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Study on mechanisms and molecular verification of Buyang Huanwu decoction in treating ischemic stroke from the perspective of cuproptosis

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Competing interests

The authors declare no conflicts of interest.

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Peer review information

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Abbreviations

BHD, Buyang Huanwu decoction; IS, ischemic stroke; TCM, traditional Chinese medicine; TCMSP, Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CC, cellular component; MF, molecular function; BP, biological process; HQ, Huangqi, *Astragali Radix*; TR, Taoren, *Persicae Semen*; HH, Honghua, *Carthami Flos*; CS, Chishao, *Paeoniae Rubra Radix*; DL, Dilong, *Pheretima*; DG, Danggui, *Angelicae Sinensis Radix*; CX, Chuanxiong, *Chuanxiong Rhizoma*.

Citation

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Abstract

Background: Ischemic stroke (IS) is a global health issue and the current treatment options for IS are inadequate. Buyang Huanwu decoction (BHD) has demonstrated effectiveness in treating IS. However, the mechanisms by which BHD treats IS remain unclear, and no studies have been conducted to analyze these mechanisms from the perspective of Cuproptosis. In order to investigate the potential of BHD to intervene in IS through Cuproptosis, this study employed a systematic pharmacological approach and molecular docking verification. **Methods:** To investigate the mechanism of BHD in treating IS through Cuproptosis, relevant information on the structure, targets, and major biological functions and pathways of compounds related to BHD was collected from databases such as PubChem, PharmMapper, UniProt, and GeneCards. The results were then visualized using Cytoscape3.6.1, Ledock, and pymol software. **Results:** BHD is composed of 7 Chinese medicines, which contain 82 compounds, including 10 core compounds. These compounds are associated with 241 genes, of which 97 are common to BHD, IS, and Cuproptosis. The 97 common genes, including 10 core genes, are involved in biological processes such as proteolysis, regulation of apoptosis and cholesterol storage, as well as cellular components and molecular functions. The common genes among BHD, IS, and Cuproptosis, including 10 core genes, participate mainly in Kyoto Encyclopedia of Genes and Genomes pathways such as pathways in cancer, PI3K-Akt signaling pathway, and Estrogen signaling pathway. According to molecular docking results, linolenic acid showed good docking scores with 9 out of 10 core genes, except for SRC. 13-hydroxy-9,11-octadecadienoic acid also demonstrated good docking scores with EGFR, MAPK14, and F2. Similarly, senkyunone also had good docking scores with EGFR, MAPK14, and F2, all of which had docking energy greater than −5 kcal. **Conclusion:** In this study, the potential of BHD for treating IS through Cuproptosis and its underlying mechanisms were explored and partially validated through molecular docking. However, due to the limitations of the systems pharmacology research method, further validation through cell experiments, animal experiments, and clinical trials may be necessary to confirm these findings.

Keywords: Buyang Huanwu decoction; ischemic stroke; network pharmacology; molecular docking

Introduction

China has a higher incidence rate of stroke, ranking in the fourth quartile globally [1]. Ischemic stroke (IS) is the most prevalent type, accounting for over 85% of all strokes [2]. The estimated incidence rate of IS in China's healthy population is approximately 0.342% [3]. IS is a significant global health concern, ranking as the second leading cause of death and the third leading cause of disability worldwide [4, 5]. When the cerebral arteries that supply blood to the brain become diseased, local blood flow decreases, and blood composition and hemodynamics are altered, ultimately resulting in neuronal necrosis and apoptosis due to cerebral ischemia and hypoxia [6]. The clinical objective in treating IS is to restore cerebral perfusion, primarily through thrombolytic therapy or endovascular intervention [7, 8]. Despite the notable effectiveness of thrombolytic therapy, there are several contraindications, such as age, genetics, and environment, leading to an increasing disability and mortality rate among patients with IS [9–11]. Our research has been dedicated to investigating the therapeutic effects of traditional Chinese medicine (TCM) in the context of IS. Previous studies have shown promising results with Buyang Huanwu decoction (BHD) in IS treatment [12, 13]. Currently, we are delving into the intricate mechanisms through which BHD exerts its therapeutic effects in treating IS, involving processes such as ferroptosis, autophagy, and apoptosis [14]. Cuproptosis, a recently discovered form of programmed cell death associated with lipid peroxidation and direct binding of copper with tricarboxylic acid cycle components, leading to protein toxicity stress and cell death, has emerged as a potential mechanism [15]. Fan et al. have identified a close association between cuproptosis and IS, potentially linked to the activation of immune infiltration mechanisms [16]. NLRP3, NFE2L2, ATP7A, LIPT1, GLS, and MTF1 have been implicated as potential key players in the intervention process [16]. Although the exact role of copper-mediated cell death in cerebral ischemic injury remains uncertain, Jiang et al. conducted experiments on mice exposed to long-term ingestion of copper in the form of copper sulfate in drinking water, and observed that mice with copper intake experienced more severe brain ischemic injury compared to the control group, suggesting that increased levels of free copper may have toxic effects on the brain [17]. Furthermore, TCM interventions in diseases may be linked to the mechanism of cuproptosis [18]. Therefore, our study aims to employ a systematic pharmacological approach to thoroughly investigate the mechanisms by which BHD intervenes in cuproptosis and IS, and validate our findings through molecular docking studies [19].

Materials and methods

Databases and software

In this study, we utilized a variety of databases and software, including Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform (TCMSP) [20], PubChem [21], PharmMapper [22], UniProt [23], String [24], GeneCards [25], DAVID [26], Protein-Ligand Interaction Profiler [27], Cytoscape 3.6.1 [28], OpenBabel-2.4.1 [29], Ledock [30], Pymol [31], and R 4.2.2 [32].

Collection and processing of BHD drug genes

To identify the relevant genes associated with BHD, we conducted a comprehensive search in the TCMSP database for seven active compounds that are present in the seven constituent herbs of BHD, namely "*Astragali Radix*, *Persicae Semen*, *Carthami Flos*, *Paeoniae Rubra Radix*, *Pheretima*, *Angelicae Sinensis Radix*, *Chuanxiong Rhizoma*". The 3D structures of these compounds were obtained from the PubChem database, and we utilized the PharmMapper database to predict their molecular gene targets. Subsequently, the predicted gene names were annotated using the UniProtKB search function in the UniProt database, allowing us to generate a comprehensive list of BHD-related genes.

Construction of the network of Chinese medicine-compound-gene

The data pertaining to the relationship between Chinese medicine and compounds, as well as compounds and genes, were organized and imported into Cytoscape 3.6.1 software to construct a comprehensive network involving Chinese medicine-compound-gene-disease relationships. Genes associated with IS and cuproptosis were identified through the GeneCards database. The common genes shared by BHD, IS, and Cuproptosis were determined by taking the intersection of genes related to BHD and those related to IS and Cuproptosis. The protein-protein interaction (PPI) network involving these common genes was then constructed using the String database. Subsequently, a network diagram depicting the relationships between Chinese medicine, compounds, BHD, IS, and Cuproptosis was created using Cytoscape 3.6.1 software for visual representation and analysis.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of common genes of BHD, IS, and Cuproptosis

The common genes among BHD, IS, and Cuproptosis were analyzed for GO and KEGG enrichment using the DAVID database. Additionally, the potential common genes of BHD, IS, and Cuproptosis for each Chinese medicine were analyzed separately using GO and KEGG enrichment analysis. The top 15 GO and KEGG items with the most significant *P* values were selected for visualization, with a significance level set at $P<0.05$, to emphasize the most relevant functional annotations and pathways associated with these common genes.

Selection of core compounds and core disease genes and molecular docking validation.

Through the Network Analysis feature of Cytoscape 3.6.1 software, we identified 10 core compounds for treating IS and 10 core genes related to BHD. The sdf files of the key compounds were obtained from the PubChem database, converted to mol files using OpenBabel-2.4.1 software, and the pdb structure files of the key disease genes were downloaded from the Protein Data Bank database. Molecular docking energies of the core compounds and core genes were calculated using Ledock software after ligand stripping and hydrogenation. Finally, visualization of the docking results was performed using Pymol software and the Protein-Ligand Interaction Profiler database.

Results

Collection and screening of BHD compounds

BHD comprises 7 Chinese medicinal herbs (*Astragali Radix*, *Persicae Semen*, *Carthami Flos*, *Paeoniae Rubra Radix*, *Pheretima*, *Angelicae Sinensis Radix*, *Chuanxiong Rhizoma*), as shown in Figure 1. Using oral bioavailability > 30% and drug-likeness > 0.2 as screening criteria, 82 compounds were retrieved from the TCMSP database and are listed in Table 1. Then, by using the PharmMapper database with Normfit > 0.7 as the screening criterion, 241 potential genes of BHD were predicted. The BHD-compound-gene network was constructed using Cytoscape software, as shown in Figure 1B. Using Genecards, 3684 IS genes and 1930 Cuproptosis genes were collected. The intersection of these genes resulted in 97 common genes of BHD, IS, and Cuproptosis, as shown in Figure 1C. The PPI network of the common genes of BHD, IS, and Cuproptosis was constructed using the STRING database, as shown in Figure 1D.

Enrichment analysis of GO and KEGG pathways for genes contained in different Chinese medicines.

A network of BHD-compounds-97 common genes of BHD, IS, and Cuproptosis was constructed using Cytoscape software, as depicted in Figure 2A. Among these common genes, *Paeoniae Rubra Radix* exhibited the highest number, followed by *Carthami Flos* and *Persicae Semen*, as shown in Figure 2B. Further analysis using GO enrichment revealed that the 97 common genes are primarily involved in biological processes such as proteolysis, negative regulation of apoptotic process, and negative regulation of cholesterol storage, as

well as cellular components such as extracellular region, extracellular exosome, and extracellular space. In terms of molecular functions, these genes are associated with enzyme binding, RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, and zinc ion binding. Additionally, KEGG pathway analysis demonstrated that these genes are mainly involved in pathways such as pathways in cancer, PI3K-Akt signaling pathway, and estrogen signaling pathway, as depicted in Figure 2C. The potential genes, GO items, KEGG pathways, and other relevant information for each Chinese medicine are illustrated in Figure 3A–3N.

Table 1 List of compounds contained in BHD

Number	Medicine	Compounds	Number	Medicine	Compounds
1	*Paeoniae Rubra Radix*	Spinasterol	42	*Carthami Flos*	quercetin
2	*Paeoniae Rubra Radix*	Stigmasterol	43	*Carthami Flos*	kaempferol
3	*Paeoniae Rubra Radix*	beta-sitosterol	44	*Carthami Flos*	lignan
4	*Paeoniae Rubra Radix*	sitosterol	45	*Carthami Flos*	lupeol-palmitate
5	*Paeoniae Rubra Radix*	Baicalin	46	*Carthami Flos*	6-Hydroxynaringenin
6	*Paeoniae Rubra Radix*	Albiflorin	47	*Carthami Flos*	baicalein
7	*Paeoniae Rubra Radix*	stigmast-7-en-3-ol	48	*Carthami Flos*	Phytoene
8	*Paeoniae Rubra Radix*	(+)-catechin	49	*Astragali Radix*	Bifendate
9	*Paeoniae Rubra Radix*	paeoniflorin	50	*Astragali Radix*	Mairin
10	*Paeoniae Rubra Radix*	campest-5-en-3beta-ol	51	*Astragali Radix*	13-hydroxy-9,11-octadecadienoic acid
11	*Paeoniae Rubra Radix*	ellagic acid	52	*Astragali Radix*	Jaranol
12	*Paeoniae Rubra Radix*	Paeoniflorigenone	53	*Astragali Radix*	astragalosideI
13	*Paeoniae Rubra Radix*	baicalein	54	*Astragali Radix*	linolenic acid
14	*Chuanxiong Rhizoma*	sitosterol	55	*Astragali Radix*	isoflavanone
15	*Chuanxiong Rhizoma*	Myricanone	56	*Astragali Radix*	isorhamnetin
16	*Chuanxiong Rhizoma*	senkyunone	57	*Astragali Radix*	7-O-methylisomucronulatol
17	*Chuanxiong Rhizoma*	Perlolyrine	58	*Astragali Radix*	astragalosideII
18	*Chuanxiong Rhizoma*	wallichilide	59	*Astragali Radix*	formononetin
19	*Angelicae Sinensis Radix*	Stigmasterol	60	*Astragali Radix*	3,9-di-O-methylnissolin
20	*Angelicae Sinensis Radix*	beta-sitosterol	61	*Astragali Radix*	quercetin
21	*Angelicae Sinensis Radix*	cis-Thujopsene	62	*Astragali Radix*	hederagenin
22	*Angelicae Sinensis Radix*	2,6-diphenylthiopyran-4-thione	63	*Astragali Radix*	kaempferol
23	*Pheretima*	cholesteryl ferulate	64	*Astragali Radix*	Calycosin
24	*Pheretima*	guanosine	65	*Persicae Semen*	gibberellin 17
25	*Pheretima*	cholesterol	66	*Persicae Semen*	GA87
26	*Pheretima*	xanthinin	67	*Persicae Semen*	2,3-didehydro GA77
27	*Pheretima*	hyrcanoside	68	*Persicae Semen*	beta-sitosterol
28	*Pheretima*	4-guanidino-1-butanol	69	*Persicae Semen*	Sitosterol alpha1
29	*Pheretima*	xanthine	70	*Persicae Semen*	GA119
30	*Pheretima*	hypoxanthine	71	*Persicae Semen*	Gibberellin A44
31	*Carthami Flos*	Stigmasterol	72	*Persicae Semen*	GA121-isolactone
32	*Carthami Flos*	beta-sitosterol	73	*Persicae Semen*	3-O-p-coumaroylquinic acid
33	*Carthami Flos*	beta-carotene	74	*Persicae Semen*	GA77
34	*Carthami Flos*	poriferast-5-en-3beta-ol	75	*Persicae Semen*	GA122-isolactone
35	*Carthami Flos*	Baicalin	76	*Persicae Semen*	GA122
36	*Carthami Flos*	6-Hydroxykaempferol	77	*Persicae Semen*	GA63
37	*Carthami Flos*	luteolin	78	*Persicae Semen*	hederagenin
38	*Carthami Flos*	Pyrethrin II	79	*Persicae Semen*	campesterol
39	*Carthami Flos*	Flavoxanthin	80	*Persicae Semen*	GA60
40	*Carthami Flos*	phytofluene	81	*Persicae Semen*	GA120
41	*Carthami Flos*	quercetagetin	82	*Persicae Semen*	GA54

BHD, Buyang Huanwu decoction.

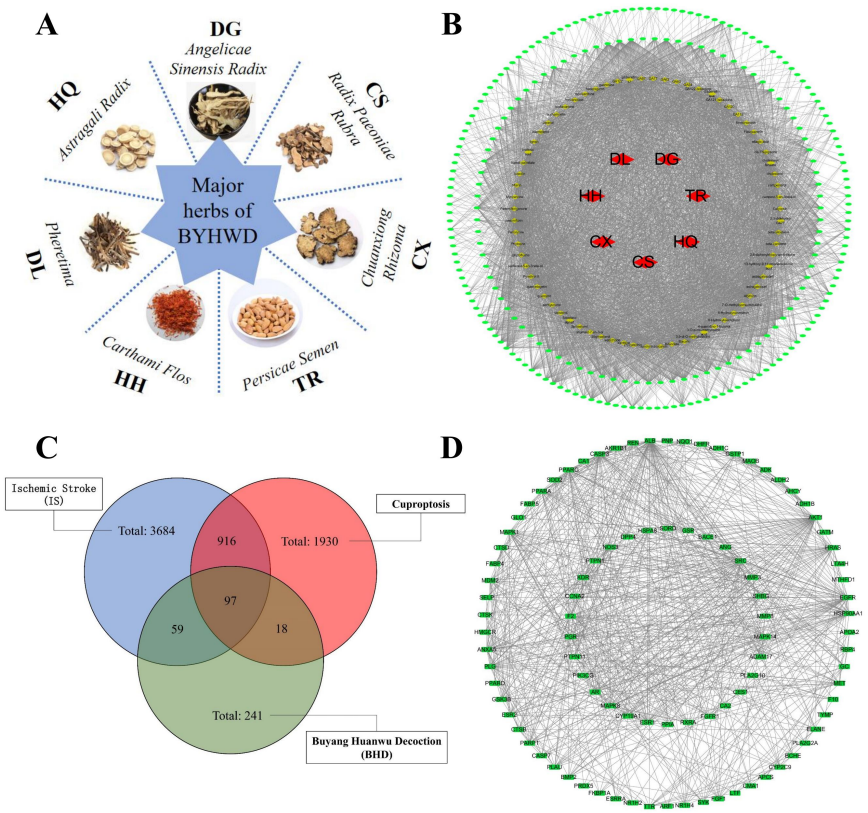

Figure 1 The graph of BHD-related information. (A) BHD drug composition; (B) BHD-Chinese medicine-compound-gene network diagram; (C) BHD, IS, Cuproptosis gene Wayne diagram; (D) PPI network diagram of common genes of BHD, IS and Cuproptosis. BHD, Buyang Huanwu decoction; IS, ischemic stroke; PPI, protein-protein interaction. HQ, Huangqi, *Astragali Radix*; TR, Taoren, *Persicae Semen*; HH, Honghua, *Carthami Flos*; CS, Chishao, *Paeoniae Rubra Radix*; DL, Dilong, *Pheretima*; DG, Danggui, *Angelicae Sinensis Radix*; CX, Chuanxiong, *Chuanxiong Rhizoma*.

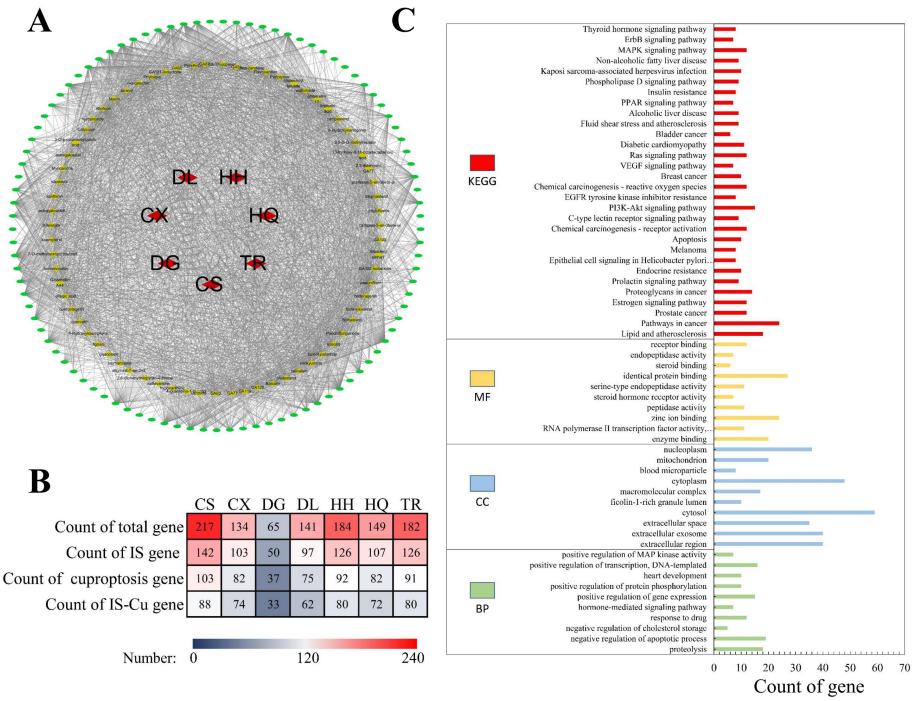

Figure 2 Gene information contained in different herbs, KEGG and GO enrichment analysis results of BHD. (A) Network diagram of BHD-Chinese medicine-compound-BHD, IS, Cuproptosis common genes; (B) Heat map of the number of genes that can be intervened by different Chinese medicines in BHD; (C) GO and KEGG enrichment analysis results of common genes of BHD, IS and Cuproptosis. BHD, Buyang Huanwu decoction; IS, ischemic stroke; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CC, cellular component; MF, molecular function; BP, biological process; HQ, Huangqi, *Astragali Radix*; TR, Taoren, *Persicae Semen*; HH, Honghua, *Carthami Flos*; CS, Chishao, *Paeoniae Rubra Radix*; DL, Dilong, *Pheretima*; DG, Danggui, *Angelicae Sinensis Radix*; CX, Chuanxiong, *Chuanxiong Rhizoma*.

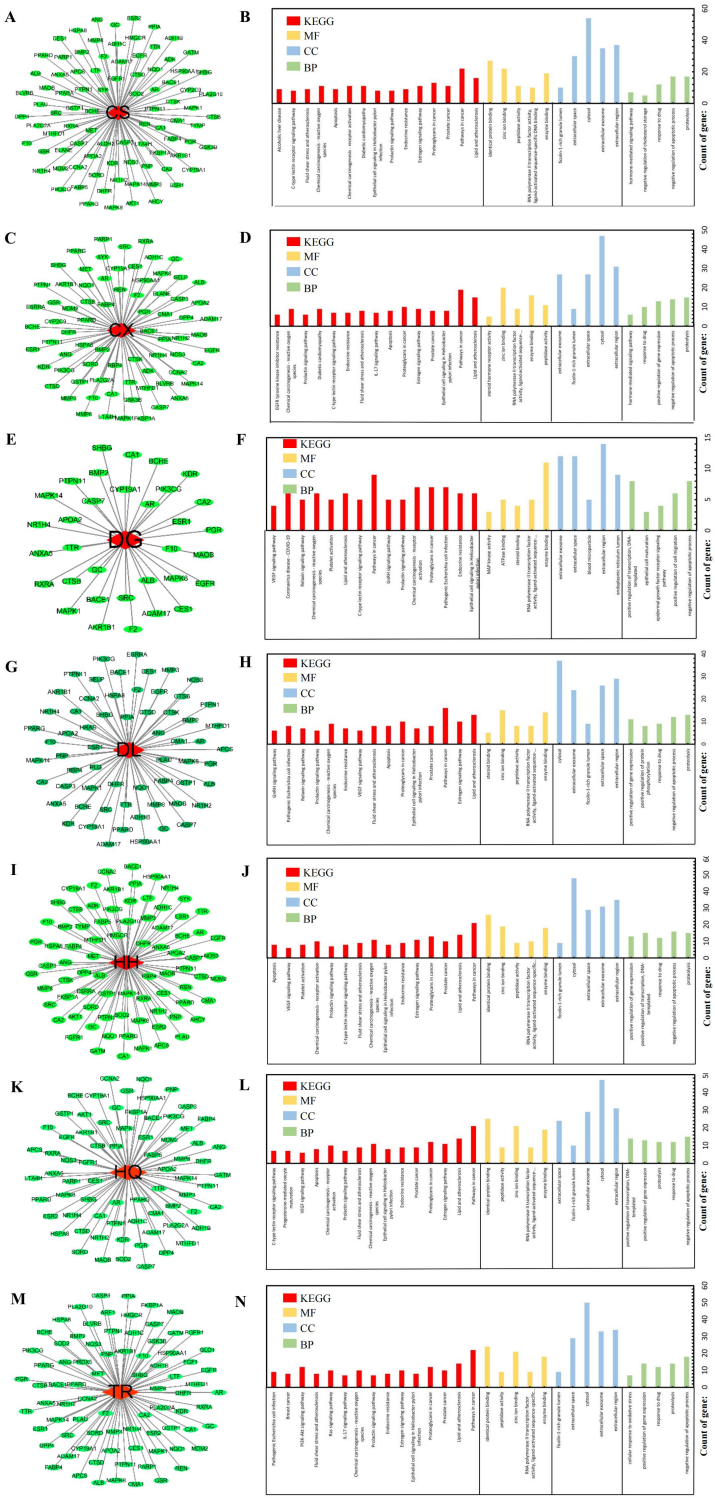

Figure 3 Gene network, KEGG and GO enrichment analysis results of different herbs. (A) The relationship network between CS and genes; (B) GO and KEGG enrichment analysis results of CS-related BHD, IS and Cuproptosis common genes; (C) CX and gene relationship network; (D) the results of GO and KEGG enrichment analysis of BHD, IS and Cuproptosis common genes related to CX; (E) Relationship network between DG and genes; (F) GO and KEGG enrichment analysis results of DG-related BHD, IS and Cuproptosis common genes; (G) DL and gene network; (H) results of GO and KEGG enrichment analysis of common genes of BHD, IS and Cuproptosis related to DL; (I) Relationship network between HH and genes; (J) GO and KEGG enrichment analysis results of BHD, IS and Cuproptosis common genes related to HH; (K) HQ and gene relationship network; (L) results of GO and KEGG enrichment analysis of common genes of BHD, IS and Cuproptosis related to HQ; (M) TR and gene network; (N) GO and KEGG enrichment analysis results of TR-related BHD, IS, and Cuproptosis common genes. HQ, Huangqi, *Astragali Radix*; TR, Taoren, *Persicae Semen*; HH, Honghua, *Carthami Flos*; CS, Chishao, *Paeoniae Rubra Radix;* DL, Dilong, *Pheretima*; DG, Danggui, *Angelicae Sinensis Radix*; CX, Chuanxiong, *Chuanxiong Rhizoma*; BHD, Buyang Huanwu decoction; IS, ischemic stroke; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CC, cellular component; MF, molecular function; BP, biological process.

Selection of core compounds

A network was constructed using Cytoscape software to link compounds and the 97 common genes of BHD, IS, and Cuproptosis, as shown in Figure 4A. Among these compounds, paeoniflorin, Albiflorin, Paeoniflorigenone, wallichilide, linolenic acid, gibberellin 17, Baicalin, 13-hydroxy-9,11-octadecadienoic acid, Pyrethrin II, and senkyunone were associated with the highest number of genes, as shown in Figure 4B. The structures of the 10 core compounds are displayed in Figure 4C–4L.

Selection of core genes and molecular docking verification

Using the network analysis function of Cytoscape software, we screened the network of compounds and 97 common genes among BHD, IS, and Cuproptosis, and selected 10 core genes, including ALB, SRC, ESR1, EGFR, AR, PGR, MAPK14, F2, MAPK1, and CA2, as shown in Figure 5A. Among them, ALB, SRC, and ESR1 were associated with the largest number of compounds, as shown in Figure 5B. We performed molecular docking of the 10 core compounds and 10 core genes. The results showed that, except for SRC, linolenic acid had a binding energy of less than −5 kcal/mol with the other 9 core genes. 13-hydroxy-9,11-octadecadienoic acid had a binding energy of less than −5 kcal/mol with EGFR, MAPK14, and F2. Senkyunone had a binding energy of less than −5 kcal/mol with EGFR, MAPK14, and F2, as shown in Figure 5C. According to previous research [33], a binding energy of less than −5.00 kcal/mol is considered stable. We selected the four docking structures with the lowest binding energies for visualization, including CA2 with Albiflorin, PGR with Albiflorin, Baicalin, and Paeoniflorin, as shown in Figure 6A–6D.

Discussion

BHD is a combination of seven Chinese herbal medicines, namely *Astragali Radix*, *Carthami Flos*, *Persicae Semen*, *Paeoniae Rubra Radix*, *Pheretima*, *Angelicae Sinensis Radix*, and *Chuanxiong Rhizoma*, known for their effects in tonifying Qi (Qi refers to the basic substance that constitutes the human body and maintains life activities, and is the unity of substance and function), activating blood circulation, and unblocking collaterals. BHD has been traditionally used for the treatment of IS, and numerous studies have explored its mechanism of action. However, there has been no research on the mechanism of action of BHD specifically from the perspective of cuproptosis.By analyzing the relationship between Chinese herbal medicines and genes, we have identified *Paeoniae Rubra Radix*, *Carthami Flos*, and *Persicae Semen* as key herbs in the potential mechanism of BHD for treating IS through cuproptosis. Previous studies have also revealed that *Paeoniae Rubra Radix* contains one compound, *Carthami Flos* contains three compounds, and *Persicae Semen* contains three compounds that may potentially intervene in cuproptosis [18]. In

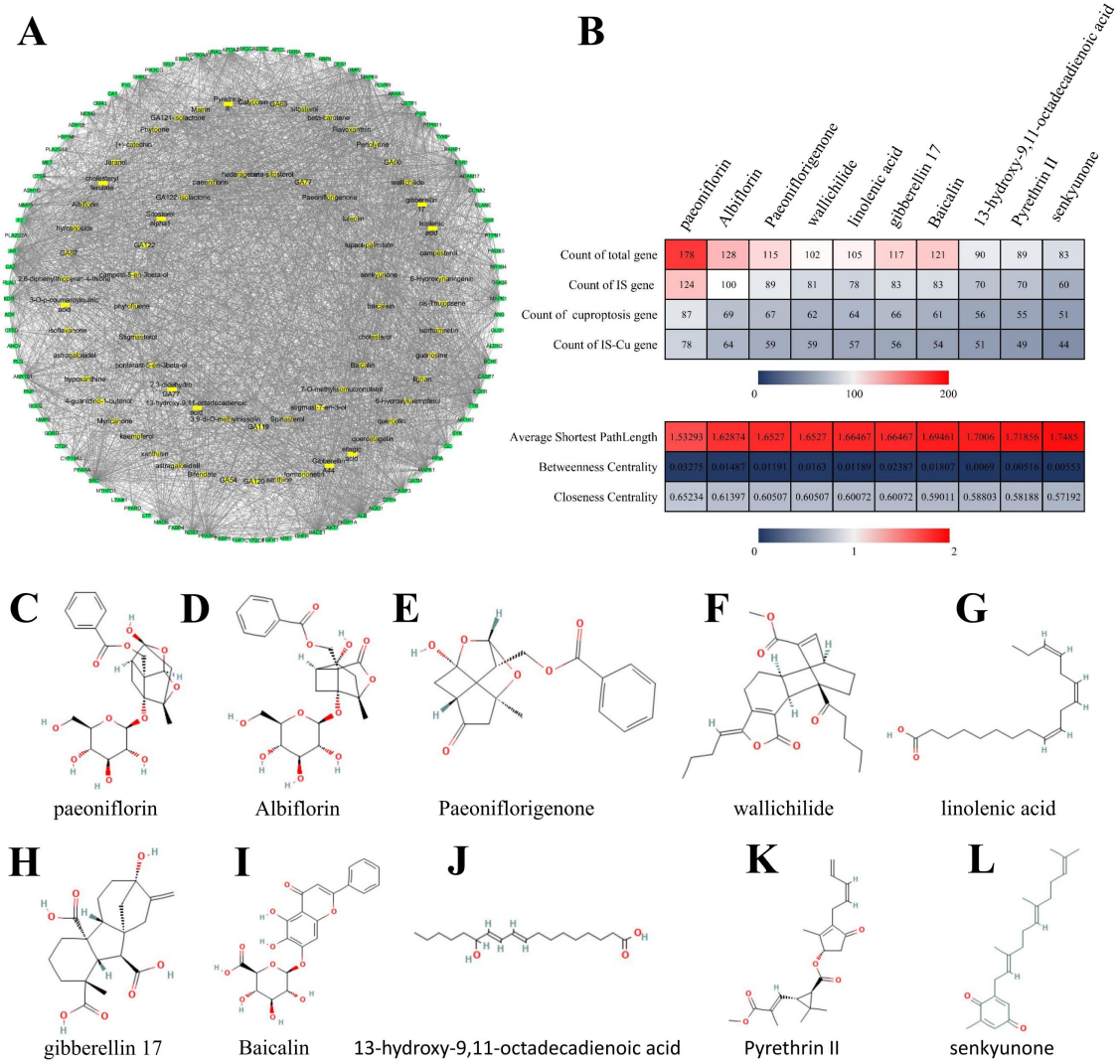

Figure 4 The information of compounds and core compounds contained in BHD. (A) Compound-BHD, IS, Cuproptosis common gene relationship network; (B) The heat map of the number of genes that can be intervened by 10 core compounds; (C–L) are the structure diagrams of paeoniflorin, Albiflorin, Paeoniflorigenone, wallichilide, linolenic acid, gibberellin 17, Baicalin, 13-hydroxy-9,11-octadecadienoic acid, Pyrethrin II and senkyunone, respectively. BHD, Buyang Huanwu decoction; IS, ischemic stroke.

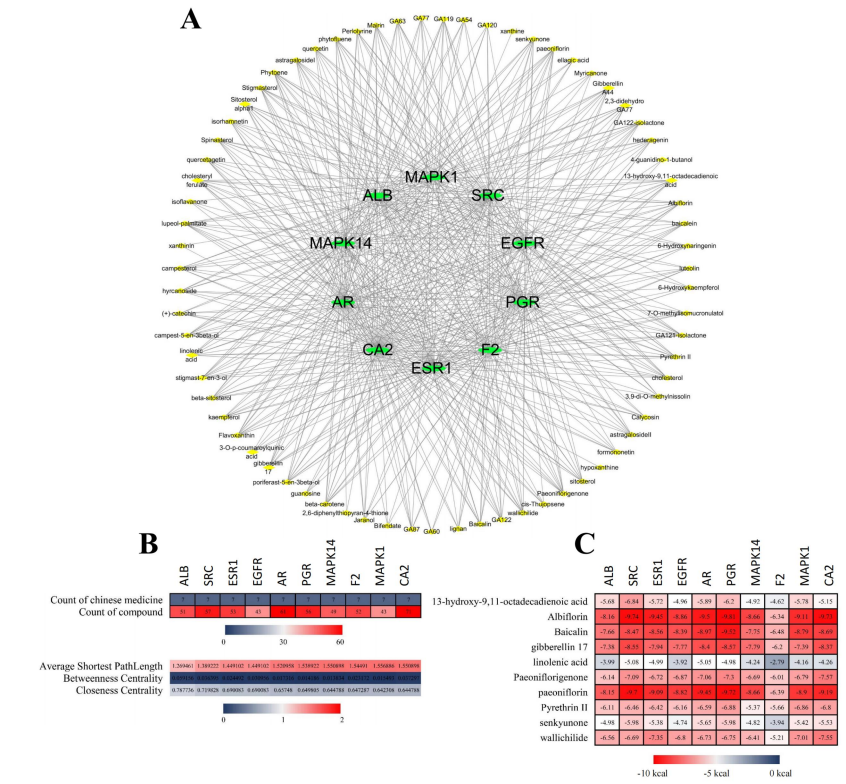

Figure 5 Gene-related compound information and molecular docking results. (A) Relationship network diagram of 10 core genes-compounds; (B) The heat map of the number of Chinese medicines and compounds associated with 10 core genes; (C) Molecular docking results of 10 core compounds and 10 core genes.

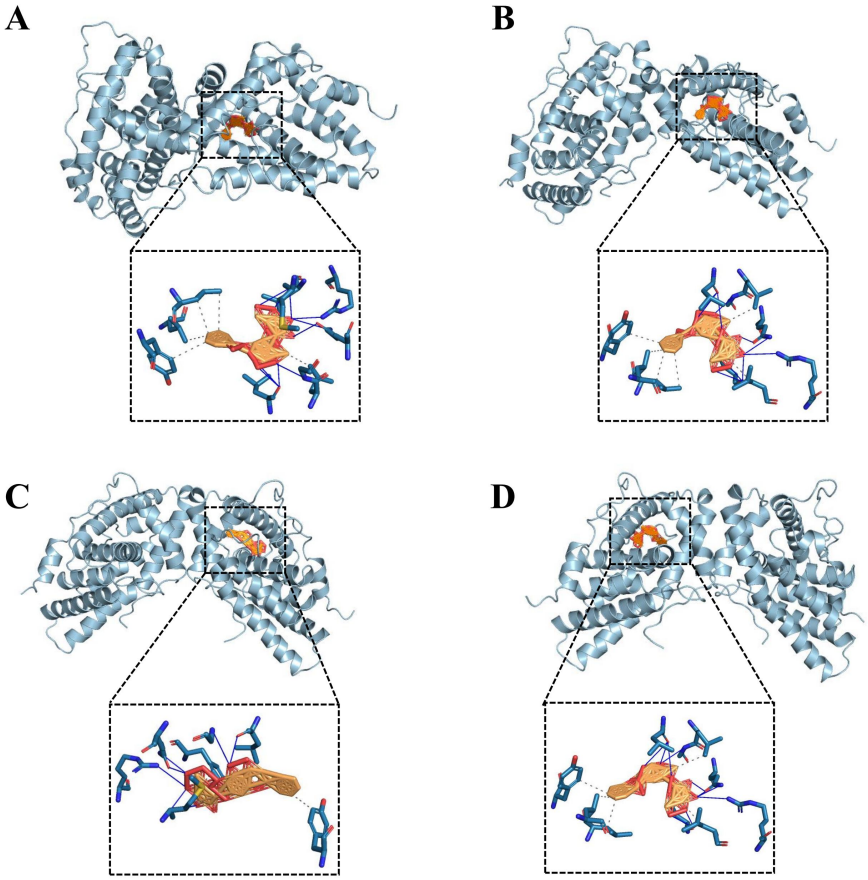

Figure 6 Structure diagram of molecular docking. (A) Molecular docking of CA2 and Albiflorin; (B) Molecular docking of PGR and Albiflorin; (C) Molecular docking of PGR and Baicalin; (D) Molecular docking of PGR and paeoniflorin.

TCM, treatment is individualized based on syndrome differentiation, with adjustments made to the combination of herbs according to the patient's condition, nature of the herbs, and treatment goals. TCM theory suggests that *Paeoniae Rubra Radix*, *Carthami Flos*, and *Persicae Semen* are known for promoting blood circulation and resolving stasis, and are commonly used for diseases associated with blood stasis. Studies on the syndrome types of IS have shown that deficiency of Qi and blood stasis is the main syndrome pattern [34]. Therefore, from the perspective of TCM theory, the inclusion of *Paeoniae Rubra Radix*, *Carthami Flos*, and *Persicae Semen* in BHD is reasonable and consistent with the research findings of Yang Z [35]. These findings provide evidence that *Paeoniae Rubra Radix*, *Carthami Flos*, and *Persicae Semen* have both material and TCM theoretical basis for their potential intervention in cuproptosis and IS.

Through our analysis of the relationship between compounds and genes, we have identified 10 compounds that are closely associated with BHD, IS, and cuproptosis. Notably, paeoniflorin, linolenic acid, baicalin, and 13-hydroxy-9,11-octadecadienoic acid have been shown to have beneficial effects on IS in previous studies [36–39]. Additionally, we have identified 10 core genes that are closely linked to BHD, IS, and cuproptosis. These findings highlight the crucial roles of these core compounds and genes, which collectively reflect the overall pharmacological effects of BHD on IS, involving the combined action of multiple compounds, genes, and pathways. Furthermore, our molecular docking results reveal strong binding affinity between 9 out of the 10 core compounds and the 10 core genes, suggesting that these 9 compounds can effectively bind to and exert functional effects on the core genes, thereby providing positive validation for the accuracy of our research results.

The results of GO enrichment analysis revealed that these genes play a significant role in cellular components (CC) such as the extracellular region, extracellular exosome, and extracellular space, as well as molecular functions (MF) including enzyme binding, RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, and zinc ion binding. These findings suggest that cellular components and functions in vivo are not isolated, but rather form an interconnected and coordinated network that contributes to the overall biological processes. Furthermore, the main involvement of these genes in biological processes (BP) is predominantly associated with negative regulation of apoptotic processes, indicating an anti-apoptotic role. Apoptosis, a programmed cell death pathway, is one of the important mechanisms in the pathogenesis of IS [40–42]. Specifically, during the occurrence of cerebral ischemia, the peri-infarct penumbra region, which sustains less damage and retains ATP, primarily undergoes cell death through the apoptotic pathway [43].

The results of KEGG enrichment analysis uncovered that BHD treatment for IS may impact pathways such as Pathways in cancer, PI3K-Akt signaling pathway, and Estrogen signaling pathway, which have been previously established to play crucial roles in IS [44–46]. Among these pathways, the PI3K-Akt signaling pathway appears to be particularly relevant. IS is a multifactorial condition involving energy depletion, excitotoxicity, neuroinflammation, neuronal apoptosis, reperfusion injury, and oxidative stress [10]. Existing research has consistently shown that apoptosis of brain cells is closely associated with IS-induced damage [47, 48]. The PI3K/Akt signaling pathway, a critical signaling mechanism in mammalian biological processes, regulates signal transduction, cell development, differentiation, cell survival, protein synthesis, and metabolism [49]. PI3K is composed of regulatory subunit P85 and catalytic subunit p110, which interacts with growth factor receptors to activate Akt [50]. Akt, the primary downstream molecule of the PI3K signaling pathway, comprises three isoforms: Akt 1, Akt 2, and Akt 3 [51]. In the context of cerebral ischemia, Akt expression increases in the penumbra region of the brain [52]. Previous studies have demonstrated that the PI3K/Akt/mTOR signaling pathway exerts neuroprotective effects in ischemic-reperfusion injury by upregulating the expression of PI3K, p-Akt, and p-mTOR in brain tissue, leading to a significant reduction in the size of cerebral infarction and pathological changes in brain tissue in rats with middle cerebral artery occlusion [53]. Akt plays a crucial role in maintaining a balance between cell survival and apoptosis, and studies have suggested that copper-induced cell death can affect apoptosis by modulating Akt phosphorylation [54]. Furthermore, the PI3K/Akt signaling pathway is involved in a series of phosphorylation cascades that can delay cell apoptosis [55]. For instance, insulin-like growth factor 1 has been found to upregulate neuronal survival in IS by activating the PI3K/Akt signaling cascade, thereby upregulating yes-associated protein/transcriptional coactivator with PDZ-binding motif [56].

Limitations

This study has several limitations. Firstly, all data were sourced from online databases, without cross-validation using real-world clinical data. The study focused solely on the relationship and quantity of targets, compounds, and herbs, without considering factors such as compound potency, compound content in different herbs, variability in compound content due to herb origins, and the impact of decoction methods on compound composition in traditional Chinese medicine. Additionally, while the study explored the molecular functions and KEGG pathways associated with these genes, there is a lack of experimental research to validate and refine the specific molecular mechanisms identified.

Conclusion

In summary, this study explored the potential of BHD in treating IS through Cuproptosis and its underlying mechanisms using a systems pharmacology approach. The results of this study suggest that BHD may potentially intervene in the mechanism of IS by activating the PI3K-Akt signaling pathway, leading to anti-apoptotic effects through cuproptosis. Multiple genes and signaling pathways are implicated in this process. However, as this study solely relies on data mining, further validation through in vitro and animal experiments is needed to confirm the research findings.

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