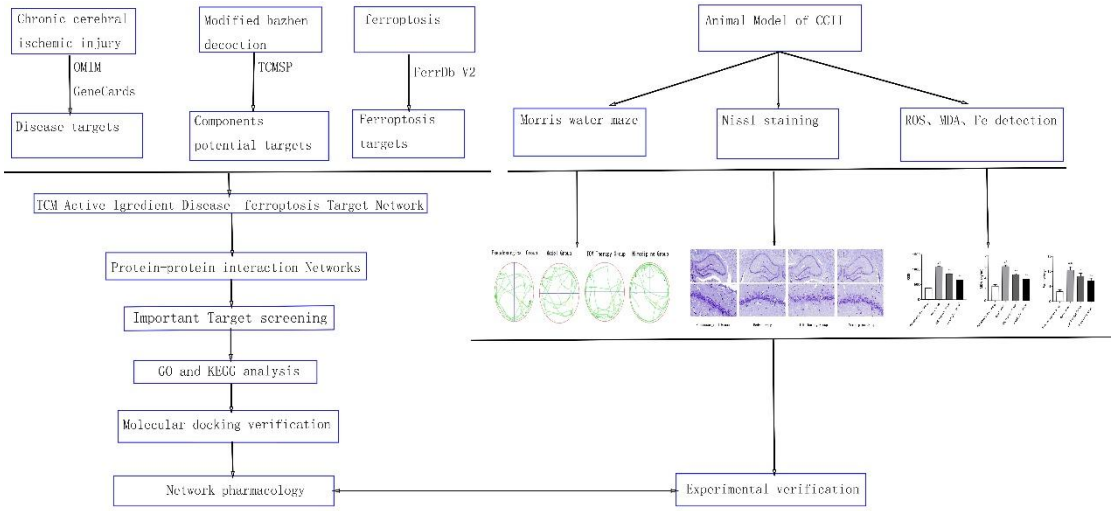

Figure 1 Workflow of the study.

Materials and Methods

Drug-disease intersection target identification

The active ingredients and associated targets of modified bazhen decoction were retrieved from the TCMSP (https://old.tcmspe.com/tcmsp.php)and UniProt (https://www.uniprot.org/) databases(Ru et al., 2014; UniProt, 2021). Targets related to chronic cerebral ischemic injury were obtained from the GeneCards (https://www.genecards. org/) database, while ferroptosis-related targets were sourced from the FerrDb (http://www.zhounan.org/ferrdb) database(Safran et al., 2010; Zhou et al., 2023). The intersecting targets of modified bazhen decoction mediating the ferroptosis pathway in chronic cerebral ischemic injury were identified using the Venny 2.1.0 tool ((https://bioinfogp.cnb.csic.es/tools/venny/).

Protein-Protein Interaction（PPI）screening of core targets

The intersecting targets were imported into the STRING database (https://string-db.org) , and PPI data with a confidence score >0.4 were extracted(Szklarczyk et al., 2021). Cytoscape 3.8.0 software was then used to construct a PPI network to analyze the role of modified bazhen decoction in mediating the ferroptosis pathway for the treatment of chronic cerebral ischemic injury(Majeed & Mukhtar, 2023).

Gene ontology and pathway enrichment analysis

Biological pathway enrichment analysis and Gene Ontology (GO) functional enrichment analysis of KEGG were performed using the DAVID database (https://davidbioinformatics.nih.gov/)(Dennis et al., 2003). These analyses focused on the targets of modified bazhen decoction mediating the ferroptosis pathway in the treatment of chronic cerebral ischemic injury.

Network construction

A component-target-pathway network diagram was constructed using Cytoscape 3.8.0 software to comprehensively analyze the mechanism of action of modified bazhen decoction in mediating the ferroptosis pathway for the treatment of chronic cerebral ischemic injury. This network was built based on the active ingredients, targets, therapeutic targets of diseases, ferroptosis-related targets, and KEGG signaling pathways of the traditional Chinese medicine (TCM) compound.

Molecular docking

The active ingredients of the herbal formula were calculated using the cytoNCA function in Cytoscape 3.8.0 software(Tang et al., 2015). Ligand files were obtained from the PubChem(https://pubchem.ncbi.nlm.nih.gov/) database and ChemBio3D software(Kim et al., 2022). Core gene-related proteins were screened through PPI analysis, and receptor files were retrieved from the UniProt database, the PDB(https://pubchem.ncbi.nlm.nih.gov/) database, and PyMOL software. AutoDock Tool software was used to identify active binding pockets, and molecular docking results were obtained using AutoDock Vina(Morris et al.,

2009). A lower binding energy indicates more stable binding between the herbal compound's active ingredients and the disease target. Finally, PyMOL and Discovery Studio 2019 software were used to visualize the docking results(Seeliger & de Groot, 2010).

Animal experiments

Drugs, reagents and instruments

modified bazhen decoction Preparation:The following herbal ingredients were used in the preparation of the formula: 30 g each of Astragalus, Salviae Miltiorrhizae, and Pueraria Mirifica; 15 g each of Radix et Rhizoma Ginseng and Radix Paeoniae Alba; and 10 g each of Rhizoma Atractylodis Macrocephalae, Rhizoma Ligustici Chuanxiong, Radix Angelicae Sinensis, Calamus Calamus, and Preparation of Gallus gallus, supplied by the Pharmacy Department of Jiangxi Province Chinese Medicine Hospital. The herbs were placed in a round-bottomed flask, with 3200 mL of 70% ethanol added (10 times the amount of the herbs). After immersion for 2 hours, the mixture was reflux-extracted for 2 hours and filtered. The herbal residue was then treated with 2560 mL of 70% ethanol (8 times the amount of the herbs) for reflux extraction for 1.5 hours, followed by filtration. The combined filtrate was concentrated using a rotary evaporator until the alcohol was removed. A water decoction-ethanol precipitation process was applied to produce a sterile decoction with a raw drug content of 1 g/mL, which was divided into sterile vials and stored in a refrigerator.

Main Reagents:The Tissue Reactive Oxygen Species (ROS) detection kit and Malondialdehyde (MDA) detection kit were purchased from Shanghai Biyuntian Biotechnology Company Limited. The Fe content detection kit was purchased from Wuhan Eimage Technology Co.

Main Instruments:The MS-1 Water Maze Instrument was obtained from Chengdu Instrument Factory. The Stabilized Pressure and Current Electrophoresis Instrument and Gel Imaging System were provided by Beijing Liuyi Biotechnology Co.

Animal Selection and Grouping:

Twenty adult SPF-grade male Sprague-Dawley (SD) rats, each weighing approximately 150 g, were obtained from the Laboratory Animal Center of Jiangxi University of Traditional Chinese Medicine (License No. SYXK (Gan) 2022-0002). The animals were housed in an environment maintained at a temperature of 20-22°C, with a 12-hour light/dark cycle. Food and water were provided ad libitum. The rats were randomly assigned to four groups (n = 5 per group) using a random number table method: the Pseudosurgical Group, the Model group, the TCM Therapy Group and Nimodipine Group. The study was approved by the Animal Ethics Committee (Approval No. JZFYLL20220727018).

Method of administration

Drug administration began on the day of surgery and continued daily until the day of sampling, for a total duration of 28 days. The doses were adjusted based on the body surface area of the animals. The groups received the following treatments:

① The Pseudosurgical Group: distilled water gavage;

② The Model group: modeling + distilled water gavage 10 mL/kg/d;

③The TCM Therapy Group: modeling + modified bazhen decoction suspension 16 g/kg/d by gavage;

④The Nimodipine Group: modeling + nimodipine 6 mg/kg/d administration.

CCII Rat Model Establishment

After a 12-hour fasting period, anesthesia was induced using 5% enflurane mixed with 70% N_2O and 30% O_2. The rats reached an atonic state within approximately 2 minutes. They were then fixed on the operating table, and anesthesia was maintained using 1%-2% enflurane with 70% N_2O and 30% O_2. Body temperature was regulated with temperature-controlled heating blankets to maintain an anal temperature of 37°C. The skin in the mid-neck region was prepared, and the bilateral common carotid arteries and vagus nerves were exposed. The left common carotid artery was ligated twice at both the proximal and distal ends with No. 4 surgical thread, completely obstructing blood flow. The skin was then sutured, anesthesia was terminated, and the rats were placed in a recovery cage. After 30 minutes, the right common carotid artery was ligated in the same manner to block blood flow, and the skin was sutured. Anesthesia was terminated, and the

rats were returned to the rearing cage with clean bedding. Penicillin (50,000 units) was administered intramuscularly daily for 3 days. In the sham-operated group, only the common carotid arteries were isolated without ligation, with the rest of the procedure identical to that of the operated group. Postoperative conditions were monitored for 3 days, and rats exhibiting acute ischemic symptoms or mortality were excluded from the study.

Sample Collection:On day 29 post-modeling, animals from each group were euthanized and sampled. Anesthesia was induced via intraperitoneal injection of 10% chloral hydrate. Hearts were perfused with 4% neutral paraformaldehyde, and the rats' heads were severed to remove the brains, which were then preserved in 4% neutral paraformaldehyde. After anesthesia, the head was swiftly removed and placed in an ice box. A craniotomy was performed in an ice bath, and the brain tissue was exposed and removed. Coronal sections were made at the anterior end of the optic chiasm, and cortical and hippocampal tissues were isolated on ice, rinsed with water containing 0.1% DEPC, and transferred to freezing tubes for rapid preservation in liquid nitrogen.

Morris Water Maze Experiment

A fixed entry point was designated for each quadrant, and a hidden platform was positioned in the center of one quadrant. Rats were placed along the pool's wall, and their escape latency was recorded over 7 days of training (twice daily). If the platform was not located within 120 seconds, the rat was guided to the platform and allowed to remain there for 30 seconds to reinforce the memory. After the platform was removed, rats were placed into the pool at a random location. The number of times they crossed the original platform location within 180 seconds and the retention time in the target platform quadrant were recorded(Lissner et al., 2021).

Nissl Staining

Rat hippocampal tissues were deparaffinized by standard methods and rinsed three times with water. The tissues were then placed in a 60°C incubator and stained with 1% toluidine blue for 40 minutes. After staining, the tissues were dehydrated sequentially in 70%, 80%, 95%, and 100% ethanol, followed by clarification with xylene. Finally, the tissue slices were mounted with neutral gum(Paul et al., 2008).

Detection of iron, ROS and MDA content in the hippocampal region

Rat brain tissue was homogenized in an ice bath with the sample preparation solution, then centrifuged at 4°C and 10,000 g for 5 minutes. The supernatant was collected, and each working solution was sequentially added and mixed. Absorbance values were measured at 450-550 nm using an automatic enzyme analyzer. The concentrations of ferric ions, reactive oxygen species (ROS), and malondialdehyde (MDA) were calculated based on the respective formulas.

Statistical Methods

The data analysis was performed using SPSS version 22.0. Measurement data are presented as mean ± standard deviation, and one-way ANOVA was used for comparisons. Tukey's test was applied for post-hoc pairwise comparisons, and a significance level of $P < 0.05$ was considered statistically significant.

Results

Intersecting Target Proteins of modified bazhen decoction Mediating the ferroptosis Pathway in the Treatment of Chronic Cerebral Ischemic Injury

Database analysis identified 121 potential active ingredients, 13 shared ingredients, 2,015 active drug targets, and 246 active targets after de-weighting from modified bazhen decoction(Table.1-2). Cytoscape 3.8.0 was used to visualize the network and construct the data, resulting in a network with 379 nodes and 2,152 edges. The disease database provided 6,111 targets related to chronic cerebral ischemic injury, 844 targets related to ferroptosis, and 564 targets after de-weighting(Fig.2). Using Venny 2.1.0, the intersection of these datasets revealed 43 intersecting targets involved in the mediation of the ferroptosis pathway by modified bazhen decoction for the treatment of chronic cerebral ischemic injury (Fig.3).

Table 1 Potential active ingredients of modified Bazhen decoction.

serial number	Name of Chinese medicine	Unique Potential Active Ingredients
BZ	Atractylodes macrocephala	3
CS	red peony	9
CX	persimmon	3
DS	Salvia miltiorrhiza	56
DG	Angelica sinensis	0
DGS	codonopsis root	12
FL	tuckahoe	5
GG	tuber of the kudzu vine	2
HQ	Astragalus membranaceus	12
SCP	Acorus calamus	3
TNX	Amanita muscaria	3

Table 2 Identical ingredients of modified Bazhen decoction

serial number	ID	identical Ingredients	origins
B1	MOL000422	kaempferol	HQ/SCP
B2	MOL007059	3-beta-Hydroxymethyllenetanshiquinone	DS/DGS
B3	MOL000392	formononetin	GG/HQ
B4	MOL000296	hederagenin	FL/HQ
B5	MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10, 13-dimethyl-17-[(2R,5S)-5-propn 14--2-yloctan-2-yl]-2,3,4,7,8,9,11,12, 15-14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	BZ/HQ
B6	MOL002776	Baicalin	CS/DS
B7	MOL004355	beta-sitosterol	CS/DGS
B8	MOL002140	Perlolyrine	CX/DGS
B9	MOL000006	luteolin	DS/DGS
C1	MOL000359	sitosterol	CS/CX/TNX
C2	MOL000433	FA	BZ/HQ/CX
D1	MOL000358	beta-sitosterol	CS/DG/GG/TNX
D2	MOL000449	Stigmasterol	CS/DG/DGS/TNX

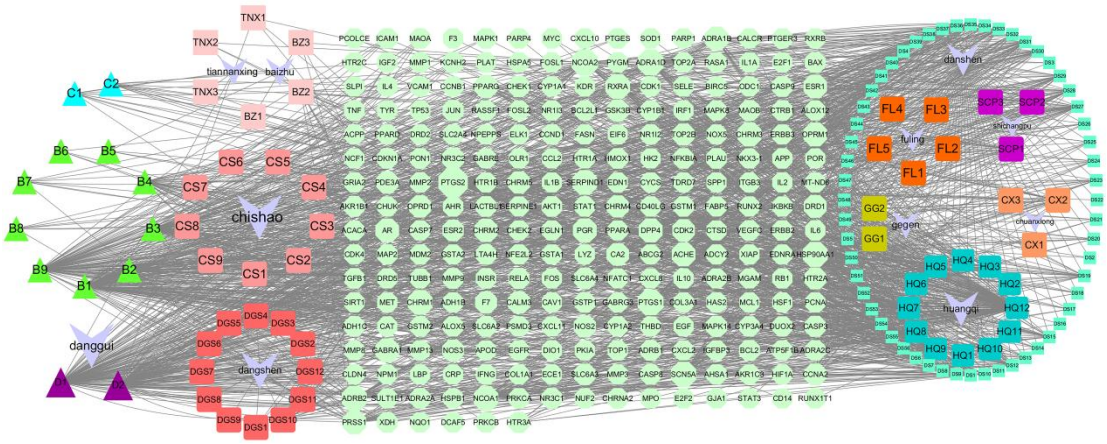

Figure 2 Network diagram of MBZD. (A) .MBZD=modified bazhen decoction.

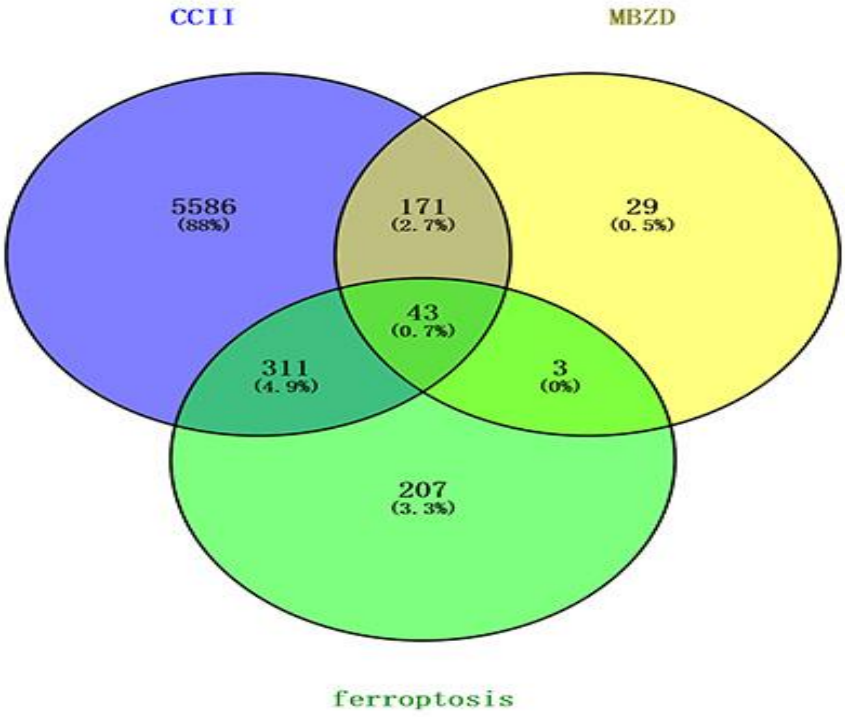

Figure 3. Venn diagram showing shared targets of CCII and MBZD and ferroptosis. CCII = Chronic cerebral ischemic injury, MBZD = modified bazhen decoction.

PPI Networks

The 43 intersecting target genes were analyzed for PPI in the STRING database, with a threshold score of >0.4. Cytoscape 3.8.0 was employed to visualize and analyze the network's node statistics The network consisted of 42 nodes and 477 edges. After two rounds of filtering, six targets with a degree value greater than the average (degree >30) were identified. These targets, ranked from highest to lowest degree, are: TP53, IL6, PTGS2, JUN, STAT3, and IL1B(Fig.4 and Table.3). These six targets, with higher-than-average degree values, may represent key mediators of the ferroptosis pathway in the treatment of chronic cerebral ischemic injury by modified bazhen decoction.

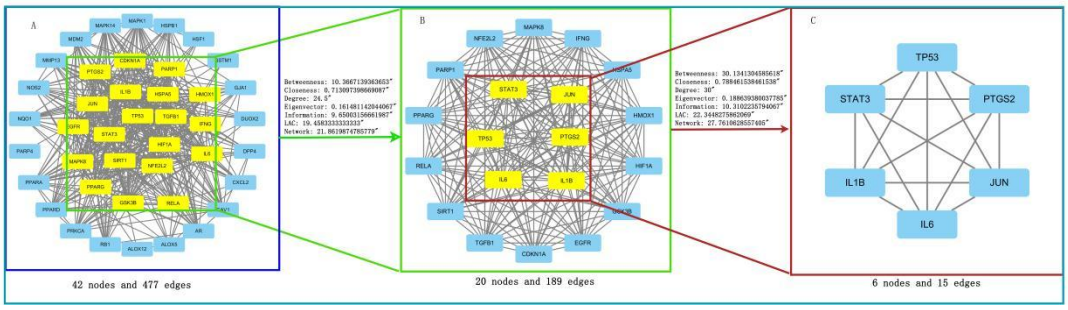

Figure 4. PPI network and cluster analysis of candidate targets of MBZD against CCII by ferroptosis. (A). The interactive PPI network of MBZD putative targets and CCII related targets by ferroptosis. (B). PPI network of significant proteins extracted from A. (C). PPI network of candidate MBZD targets for CCII by ferroptosis treatment extracted from B. CCII = Chronic cerebral ischemic injury, MBZD = modified bazhen decoction.

Table 3 Specific information of the 6 key target genes.

Name	Uniprot ID	Degree	Betweenness	Closeness
TP53	P04637	36	71.57448895875027	0.8913043478260869

IL6	P05231	36	49.45672728113189	0.8913043478260869
PTGS2	P35354	35	80.00185394563076	0.8723404255319149
JUN	P05412	35	40.772064430405436	0.8723404255319149
STAT3	P40763	35	38.55989701113088	0.8723404255319149
IL1B	P01584	35	45.65446957152124	0.8723404255319149

Functional Enrichment Analysis

The DAVID data platform was used to perform GO functional enrichment analysis on 43 key targets of modified bazhen decoction mediating the ferroptosis pathway in the treatment of chronic cerebral ischemic injury. The analysis focused on biological processes (BP), cellular components (CC), and molecular functions (MF) associated with gene functions. Using a significance threshold of $P<0.05$, 460 GO terms were identified, and bubble plots were generated for the top 20 terms in each category (BP, CC, and MF).The BP analysis revealed 360 significant biological processes, indicating that the therapeutic effects of modified bazhen decoction are primarily linked to processes such as the positive regulation of vascular endothelial growth factor production, the positive regulation of RNA polymerase II-mediated transcription, the regulation of miRNA transcription, the production of interleukin-6, the positive regulation of classical NF-kappaB signaling, and the positive regulation of interleukin-8 production. Additionally, processes like the negative regulation of DNA template transcription, astrocyte activation, the DNA damage response, and p53-mediated cell cycle arrest were also identified as relevant.The MF analysis identified 67 molecular functions, suggesting that the molecular mechanisms underlying the effects of modified bazhen decoction are related to protein structural domain binding, enzyme binding, protein binding, RNA polymerase II-specific DNA-binding transcription factors, NAD+-dependent ADP-ribosyltransferase activity, protein serine/threonine kinase activity, zinc ion binding, chromatin binding, and R-SMAD binding.The CC analysis identified 33 cellular components, revealing that the therapeutic effects of modified bazhen decoction are primarily associated with cellular locations such as Ficolin-1-rich granules, protein-containing complexes, the cytoplasm, chromatin, nucleoplasm, protein-DNA complexes, the perinuclear region, and glutamatergic synapses.

Additionally, KEGG pathway enrichment analysis was performed using the DAVID platform, identifying 130 relevant signaling pathways. These included the IL-17, HIF-1, FoxO, TNF, MAPK, ErbB, GnRH, VEGF, PI3K-Akt, cAMP, TGF-β pathways, among others (Fig.5).

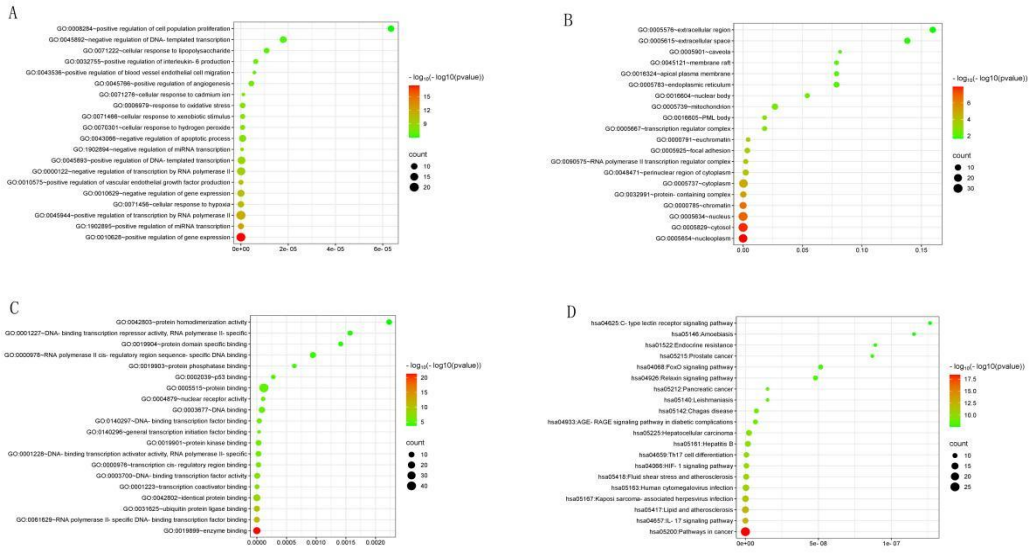

Fig.5. Functional enrichment analysis and Bubble chart of GO enrichment:BP (A);CC(B) ;MF(C).Bubble chart of KEGG pathways(D). BP=biological process;CC = cellular

component;MF=molecular function;KEGG=Kyoto Encyclopedia of Genes and Genomes.(The color of the bubbles represents the *P* value, while the size denotes the number of genes).

Network Construction

Cytoscape 3.8.0 was used to construct a component-target-pathway map to comprehensively illustrate the mechanism of action of modified bazhen decoction in mediating the ferroptosis pathway for the treatment of chronic cerebral ischemic injury. The cytoNCA function was employed to calculate the statistics of nodes in the network, resulting in a total of 389 nodes and 2,388 edges(Fig.6). The analysis identified the key components of modified bazhen decoction that may mediate the ferroptosis pathway in chronic cerebral ischemic injury, including quercetin, β-sitosterol, kaempferol, lignans, and stigmasterol (Table.4).

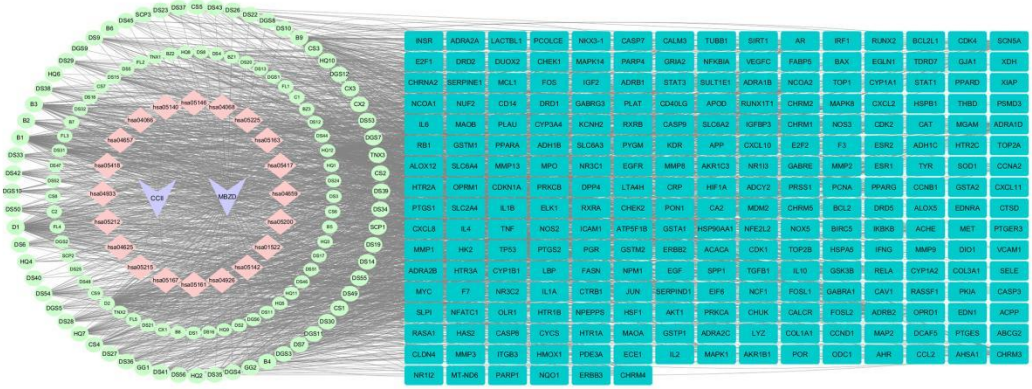

Fig.6. Network diagram of MBZD against CCII by ferroptosis.MBZD = modified bazhen decoction.CCII = Chronic cerebral ischemic injury.

Table 4 Top 10 compounds information.

number	ID	compound	OB	DL	Degree
HQ12	MOL000098	Quercetin	46.43	0.28	147
D1	MOL000358	beta-sitosterol	36.91	0.75	125
B1	MOL000422	Kaempferol	41.88	0.24	118
D2	MOL000449	Stigmasterol	43.83	0.76	112
B9	MOL000006	luteolin	36.16	0.25	112
B3	MOL000392	formononetin	69.67	0.21	72
HQ7	MOL000378	7-O-methylisomucronulatol	74.69	0.3	43
DS54	MOL007154	tanshinone iia	49.89	0.4	41
DGS4	MOL003896	7-Methoxy-2-methyl isoflavone	42.56	0.2	40
CS4	MOL002714	baicalein	33.52	0.21	36

Molecular Docking

Six targets with a degree value greater than the average were selected from the PPI network: TP53, IL6, PTGS2, JUN, STAT3, and IL1B. These targets were molecularly docked with the five top-ranked molecules: quercetin, β-sitosterol, kaempferol, stigmasterol, and luteolin. The docking analysis revealed that β-sitosterol only formed hydrogen bonds with STAT3, while stigmasterol interacted with all six targets without forming hydrogen bonds. Kaempferol docked with IL1B but did not form hydrogen bonds. The docking results indicated that the binding energies for all interactions were ≤ -5.2 kCal/mol, suggesting that the compounds in modified bazhen decoction are relatively stable when binding to core target proteins in the ferroptosis pathway(Table.5 and Fig.7). These interactions support the therapeutic potential of modified bazhen decoction in treating chronic cerebral ischemic injury by targeting key proteins.

Table 5 The binding energy of compound and core targets.

	Binding free energy (kcal·mol − 1)					
Active compound	TP53	IL6	PTGS2	JUN	STAT3	IL1B
Quercetin	-6.3	-7.4	-9.6	-5.5	-8.1	-7.3
beta-sitosterol	-6.1	-6.9	-8.9	-5.2	-6.9	-6.6
Kaempferol	-5.9	-7.4	-9.2	-5.5	-8.2	-7.2
Stigmasterol	-6.7	-7.4	-9.6	-5.8	-7.2	-7.3
luteolin	-6.4	-7.4	-10.0	-5.6	-9.0	-7.4

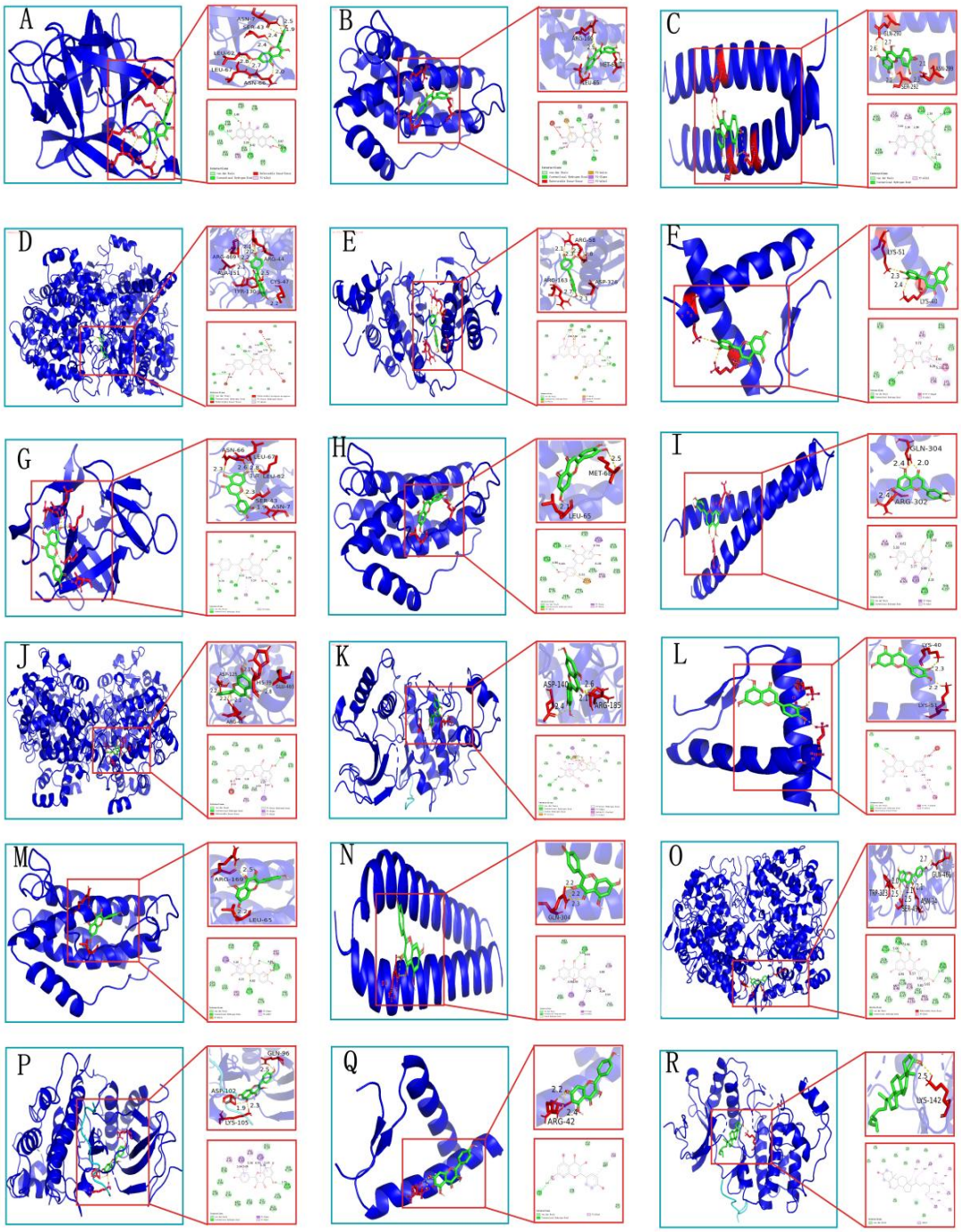

Fig.7. Diagram of molecular docking.Quercetin-IL1B(A).Quercetin-IL6(B). Quercetin- JUN(C). Quercetin-PTGS2(D). Quercetin-STAT3(E).Quercetin-TP53(F).luteolin-IL1B(G). luteolin-IL6(H). luteolin- JUN(I). luteolin-PTGS2(J). luteolin-STAT3(K). luteolin-TP53(L). Kaempferol-IL6(M). Kaempferol- JUN(N). Kaempferol-PTGS2(O). Kaempferol-STAT3(P). Kaempferol-TP53(Q). beta-sitosterol-STAT3(R).

Animal Experiments

Behavioral Indicators (Morris Water Maze Test)

In the Morris water maze test, compared to the Pseudosurgical Group, rats in the Model group exhibited significantly longer escape latencies ($P < 0.05$), as well as significantly reduced target platform quadrant retention times and fewer platform crossings ($P < 0.05$). These results indicate a significant impairment in learning and memory functions in the Model group. In contrast, the TCM Therapy Group and the Nimodipine Group showed significant improvements in escape latency, target platform quadrant retention time, and number of platform crossings ($P < 0.05$). These changes were characterized by a reduction in escape latency, an increase in retention time, and a higher number of platform crossings. These findings suggest that modified bazhen decoction effectively alleviates cognitive dysfunction induced by chronic cerebral ischemia injury(Table.6 and Fig.8).

Table 6 The escape latency, time spent in the target platform quadrant, and number of platform crossings for rats in each group were measured.（n=5，$\bar{x}$±s）

group	escape latency/s	time spent in the target platform quadrant/s	Number of platform crossings/times
Pseudosurgical Group	23.25±7.88	2.40±0.70	3.40±1.14
Model Group	111.60±13.74*	0.16±0.23*	0.20±0.45*
TCM Therapy Group	88.64±12.57△	0.90±0.25△	1.60±0.55△
Nimodipine Group	78.12±10.53△	0.93±0.20△	1.80±0.45△
F-values	54.090	27.360	17.170
P-values	0.000	0.000	0.000

Note: *P<0.05 compared with the pseudosurgical group; △P<0.05 compared with the model group.

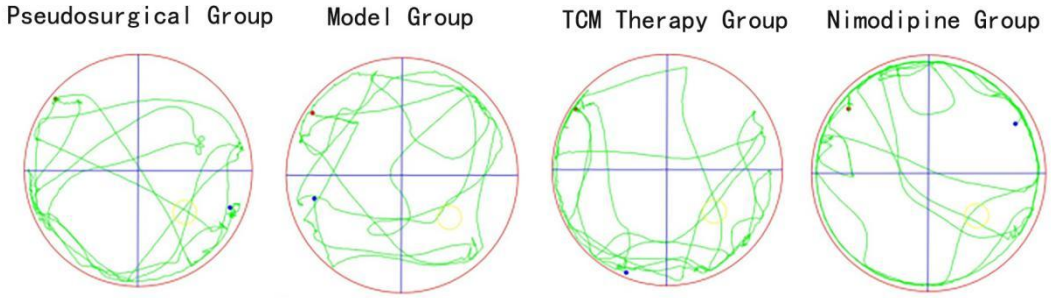

Figure 8. Swimming paths of rats in the Morris water maze experiment

Nissl Staining

Nissl staining revealed that neurons in the CA1 region of the hippocampus in the Model group exhibited disorganization, cytosolic atrophy, and a reduction in Nissl bodies, representing significant morphological damage compared to the Pseudosurgical Group. In the TCM Therapy Group and the Nimodipine Group, neuronal morphology was notably improved, with fuller cytosol and a more uniform distribution of Nissl bodies, suggesting that modified bazhen decoction helps maintain the structural integrity and metabolic function of neurons(Fig.9).

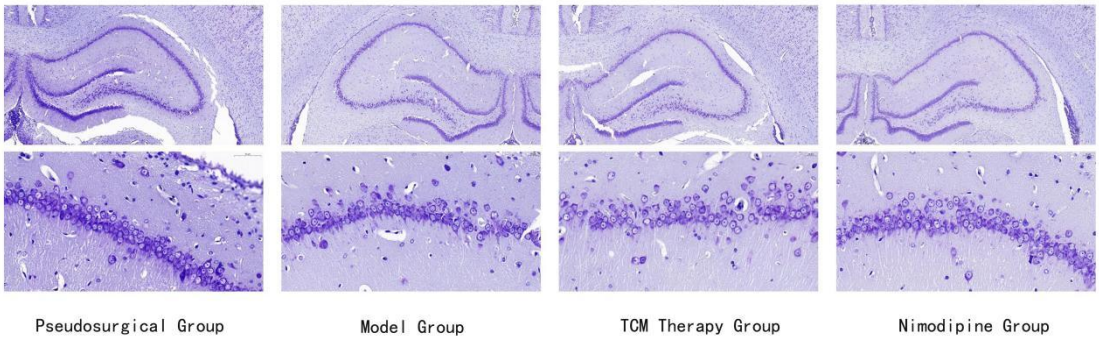

Figure 9. Nissl microsomal morphology

Oxidative Stress and Iron Ion Accumulation (ROS, MDA, Fe)

Compared to the Pseudosurgical Group, the Model Group showed significantly higher levels of ROS fluorescence intensity (1103.00 ± 6.44), MDA concentration (6.10 ± 0.22 ng/mL), and Fe content (10.54 ± 1.01 nmol/mg, $P < 0.05$). These findings indicate a significant increase in oxidative stress and iron deposition. In contrast, the TCM Therapy Group and the Nimodipine Group exhibited significantly lower levels of ROS, MDA, and Fe content ($P < 0.05$) compared to the Model Group, suggesting that modified bazhen decoction alleviates oxidative stress injury and reduces iron ion accumulation(Table.7 and Fig.10).

Table 7 ROS, MDA and Fe content of rats in each group（n=5，$\bar{x}$±s）

group	ROS fluorescence intensity	MDA ng/mL	Fe nmol/mg
Pseudosurgical Group	386.50±3.95	2.62±0.21	3.34±0.50
Model Group	1103.00±6.44*	6.10±0.22*	10.54±1.01*
TCM Therapy Group	865.10±9.77△	4.67±0.11△	8.15±0.46△
Nimodipine Group	654.60±11.04△	3.87±0.02△	6.89±0.93△
F-values	4063.01	237.90	46.06
P-values	0.000	0.000	0.000

Note: *P<0.05 compared with the pseudosurgical group; △P<0.05 compared with the model group.

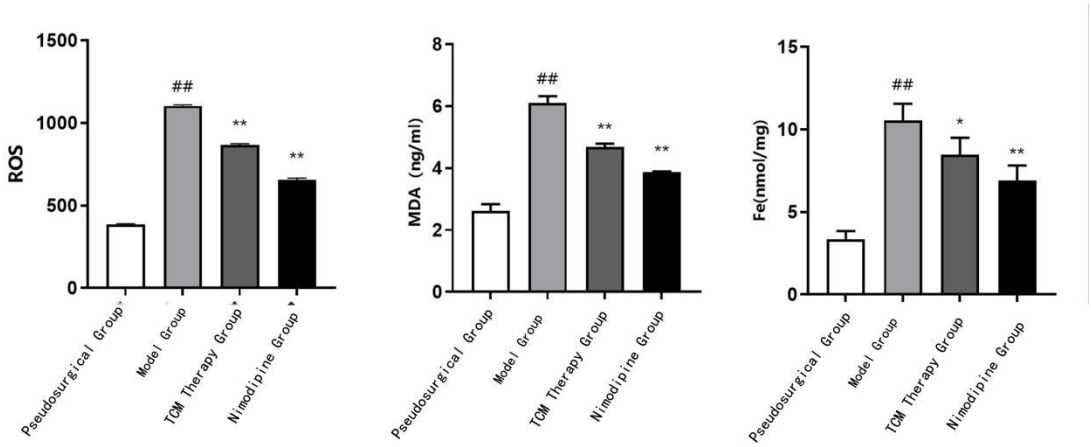

Figure10. ROS, MDA and Fe content of rats in each group

Discussion

Chronic cerebral ischemic injury serves as a precursor to various neurological disorders, with its etiology and underlying mechanisms being highly complex. In this study, we systematically explored the therapeutic mechanism of modified bazhen decoction in treating chronic cerebral ischemic injury via ferroptosis pathways, utilizing network pharmacology, molecular docking, and animal experiments. The results indicate that modified bazhen decoction exerts therapeutic

effects on chronic cerebral ischemic injury by targeting key molecules such as TP53, IL6, PTGS2, JUN, STAT3, and IL1B through lipid metabolism and atherosclerosis pathways. These findings provide a foundation for the clinical application of modified bazhen decoction and offer new insights into the treatment of chronic cerebral ischemic injury.

The top five active components in the PPI network are quercetin, β-sitosterol, kaempferol, luteolin, and stigmasterol. The core components of the Modified Bazhen Decoction include these traditional herbal ingredients, suggesting that the findings from the network pharmacology study are in agreement with its clinical use. Quercetin, a naturally occurring flavonoid, demonstrates various biological activities, such as anti-tumor, anti-inflammatory, antioxidant, and anti-apoptotic properties. Josiah and colleagues discovered that quercetin prevents the buildup of oxidants within cells and deactivates phosphorylated p53 in the nucleus, which in turn reduces oxidative stress-induced apoptosis in endothelial cells.(Josiah et al., 2022). Moreover, quercetin alleviates atherosclerosis by inhibiting the activation of dendritic cells, which in turn helps reduce endothelial cell dysfunction. Additionally, Park and colleagues found that quercetin might enhance neuroprotection in focal cerebral ischemia by regulating thioredoxin expression.(Park et al., 2020). Luteolin, a natural compound, has been shown to alleviate oxidative damage, counteract inflammatory responses, reduce tumor heterogeneity, and possess antimicrobial, cardiovascular, cerebrovascular, and respiratory protective effects(Vongthip et al., 2024). Kaempferol, a flavonoid found primarily in the rhizomes of the ginger family plant Kaempferia, is widely present in various traditional herbs. Research by Yu et al. demonstrated that kaempferol glycoxib inhibits the expression of NF-κB and phosphorylated STAT3, providing neuroprotection in rat brain tissue following ischemia-reperfusion injury(Khanam et al., 2024). Similarly, experimental animal studies indicate that kaempferol significantly reduces cerebral infarct volume, improves pathological tissue changes, and decreases the expression of infarction and apoptosis factors, offering protective effects in brain tissue(Zhang & Jiao, 2024). β-Sitosterol, a type of plant sterol, helps to decrease the release of pro-inflammatory factors by blocking the NF-κB pathway, thus reducing inflammation. In the context of cerebral ischemia-reperfusion injury, β-sitosterol has been shown to reduce the occurrence of neuroinflammation, alleviate brain edema, and improve neurological function(Zheng et al., 2023). Stigmasterol mitigates oxidative stress-induced damage to brain tissue by inhibiting hydrogen peroxide production and reducing the accumulation of free radicals(Pratiwi et al., 2021). These active molecules may play a key role in the therapeutic effects of modified bazhen decoction.

Six key potential targets were identified, including TP53, IL6, PTGS2, JUN, STAT3, and IL1B. Jianbin et al. suggest that TP53-induced glycolysis and apoptosis regulator (TIGAR) may promote the production of NADPH via the pentose phosphate pathway (PPP), which helps to inhibit oxidative stress and neuroinflammation(Ge et al., 2021). In addition, p53 serves as an activator of ferroptosis by inhibiting the expression of solute carrier family 7 member 11 (SLC7A11). Furthermore, prostaglandin-endoperoxide synthase 2 (PTGS2), which is directly activated as a downstream gene in response to cell death, serves as a marker of ferroptosis. By inhibiting the p53/PTGS2 pathway, ferroptosis in hippocampal neurons can be reduced, which helps to improve injury caused by chronic cerebral ischemia. (Hou et al., 2024). IL-6, a pro-inflammatory cytokine. Adi et al. found that to induce differentiation of rat pheochromocytoma (PC12) cells into neuron-like cells, supporting cell survival and axonal extension. It also maintains the survival of cholinergic and catecholaminergic neurons in culture and increases astrocytic secretion of nerve growth factor (NGF) and other growth factors(Lahiani et al., 2015).Additionally, research by Armstead William M et al. has demonstrated that IL-6 activation of the NMDA receptor, along with the upregulation of ET-1 and JNK, leads to impaired brain autoregulation and hippocampal neuron necrosis(Armstead et al., 2019). P et al. have demonstrated that c-Jun N-terminal kinase (JNK), a member of the MAPK family, promotes neuronal apoptosis in ischemic stroke and neurodegenerative diseases(Xiao et al., 2018). JNK1, its major subunit, is highly expressed in brain tissue, and phosphorylated JNK1 further activates downstream c-Jun. Gengyin et al. have

shown that phosphorylated c-Jun enters the nucleus, binds to DNA, and promotes cell death through transcriptional regulation of target genes. Inhibition of JNK alleviates brain injury induced by ischemia-reperfusion in rats, exerting neuroprotective effects(Wang et al., 2022). Moreover, Chenghe et al. discovered that JNK is involved in regulating neuronal autophagy in ischemia-reperfusion injury by inhibiting Beclin1-induced autophagy. The JNK pathway may regulate the interaction between Mcl-1 and Beclin1, and inhibition of JNK promotes Mcl-1 suppression of Beclin1-induced autophagic activation, reducing Bax protein expression and protecting neurons(Fan et al., 2022). Recent studies have reported that downregulating the JAK2/STAT3 signaling pathway can regulate the conversion of cortical astrocytes in rats from A1 to A2 phenotype, enhancing angiogenesis, reducing the release of inflammatory factors, and mitigating ischemic damage. Numerous studies indicate that modulation of the STAT3 pathway can alleviate neuroinflammation and improve synaptic plasticity. Targeting the JAK2/STAT3 signaling pathway to reduce its phosphorylation may be an effective approach to inhibiting neuronal apoptosis following ischemic stroke. Previous research has shown that inhibiting the phosphorylation of the JAK2/STAT3 pathway effectively reduces activation of microglia and astrocytes after oxygen-glucose deprivation/ reoxygenation (OGD/R), thus inhibiting neuroinflammatory responses induced by ischemia and hypoxia, reducing neuronal damage, and promoting neural repair(S. Jiang et al., 2021). Omer Asma B and colleagues discovered that during brain ischemic injury, activated endothelial cells secrete significant amounts of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, as well as factors from glial cells, leukocytes, and endotoxins. These molecules further stimulate the expression of chemokines and adhesion molecules.This leads to the aggregation and adhesion of inflammatory cells at the capillary ends in ischemic regions, further reducing cerebral blood flow and exacerbating brain edema. Recruited inflammatory cells can also synthesize secondary injury-related cytokines, such as leukotrienes and prostaglandins, leading to structural and functional damage to endothelial cells, disrupting the blood-brain barrier, and thereby exerting neuroprotective effects by inhibiting the expression of IL-1β, IL-6, and COX-2(Omer et al., 2023).

The results of molecular docking suggest that the interaction between the core target PTGS2 and the compound is the most stable. Considering that ferroptosis is characterized by widespread lipid peroxidation, and based on KEGG pathway enrichment analysis, the number of enriched genes in lipid metabolism and atherosclerosis pathways, along with the involvement of the p53/PTGS2 pathway, suggest that this pathway plays a regulatory role in the occurrence and progression of ferroptosis. In addition, animal studies demonstrated that the modified Bazhen Decoction notably improved spatial memory in CCII rats and facilitated the recovery of cerebral blood flow.Additionally, both Nissl staining and Western blot analysis showed that modified bazhen decoction alleviated neuronal cell damage, reduced oxidative stress, and decreased iron ion accumulation, further confirming its therapeutic efficacy.

Conclusion

In summary, this study is the first to describe the neuroprotective effects and underlying mechanisms of modified bazhen decoction in chronic ischemic injury mice, specifically through the ferroptosis pathway. These findings provide a scientific basis for the potential use of modified bazhen decoction in preventing and treating chronic cerebral ischemic injury. Additionally, the study opens new avenues for the development of neuroprotective drugs targeting cerebral ischemic injury. However, the primary pharmacological components of modified bazhen decoction and its multi-pathway, multi-targeted mechanisms for combating chronic cerebral ischemic neurological injury require further investigation.

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Conflict of interest

The authors declare that there is no conflict of interest.

Data Availability

Data will be made available from the corresponding author on reasonable request.

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References

1. Armstead, W. M., Hekierski, H., Pastor, P., Yarovoi, S., Higazi, A. A., & Cines, D. B. (2019). Release of IL-6 After Stroke Contributes to Impaired Cerebral Autoregulation and Hippocampal Neuronal Necrosis Through NMDA Receptor Activation and Upregulation of ET-1 and JNK. *Transl Stroke Res*, *10*(1), 104-111. https://doi.org/10.1007/s12975-018-0617-z
2. Daulatzai, M. A. (2017). Cerebral hypoperfusion and glucose hypometabolism: Key pathophysiological modulators promote neurodegeneration, cognitive impairment, and Alzheimer's disease. *J Neurosci Res*, *95*(4), 943-972. https://doi.org/10.1002/jnr.23777
3. Dennis, G., Jr., Sherman, B. T., Hosack, D. A., Yang, J., Gao, W., Lane, H. C., & Lempicki, R. A. (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol*, *4*(5), P3. https://www.ncbi.nlm.nih.gov/pubmed/12734009
4. Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., Patel, D. N., Bauer, A. J., Cantley, A. M., Yang, W. S., Morrison, B., 3rd, & Stockwell, B. R. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*, *149*(5), 1060-1072. https://doi.org/10.1016/j.cell.2012.03.042
5. Fan, C., Zhang, Y., Tian, Y., Zhao, X., & Teng, J. (2022). Phloretin enhances autophagy by impairing AKT activation and inducing JNK-Beclin-1 pathway activation. *Exp Mol Pathol*, *127*, 104814. https://doi.org/10.1016/j.yexmp.2022.104814
6. Ge, J., Lin, H., Yang, J., Li, Q., Zhou, J., Qin, Z., & Wu, F. (2021). TP53-induced glycolysis and apoptosis regulator (TIGAR) ameliorates lysosomal damage in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-mediated mouse model of Parkinson's disease. *Toxicol Lett*, *339*, 60-69. https://doi.org/10.1016/j.toxlet.2020.12.011
7. Hou, D., Hu, Y., Yun, T., Li, H., Yang, G., & Yu, D. (2024). The deubiquitinase OTUD3 stabilizes IRP2 expression to reduce hippocampal neuron ferroptosis via the p53/PTGS2 pathway to ameliorate cerebral ischemia-reperfusion injury. *Eur J Med Res*, *29*(1), 498. https://doi.org/10.1186/s40001-024-02095-w
8. Jiang, S., Wang, H., Zhou, Q., Li, Q., Liu, N., Li, Z., Chen, C., & Deng, Y. (2021). Melatonin Ameliorates Axonal Hypomyelination of Periventricular White Matter by Transforming A1 to A2 Astrocyte via JAK2/STAT3 Pathway in Septic Neonatal Rats. *J Inflamm Res*, *14*, 5919-5937. https://doi.org/10.2147/JIR.S337499
9. Jiang, X., Stockwell, B. R., & Conrad, M. (2021). Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol*, *22*(4), 266-282. https://doi.org/10.1038/s41580-020-00324-8
10. Josiah, S. S., Famusiwa, C. D., Crown, O. O., Lawal, A. O., Olaleye, M. T., Akindahunsi, A. A., & Akinmoladun, A. C. (2022). Neuroprotective effects of catechin and quercetin in experimental Parkinsonism through modulation of dopamine metabolism and expression of IL-1beta, TNF-alpha, NF-kappaB, IkappaKB, and p53 genes in male Wistar rats. *Neurotoxicology*, *90*, 158-171. https://doi.org/10.1016/j.neuro.2022.03.004
11. Khanam, N., Ghosh, A., & Nath, D. (2024). Synergistic effect of chrysin and kaempferol in ameliorating Cerebral Ischemic Reperfusion injury in rat by controlling expression of proinflammatory mediators NF-kappaB and STAT3. *Nutr Neurosci*, 1-11. https://doi.org/10.1080/1028415X.2024.2358686
12. Kim, S., Cheng, T., He, S., Thiessen, P. A., Li, Q., Gindulyte, A., & Bolton, E. E. (2022). PubChem Protein, Gene, Pathway, and Taxonomy Data Collections: Bridging Biology and Chemistry through Target-Centric Views of PubChem Data. *J Mol Biol*, *434*(11), 167514. https://doi.org/10.1016/j.jmb.2022.167514
13. Kulaszynska, M., Kwiatkowski, S., & Skonieczna-Zydecka, K. (2024). The Iron Metabolism with a Specific Focus on the Functioning of the Nervous System. *Biomedicines*, *12*(3). https://doi.org/10.3390/

biomedicines12030595
14. Lahiani, A., Zahavi, E., Netzer, N., Ofir, R., Pinzur, L., Raveh, S., Arien-Zakay, H., Yavin, E., & Lazarovici, P. (2015). Human placental eXpanded (PLX) mesenchymal-like adherent stromal cells confer neuroprotection to nerve growth factor (NGF)-differentiated PC12 cells exposed to ischemia by secretion of IL-6 and VEGF. *Biochim Biophys Acta*, *1853*(2), 422-430. https://doi.org/10.1016/j.bbamcr.2014.11.009
15. Lissner, L. J., Wartchow, K. M., Toniazzo, A. P., Goncalves, C. A., & Rodrigues, L. (2021). Object recognition and Morris water maze to detect cognitive impairment from mild hippocampal damage in rats: A reflection based on the literature and experience. *Pharmacol Biochem Behav*, *210*, 173273. https://doi.org/10.1016/j.pbb.2021.173273
16. Majeed, A., & Mukhtar, S. (2023). Protein-Protein Interaction Network Exploration Using Cytoscape. *Methods Mol Biol*, *2690*, 419-427. https://doi.org/10.1007/978-1-0716-3327-4_32
17. Merelli, A., Repetto, M., Lazarowski, A., & Auzmendi, J. (2021). Hypoxia, Oxidative Stress, and Inflammation: Three Faces of Neurodegenerative Diseases. *J Alzheimers Dis*, *82*(s1), S109-S126. https://doi.org/10.3233/JAD-201074
18. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem*, *30*(16), 2785-2791. https://doi.org/10.1002/jcc.21256
19. Omer, A. B., Afzal, O., Altamimi, A. S. A., Patil, S., AlGhamdi, S. A., Alghamdi, A. M., Alzarea, S. I., Almalki, W. H., & Kazmi, I. (2023). Neuroprotective Effect of Barbaloin on Streptozotocin-Induced Cognitive Dysfunction in Rats via Inhibiting Cholinergic and Neuroinflammatory Cytokines Pathway-TNF-alpha/IL-1beta/IL-6/NF-kappaB. *ACS Omega*, *8*(8), 8110-8118. https://doi.org/10.1021/acsomega.2c08277
20. Park, D. J., Kang, J. B., Shah, F. A., Jin, Y. B., & Koh, P. O. (2020). Quercetin Attenuates Decrease of Thioredoxin Expression Following Focal Cerebral Ischemia and Glutamate-induced Neuronal Cell Damage. *Neuroscience*, *428*, 38-49. https://doi.org/10.1016/j.neuroscience.2019.11.043
21. Paul, C. A., Beltz, B., & Berger-Sweeney, J. (2008). The nissl stain: a stain for cell bodies in brain sections. *CSH Protoc*, *2008*, pdb prot4805. https://doi.org/10.1101/pdb.prot4805
22. Pratiwi, R., Nantasenamat, C., Ruankham, W., Suwanjang, W., Prachayasittikul, V., Prachayasittikul, S., & Phopin, K. (2021). Mechanisms and Neuroprotective Activities of Stigmasterol Against Oxidative Stress-Induced Neuronal Cell Death via Sirtuin Family. *Front Nutr*, *8*, 648995. https://doi.org/10.3389/fnut.2021.648995
23. Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C., Li, P., Guo, Z., Tao, W., Yang, Y., Xu, X., Li, Y., Wang, Y., & Yang, L. (2014). TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform*, *6*, 13. https://doi.org/10.1186/1758-2946-6-13
24. Safran, M., Dalah, I., Alexander, J., Rosen, N., Iny Stein, T., Shmoish, M., Nativ, N., Bahir, I., Doniger, T., Krug, H., Sirota-Madi, A., Olender, T., Golan, Y., Stelzer, G., Harel, A., & Lancet, D. (2010). GeneCards Version 3: the human gene integrator. *Database (Oxford)*, *2010*, baq020. https://doi.org/10.1093/database/baq020
25. Seeliger, D., & de Groot, B. L. (2010). Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *J Comput Aided Mol Des*, *24*(5), 417-422. https://doi.org/10.1007/s10822-010-9352-6
26. She, X., Lan, B., Tian, H., & Tang, B. (2020). Cross Talk Between Ferroptosis and Cerebral Ischemia. *Front Neurosci*, *14*, 776. https://doi.org/10.3389/fnins.2020.00776
27. Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L. J., & von Mering, C. (2021). Correction to 'The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets'. *Nucleic Acids Res*, *49*(18), 10800. https://doi.org/10.1093/nar/gkab835
28. Tang, Y., Li, M., Wang, J., Pan, Y., & Wu, F. X. (2015). CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein

interaction networks. *Biosystems*, *127*, 67-72. https://doi.org/10.1016/j.biosystems.2014.11.005

29. UniProt, C. (2021). UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res*, *49*(D1), D480-D489. https://doi.org/10.1093/nar/gkaa1100
30. Vongthip, W., Nilkhet, S., Boonruang, K., Sukprasansap, M., Tencomnao, T., & Baek, S. J. (2024). Neuroprotective mechanisms of luteolin in glutamate-induced oxidative stress and autophagy-mediated neuronal cell death. *Sci Rep*, *14*(1), 7707. https://doi.org/10.1038/s41598-024-57824-2
31. Wang, G., Zhao, Z., Ren, B., Yu, W., Zhang, X., Liu, J., Wang, L., Si, D., & Yang, M. (2022). Exenatide exerts a neuroprotective effect against diabetic cognitive impairment in rats by inhibiting apoptosis: Role of the JNK/c-JUN signaling pathway. *Mol Med Rep*, *25*(4). https://doi.org/10.3892/mmr.2022.12627
32. Xiao, P., Liu, X. W., Zhao, N. N., Fang, R., Wen, Q., Zeng, K. X., & Wang, Y. L. (2018). Correlations of neuronal apoptosis with expressions of c-Fos and c-Jun in rats with post-ischemic reconditioning damage. *Eur Rev Med Pharmacol Sci*, *22*(9), 2832-2838. https://doi.org/10.26355/eurrev_201805_14984
33. Xu, Z., Feng, X., Li, L., Hu, Z., Xiao, Y., Jin, G., & Liao, W. (2019). Efficacy and safety of oral traditional Chinese patent medicine for chronic cerebral circulation insufficiency patients: A protocol for a systematic review and network meta-analysis. *Medicine (Baltimore)*, *98*(27), e16175. https://doi.org/10.1097/MD.0000000000016175
34. Zhang, A. S., & Enns, C. A. (2009). Iron homeostasis: recently identified proteins provide insight into novel control mechanisms. *J Biol Chem*, *284*(2), 711-715. https://doi.org/10.1074/jbc.R800017200
35. Zhang, S., & Jiao, H. (2024). Kaempferol regulates apoptosis and migration of neural stem cells to attenuate cerebral infarction by O-GlcNAcylation of beta-catenin. *Open Life Sci*, *19*(1), 20220829. https://doi.org/10.1515/biol-2022-0829
36. Zheng, Y., Zhao, J., Chang, S., Zhuang, Z., Waimei, S., Li, X., Chen, Z., Jing, B., Zhang, D., & Zhao, G. (2023). beta-Sitosterol Alleviates Neuropathic Pain by Affect Microglia Polarization through Inhibiting TLR4/NF-kappaB Signaling Pathway. *J Neuroimmune Pharmacol*, *18*(4), 690-703. https://doi.org/10.1007/s11481-023-10091-w
37. Zhou, N., Yuan, X., Du, Q., Zhang, Z., Shi, X., Bao, J., Ning, Y., & Peng, L. (2023). FerrDb V2: update of the manually curated database of ferroptosis regulators and ferroptosis-disease associations. *Nucleic Acids Res*, *51*(D1), D571-D582. https://doi.org/10.1093/nar/gkac935