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Study on the mechanism of Gualou Xiebai Guizhi decoction (GLXBGZD) in the treatment of coronary heart disease based on network pharmacology

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Abstract

Background: This study aims to analyze the mechanism of Gualou Xiebai Guizhi decoction (GLXBGZD) in treating coronary heart disease (CHD) utilizing network pharmacology.

Methods: The GLXBGZD effective components were searched on the pharmacological database platform of the Traditional Chinese Medicine Systems Pharmacol, and its potential target was predicted. The Online Mendelian Inheritance obtained CHD disease target in Man and GeneCards database. The Venn map of the intersection target for GLXBGZD and CHD was constructed by using Venn online website. The "drug-component-target-disease" network map was constructed by Cytoscape 3.7.2 software. The DAVID online platform was used to analyze the function of Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) at the intersection of targets of drugs and diseases.

Results: A total of 27 articles were searched for GLXBGZD, including 111 potential targets, 5521 disease targets, 100 drug and disease intersection targets. The core target network map shows that Interleukin (IL)-6, TNF, vascular endothelial growth factor (VEGFA), TP53, EGF, JUN, MAPK1, Catalase (CAT), and prostaglandin-endoperoxide synthase 2 (PTGS2) may be the key targets in CHD therapy. GO functional enrichment analysis revealed that the biological functions of GLXBGZD involved biological processes such as response to drugs, positive regulation of nitric oxide biosynthesis process, and response to hypoxia. KEGG pathway enrichment analysis showed that GLXBGZD might participate in CHD treatment through Hypoxia-inducible factor-1 (HIF-1), Tumor necrosis factor (TNF), PhosphoInositide-3 Kinase--Threonine protein kinase (PI3K-Akt), and the calcium signal pathway.

Conclusions: This study reveals that the GLXBGZD mechanism in CHD treatment has the characteristics of multi-components, multi-targets, and multi-pathways, which provides a theoretical basis for its clinical application and subsequent experimental verification.

Abbreviations: CHD = coronary heart disease, DL = drug-like properties, GLXBGZD = Gualou Xiebai Guizhi decoction, OB = oral bioavailability, OMIM = Online Mendelian Inheritance in Man, TCM = Traditional Chinese medicine, TCMSP = TCM Systems Pharmacol, VEGFA = vascular endothelial growth factor A.

Keywords: coronary heart disease, Gualou Xiebai Guizhi decoction, mechanism of action, network pharmacology

1. Introduction

Coronary heart disease (CHD) is a cardiac disease caused by the interruption of blood and oxygen supply to myocardial cells due to narrowing and occlusion of the coronary artery lumen, which eventually leads to necrosis of myocardial cells.[1] With the accelerated rate of population aging in China, CHD has become one of the important causes of cardiovascular disease mortality.[2] The exact pathogenesis of CHD is complex, in terms of lifestyle, such as obesity, type 2 diabetes, hypertension, sedentary lifestyle, and genetic factors, are all critical factors in its development.[3] The routine clinical use of drugs or surgery to treat CHD often disadvantages higher costs to patients, extensive physical trauma, and inconsistent treatment outcomes. In recent years, the advantages of Chinese medicine in the treatment of CHD have been widely appreciated because of its effective improvement of clinical symptoms, low rate of adverse reactions, and higher safety profile.[4]

Traditional Chinese medicine (TCM) formulations are challenging to elucidate the mechanism of action due to their complex compound composition and inadequate research methods. In recent years, with the development of bioinformatics, the emerging network pharmacology is based on large databases,[5] and the traditional "one drug, one target" model has been upgraded to a "multi-component, multi-target" model by constructing the network relationship between active drug components and targets. This is also in line with the theory of TCM that the human body itself can act as a complete system, with each system influencing the other.[6] Network pharmacology is a novel therapeutic approach in drug research and effectively identifies new active ingredients and mechanisms of action for TCM, which is expected to provide new strategies for TCM study.[7]

Modern pharmacological studies have shown the effectiveness of GLXBGZD in CHD treatment.[8–10] Gualou (*Trichosanthes kirilowii* Maxim) is the dried ripe fruit of *Trichosanthes kirilowii* Maxim or *T. rosthornii* Harm, family Cucurbitaceae. The chemical components of Gualou mainly include triterpenoids, flavonoids, phytosterols, fatty acids, amino acids and proteins, alkaloids, polysaccharides, and so on. The extract of Gualou has the effects of dilating coronary arteries, increasing coronary blood flow, enhancing ischemia-reperfusion, and reducing the amount of propylene glycol.[11] Xiebai (*Allium macrostemon* Bunge) is the dried bulb of allium macrostemon Bge or allium Chinese G. Don in Liliaceae. The main bioactive components of Xiebai include steroidal saponins, volatile oils, nitrogenous compounds, polysaccharides, fatty acids etc. Xiebai has the effects of inhibiting platelet aggregation, improving microcirculation, antiatherosclerosis, myocardial ischemia, hypoxia and reperfusion of myocardium in patients, and significantly elevating bone density lipoprotein cholesterol, lowering the content of serum cholesterol, triacylglycerol, and low-density lipoprotein.[12] Guizhi (Cinnamomum cassia Presl) is the dried shoots of Cinnamomum cassia Presl, mainly containing volatile oils and organic acids, which include cinnamaldehyde, cinnamic alcohol, methoxycinnamaldehyde, benzenepropanal, 3-hydroxy-ben-zaldehyde, phenylpropyl aldehyde, 1, 8-cineole-β-cymene, protocatechuic aldehyde, α-copane, etc. The cinnamaldehyde in Guizhi promotes coronary blood flow, reducing the viscosity of pulmonary secretion.[13] The adjuvants and messengers medicine can increase myocardial contractility, increase coronary blood flow, improve myocardial ischemia and hypoxia, improve myocardial hypoxia resistance, and inhibit platelet aggregation.[14] However, the mechanism of action of GLXBGZD in the treatment of CHD at the molecular level is not clear.

In this study, we used the network pharmacology method to reveal the mechanism of action of GLXBGZD in treating CHD at the molecular level. We also provide a theoretical basis for the better application of TCM in the clinical treatment of the disease.

2. Methods

2.1. Acquisition of active ingredients and targets of GLXBGZD

All the active ingredients of GLXBGZD were searched by TCM Systems Pharmacol (TCMSP) using the keywords of "Guahu Bai", "Allium" and "Gui Zhi," respectively, and the active ingredients were screened by drug-like properties (DL) ≥ 0.18 and oral bioavailability (OB ≥ 30%). The active ingredients were screened with the screening criteria of drug-like properties (DL) ≥ 0.18 and oral bioavailability (OB ≥ 30%), and the potential targets of all compounds were predicted by the TCMSP database. The obtained targets were imported into the Uniprot database for gene normalization. This study is a summary of data from published articles and does not address issues related to patient ethics, etc. Therefore, this study does not require approval by the ethics committee.

Table 1

The active ingredients of GLXBGZD.

Drugs	MOL_ID	Molecule_name	OB	DL
Gualou	MOL001494	Mandenol	41.99	0.19
	MOL002881	Diosmetin	31.13	0.27
	MOL004355	Spinasterol	42.97	0.75
	MOL005530	Hydroxygenkwanin	36.46	0.27
	MOL006756	Schottenol	37.42	0.75
	MOL007165	10α-cucurbita-5,24-diene-3β-ol	44.01	0.74
	MOL007171	5-dehydrokarounidiol	30.22	0.77
	MOL007172	7-oxo-dihydrokaro-unidiol	36.85	0.75
	MOL007175	Karounidiol 3-o-benzoate	43.99	0.49
	MOL007179	Linolenic acid ethyl ester	46.10	0.19
	MOL007180	Vitamin-e	32.28	0.69
Xiebai	MOL000098	Quercetin	46.43	0.27
	MOL000332	n-coumaroyltyramine	85.62	0.20
	MOL000358	Beta-sitosterol	36.91	0.75
	MOL000483	(Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-hydroxyphenyl) ethyl]acrylamide	118.34	0.26
	MOL000631	Coumaroyltyramine	112.90	0.20
	MOL001973	Sitosteryl acetate	40.38	0.85
	MOL002341	Hesperetin	70.31	0.27
	MOL004328	Naringenin	59.29	0.21
	MOL007640	Macrostemonoside e_qt	35.25	0.87
	MOL007650	PGA(sup 1)	43.98	0.25
	MOL007651	Prostaglandin B1	40.20	0.25
Guizhi	MOL000073	ent-Epicatechin	48.95	0.24
	MOL000358	beta-sitosterol	36.91	0.75
	MOL000359	Sitosterol	36.91	0.75
	MOL000492	(+)-catechin	54.82	0.24
	MOL001736	(−)-taxifolin	60.50	0.27

DL = drug-like properties, GLXBGZD = Gualou Xiebai Guizhi decoction, OB = oral bioavailability.

2.2. Collection of CHD disease targets

The keyword "coronary heart disease" was used to search the database, and the selected databases were the Online Mendelian Inheritance in Man (OMIM) database and GeneCards database to obtain the relevant targets of CHD disease. The values of the 2 databases were merged, and the duplicate values were removed to obtain the disease targets of CHD.

2.3. Construction of intersection targets

The targets of GLXBGZD and the disease targets of CHD were imported into Venn (https://bioinfogp.cnb.csic.es/tools/venny/) online platform to construct drug-disease intersection targets,
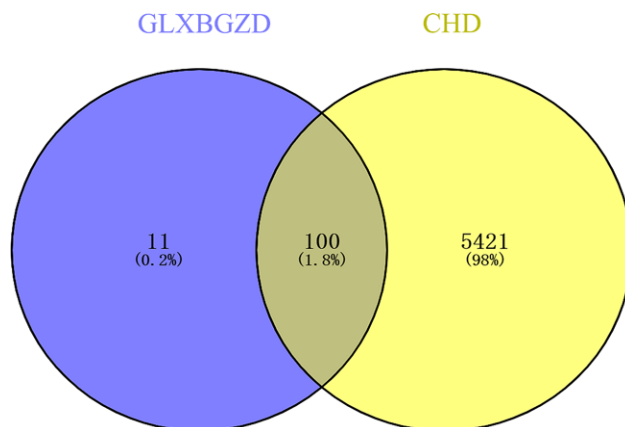

Figure 1. Venn diagram of GLXBGZD and CHD intersection target. BP = Biological process, CC = Cellular component, CHD = coronary heart disease, GLXBGZD = Gualou Xiebai Guizhi decoction, MF = Molecular function.

and the obtained intersection targets may be the potential action targets of GLXBGZD for the treatment of CHD.

2.4. Construction of drug-component-target-disease network diagram

The active ingredients and targets of GLXBGZD have been imported into Cytoscape 3.7.2 software, and the network diagram of "GLXBGZD-ingredient-target-CHD" was constructed based on Cytoscape software. The nodes in the diagram represent the active ingredients and targets; the edges represent the interactions between the drug and active ingredients, active ingredients and targets, and disease and target[15]; the Degree value of the nodes represents the number of edges connected to the nodes. A higher degree value means a more significant role in treating CHD with GLXBGZD.[16]

2.5. PPI network graph construction and core target screening

The drug-disease intersection targets obtained in 1.3 were imported into STING (HTTP://string-db.org) online database
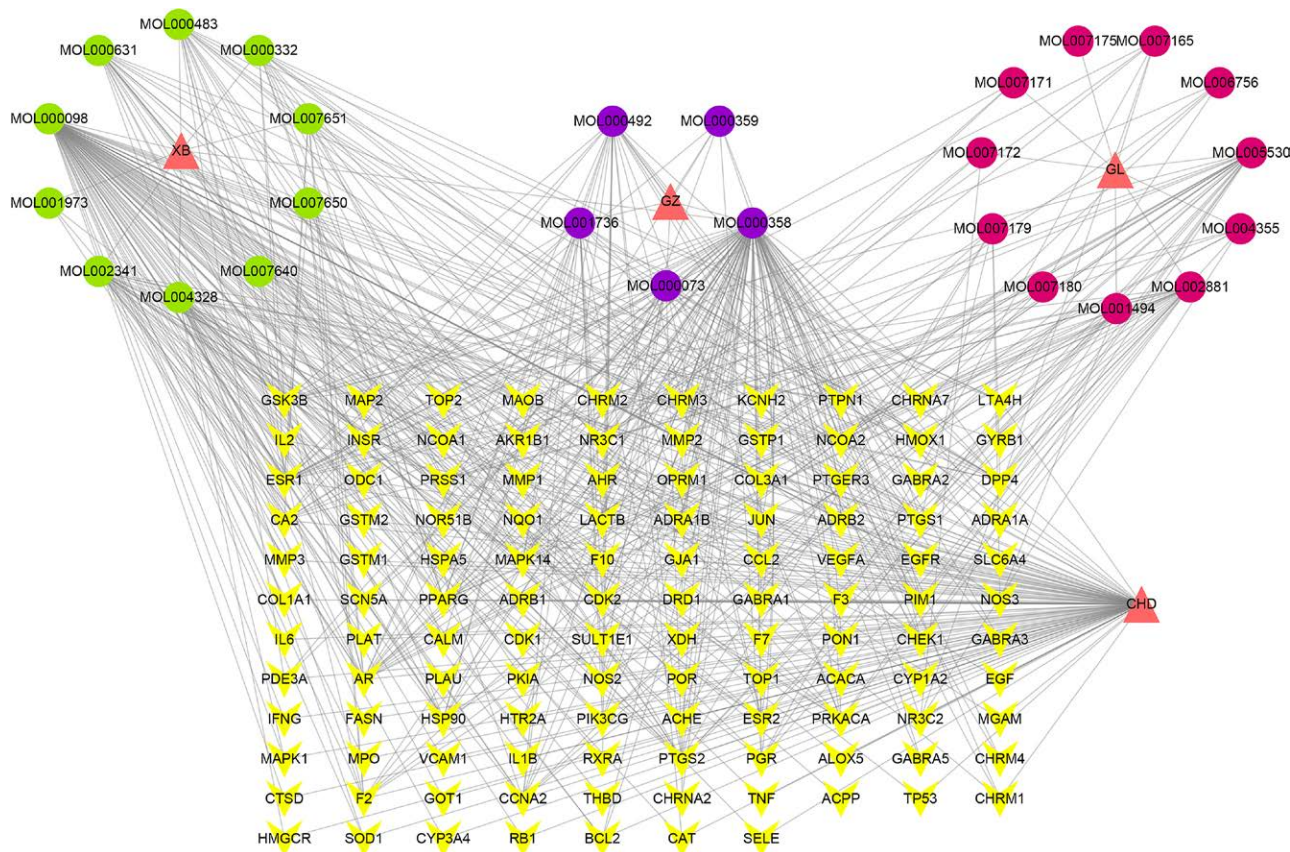

Figure 2. The Drug-component-target-disease network diagram. CHD = coronary heart disease, VEGFA = vascular endothelial growth factor A; GSK3B: Gycogen synthase kinase-3β; MAP2: Microtubule associated protein-2; TOP2: Topoisomerase (DNA) II 170kDa; MAOB: Monoamine oxidase-B; CHRM 1/2/3/4: Cholinergic receptor muscarinic 1/2/3/4; KCNH2: Potassium voltage-gated channel, subfamily H (eag-related), member 2 Human Potassium voltage-gated channel subfamily H member 2; PTPN1: Protein Tyrosine Phosphatase Non-receptor type 1; CHRNA 2/7: Alpha 2/7-nicotinic receptor gene; LTA4H: Leukotriene A4 Hydrolase; IL-1B/2/6: Interleukin 1B/2/6; INSR: Insulin receptor; NCOA1: Nuclear Receptor Coactivator 1; AKR1B1: Aldo-keto reductase family 1 member B1; NR3C1: Nuclear receptor subfamily 3, group C, member 1; MMP 1/2/3: Matrixmetalloproteinase 1/2/3; GSTP1: Glutathione S-transferase pi-1; NCOA2: Homo sapiens nuclear receptor coactivator 2; HMOX1: Heme oxygenase 1; GYRB1: Gyrase subunit B1; ESR1/2: Estrogen receptor 1/2; ODC1: Ornithine decarboxylase 1; PRSS1: serine proteinase 1; AHR: Aryl hydrocarbon receptor; OPRM1: Opioid receptor mu 1; COL3A1: Type III collagen protein-encoding gene; PTGER3: Prostaglandin E receptor 3; DPP4: Dipeptidyl peptidase-4; CA2: Carbohydrate antigen 2; GSTM1/2: Glutathione S-transferase M1/2; NQO1: NAD(P)H: quinoneoxidoreductase; LACTB: Human Lactamase Beta; ADRA1A/B: α-adrenergic receptor-1a/b; JUN: v-jun sarcoma virus 17 oncogene homolog; ADRB2: Beta 2-adrenergic receptor; PTGS1/2: Prostaglandin-endoperoxide synthase 1/2; HSPA5: Heat Shock Protein Family A (Hsp70) Member 5; MAPK1/14: Mitogen activated protein kinase 1/14; F7/10: Coagulation factor VII/X; GJA1: Gap Junction Alpha-1; CCL2: Chemokine ligand 2; VEGFA: Vascular endothelial growth factor A; EGFR: Epidermal growth factor receptor; EGF: Epidermal growth factor; SLC6A4: Solute carrier family 6, member 4; COL1A1: Collagen, type I, alpha 1; SCN5A: Sodium voltage-gated channel alpha subunit 5; PPARG: Peroxisome proliferator-activated receptor gamma; ADRB1: Adrenergic receptor beta-1; CDK2: Cyclin-dependent kinase 2; DRD1: Recombinant Dopamine Receptor D1; GABRA1/2/3/5: Gamma-aminobutyric acid type A receptor subunit 1/2/3/5; F2/3: Human tissue factor gene 2/3; PIM1: Proviral Insertion site in Murine leukemia virus kinase 1; NOS 2/3: Nitric oxide synthase 2/3; PLAT: Platlet factor; CALM: calmodulin; CDK1: Cyclin-dependent kinases; SULT1E1: Sulfotransferase family 1E; XDH: Xanthine dehydrogenase; PON1: Paraoxonase 1; CHEK1: Checkpoint kinase 1; PDE3A: Phosphodiesterase 3A; AR: Androgen receptor; PLAU: Plasminogen activator urokinase; PKIA: Protein kinase inhibitor alpha; POR: Cytochrome p450 oxidoreductase; TOP1: Topoisomerase (DNA) I; ACACA: Acetyl-Coenzyme A carboxylase alpha; CYP1A2: Cytochrome P450 1A2; IFNG: Active interferon gamma; FASN: Fatty acid synthase; HSP90: Heat shock protein 90kDa; HTR2A: 5-hydroxytryptamine (serotonin) receptor 2A; PIK3CG: Phosphoinositide-3- kinase, catalytic, gamma polypeptide; ACHE: Acetylcholinesterase; PRKACA: Protein kinase cAMP-activated catalytic subunit alpha; NR3C2: Nuclear receptor subfamily 3, group C, member 2; MGAM: Maltaseglucoamylase; MPO: Myeloperoxidase; VCAM1: Vascular cell adhesion molecule 1; RXRA: Retinoid X receptor alpha; PGR: Progesterone receptor; ALOX5: Arachidonate 5-lipoxygenase; CTSD: Cathepsin D; GOT1: Glutamic- oxaloacetic transaminase 1; CCNA2: Cyclin A2; THBD: Thrombomodulin; TNF: Tumor necrosis factor; ACPP: Acid phosphatase prostate; TP53: Tumor protein p53; HMGCR: 3-hydroxy-3-methylglutaryl-Coenzyme a reductase; SOD1: Superoxide dismutase 1; CYP3A4: Cytochrome P450 3A4; RB1: Retinoblastoma 1; BCL2: B-cell CLL/lymphoma 2; CAT: C-acetyltransferase; SELE: Selectin E.

platform, selected "Multiple-Proteins", limited the species to "human" (*Homo sapiens*), and constructed a PPI network between proteins. The obtained data were imported into Cytoscape 3.7.2 software in TSV format, and the mean value more significant than the degree was used as the screening criterion to obtain the core targets for CHD treatment with GLXBGZD.

2.6. Gene Ontology function and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis

The intersecting genes of drugs and diseases obtained in 1.3 were imported into the DAVID (https://david.ncifcrf.gov/) database, and the species was restricted to "human" (*Homo sapiens*). The genes were corrected to official genes, and the top 10 Gene Ontology (GO) biological functions were selected with *P* < .05 and False discovery rate (FDR) < 0.05 as screening criteria and visualized in a bar chart; the top 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were selected.

3. Results

3.1. Acquisition of active ingredients and targets of GLXBGZD

Using OB ≥ 30% and DL ≥ 0.18 as the screening criteria, a total of 11 active ingredients of GuaLou, 11 active ingredients of XieBai, and 5 active ingredients of Guizhi were retrieved from the TCMSP database. A total of 111 potential action targets were predicted after deleting duplicate values. The primary active ingredient information is shown in **Table 1**.

3.2. Venn diagram construction of intersecting targets

A total of 5521 CHD-related disease targets were retrieved from OMIM and GeneCards databases, and 100 mapping targets were obtained by intersecting CHD disease targets with the drug targets of GLXBGZD and making Venn diagrams for visualization and analysis (**Fig. 1**).
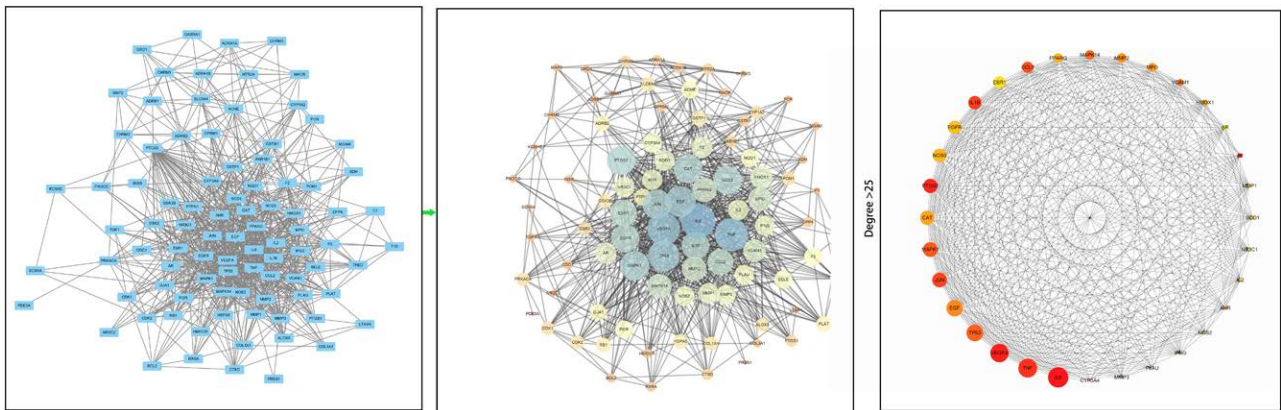

Figure 3. The Protein protein interaction (PPI) network diagram and core target network diagram.
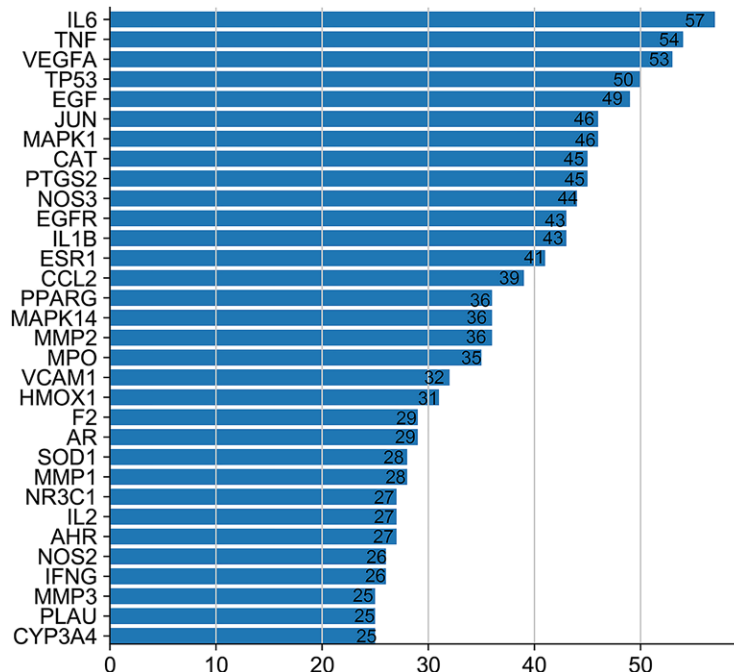

Figure 4. Degree values of the core target network graph for GLXBGZD. GLXBGZD = Gualou Xiebai Guizhi decoction, NOS = nitric oxide synthase, VEGFA = vascular endothelial growth factor. For abbreviations not defined here, please refer to Fig. 2.
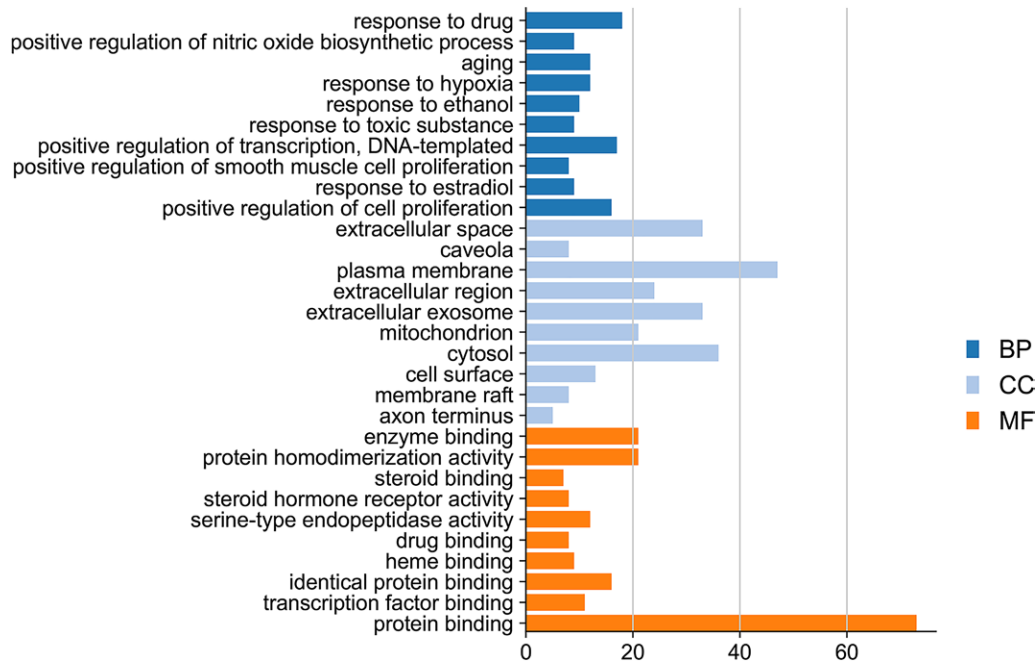
Figure 5. The results of GO functional enrichment analysis of GLXBGZD. BP = Biological process, CC = Cellular component, GLXBGZD = Gualou Xiebai Guizhi decoction, GO = Gene Ontology, MF = Molecular function. For abbreviations not defined here, please refer to Fig. 2.

3.3. Drug-component-target-disease network diagram

The above obtained active ingredients and targets of GLXBGZD and the intersection targets of drugs and diseases were imported into Cytoscape 3.7.2 software to construct the "drug-ingredient-target-disease" network diagram, which has 147 nodes (one disease, 26 active ingredients, 3 drugs, 117 targets) and 590 edges. This suggests that the above nodes may be the key active ingredients of GLXBGZD for CHD treatment (**Fig. 2**).

3.4. PPI network diagram and core target network diagram construction

The intersection targets obtained in 2.3 were imported into the STING database, the species was selected as human, and the PPI network graph between the proteins was constructed. The graph includes 100 nodes, 1002 edges, and the average node value is 20, and the detailed results are shown in **Figure 3**. The obtained data were imported into Cytoscape 3.7.2 software in Tab separated values File (TSV) format, and the core targets of drug treatment diseases were obtained with Degree > 25 as the screening criterion, and the histogram was made to display, see **Figure 4**.

3.5. GO functional enrichment analysis

The intersecting targets obtained in 2.3 were imported into the DAVID database. When $P < .05$ and FDR < 0.05 were used as screening criteria. The screening yielded 102 biological processes, 22 cell compositions, and 29 molecular functions. The top 10 items were selected separately and made into histograms for visual analysis, and the detailed results are shown in **Figure 5**.

3.6. Results of KEGG pathway enrichment analysis

Eighty-four KEGG pathways were screened by the same method as in 2.5, and the top pathways were mainly involved in the HIF-1 signaling pathway, TNF signaling pathway, pathways in cancer, PI3K-Akt signaling pathway, calcium signaling pathway, and estrogen signaling pathway (**Fig. 6** and **Table 2**). PI3K-Akt signaling pathway, calcium signaling pathway, estrogen signaling pathway, etc. The present results suggest that GLXBGZD may be involved in the therapeutic process of CHD through several of these pathways.

4. Discussion

Chinese medical theory classifies CHD under the categories of "thoracic obstruction" and "heartache," and believes that its primary pathogenic mechanism is related to irregular diet and emotional disorders.[17] According to TCM theory, the occurrence of CHD is primarily due to the rich diet of modern people coupled with high life stress leading to the patient's body with dampness endogenous and emotional and mental disorders, and Qi-blood deficiency in the patient's body.[4] In Chinese medicine, clinical treatment for CHD mainly uses GLXBGZD, which can move Qi and dispel phlegm, open the veins and relieve pain. Yang,[17] by observing 80 CHD patients with phlegm and blood stasis, found that the GLXBGZD could significantly improve the myocardial metabolic function of patients. In the treatment of 88 patients with acute myocardial infarction combined with left heart failure, Wang[18] found that the addition of GLXBGZD to conventional treatment could significantly improve the cardiac function of patients. All of the above studies suggest that GLXBGZD can be used to treat patients with CHD. However, the detailed mechanism of how the drug works is not well understood. Therefore, this study investigated the molecular mechanism of GLXBGZD in the treatment of CHD by using the network pharmacology method to provide methods and strategies for the clinical application of this drug in patients with CHD.

Our analysis by Cytoscape software yielded high values of active ingredients such as β-sitosterol, quercetin, naringenin, and hesperidin in GLXBGZD. Studies have shown that quercetin, as a flavonoid, has been widely reported in preventing and treating coronary atherosclerosis. Quercetin can limit the formation of atherosclerotic plaques in the aorta by upregulating the expression of nitric oxide synthase; quercetin can also
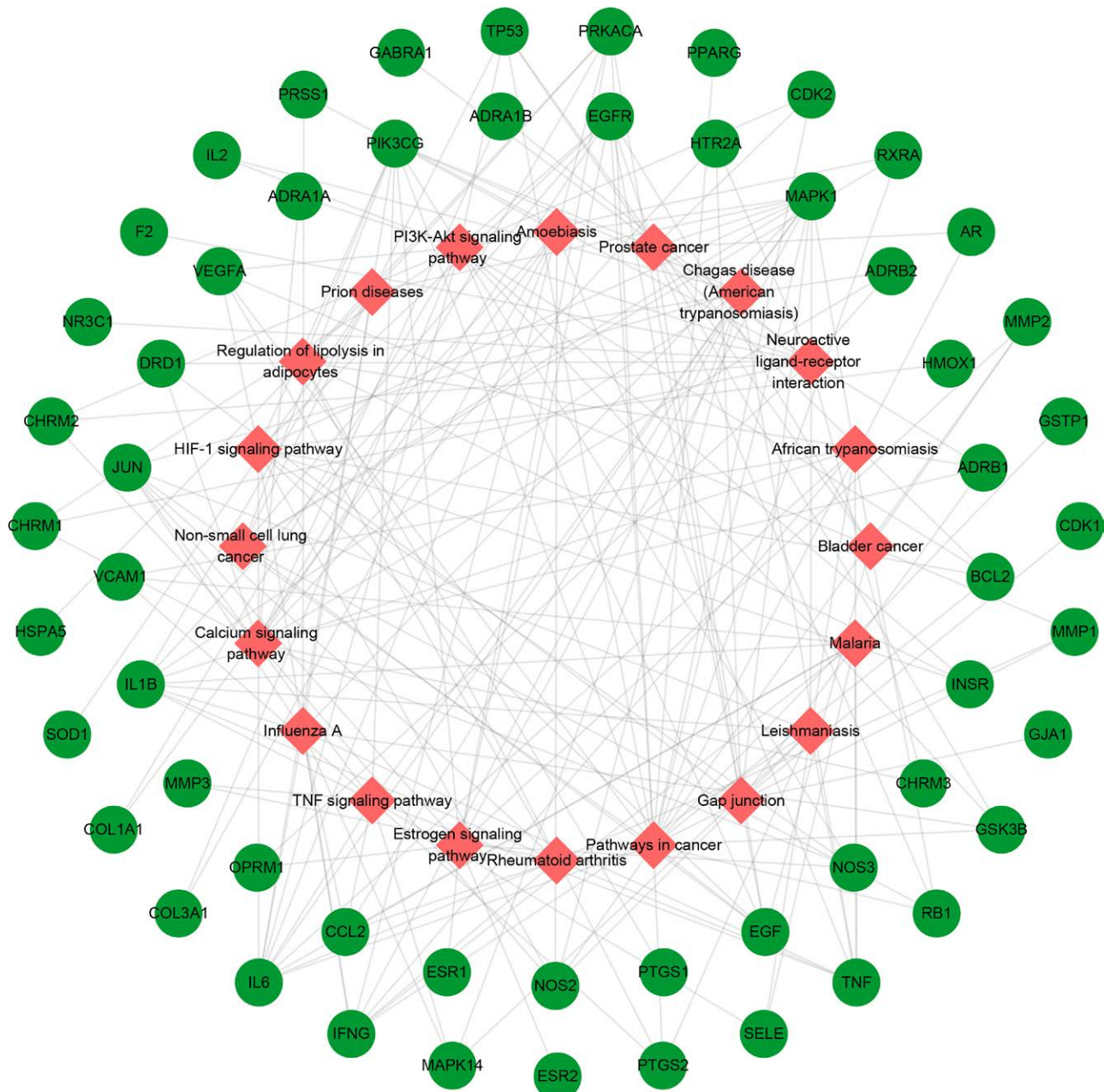
Figure 6. The key pathway-target map of GLXBGZD for the treatment of CHD. CHD = coronary heart disease, GLXBGZD = Gualou Xiebai Guizhi decoction.

stabilize atherosclerotic endothelial plaques by downregulating the expression of MMP1.[19] Therefore, understanding the relationship between quercetin metabolic activity and coronary atherosclerosis can provide new ideas for this drug in treating CHD. β-sitosterol has beneficial effects on the treatment of cardiovascular diseases.[20] Studies have shown that β-sitosterol can effectively enhance the cellular glutathione redox cycle, thereby reducing oxidative damage in rat cardiomyocytes and thus slowing the progression of coronary heart disease.[21] In addition, β-sitosterol could protect hypoxia/reoxygenation-injured cardiomyocytes and ischemia/reperfusion injury of cardiomyocytes by regulating the expression of PPARγ protein levels.[22] This study further indicates that GLXBGZD may be involved in the therapeutic process of patients with CHD through a complex mechanism of action.

The core target network map shows high IL-6, TNF, vascular endothelial growth factor (VEGFA), Tumor Protein 53 (TP53), Endothelial growth factor (EGF), v-jun sarcoma virus

17 oncogene homolog (JUN), and Mitogen-activated protein kinase (MAPK1), which may be the key targets of GLXBGZD in the treatment of CHD. There is increasing evidence that inflammation has an essential role in the development and progression of coronary atherosclerosis. Active inflammatory processes may trigger plaque rupture, thereby increasing the incidence of coronary atherosclerotic events.[23] Activating proinflammatory factors, such as IL-6 and TNF, can disrupt the endothelial function of blood vessels and accelerate the rupture of plaques, thus accelerating the onset of coronary atherosclerosis.[24] Therefore, inhibition of inflammatory factor activation can reduce the adverse incidence of cardiovascular events in CHD. VEGFA has been shown to promote microvessel formation and development by binding to vascular endothelial cell receptors to induce their differentiation, proliferation, and migration.[25] VEGFA promotes revascularization and the establishment of collateral circulation and enhances endothelium-dependent vasodilation, which is closely associated with CHD.[26] VEGFA may play a

Table 2

Results of enrichment analysis of KEGG pathway.

Number	Term	Count	Genes	P value
hsa04066	HIF-1 signaling pathway	12	IL-6, IFNG, NOS2, EGF, NOS3, INSR, BCL2, HMOX1, MAPK1, EGFR, PIK3CG, VEGFA	4.62E-08
hsa04668	TNF signaling pathway	12	IL-6, JUN, VCAM1, IL1B, MMP3, MAPK1, CCL2, MAPK14, PTGS2, SELE, TNF, PIK3CG	1.44E-07
hsa05200	Pathways in cancer	21	RB1, GSK3B, JUN, NOS2, MMP1, EGF, GSTP1, MMP2, PTGS2, EGFR, PIK3CG, VEGFA, AR, IL-6, RXRA, CDK2, BCL2, MAPK1, PPARG, PRKACA, TP53	1.56E-07
hsa05219	Bladder cancer	8	RB1, MMP1, EGF, MMP2, MAPK1, TP53, EGFR, VEGFA	9.43E-07
hsa05142	Chagas disease (American trypanosomiasis)	11	IL-6, JUN, IFNG, NOS2, IL1B, MAPK1, CCL2, MAPK14, TNF, IL2, PIK3CG	1.03E-06
hsa05215	Prostate cancer	10	RB1, GSK3B, AR, EGF, CDK2, BCL2, MAPK1, TP53, EGFR, PIK3CG	2.21E-06
hsa04151	PI3K-Akt signaling pathway	18	CHRM2, GSK3B, CHRM1, NOS3, EGF, INSR, EGFR, IL2, PIK3CG, VEGFA, COL1A1, COL3A1, IL-6, RXRA, CDK2, BCL2, MAPK1, TP53	2.43E-06
hsa04020	Calcium signaling pathway	13	CHRM2, CHRM3, CHRM1, NOS2, NOS3, ADRB1, ADRB2, HTR2A, ADRA1B, ADRA1A, EGFR, DRD1, PRKACA	3.94E-06
hsa04915	Estrogen signaling pathway	10	JUN, NOS3, MMP2, MAPK1, OPRM1, PRKACA, ESR1, ESR2, EGFR, PIK3CG	5.94E-06
hsa05323	Rheumatoid arthritis	9	IL-6, JUN, IFNG, MMP1, IL1B, MMP3, CCL2, TNF, VEGFA	2.01E-05
hsa04540	Gap junction	9	GJA1, EGF, CDK1, MAPK1, ADRB1, DRD1, HTR2A, PRKACA, EGFR	2.01E-05
hsa05140	Leishmaniasis	8	JUN, IFNG, NOS2, IL1B, MAPK1, MAPK14, PTGS2, TNF	4.03E-05
hsa05144	Malaria	7	IL-6, VCAM1, IFNG, IL1B, CCL2, SELE, TNF	4.27E-05
hsa05143	African trypanosomiasis	6	IL-6, VCAM1, IFNG, IL1B, SELE, TNF	6.77E-05
hsa04080	Neuroactive ligand-receptor interaction	14	CHRM2, CHRM3, GABRA1, PRSS1, CHRM1, ADRB1, ADRB2, HTR2A, OPRM1, F2, ADRA1B, NR3C1, ADRA1A, DRD1	7.08E-05
hsa05146	Amebiasis	9	COL1A1, COL3A1, IL-6, IFNG, NOS2, IL1B, PRKACA, TNF, PIK3CG	7.77E-05
hsa05020	Prion diseases	6	IL-6, HSPA5, IL1B, MAPK1, PRKACA, SOD1	7.85E-05
hsa04923	Regulation of lipolysis in adipocytes	7	INSR, ADRB1, ADRB2, PRKACA, PTGS2, PIK3CG, PTGS1	9.20E-05
hsa05223	Non–small cell lung cancer	7	RB1, RXRA, EGF, MAPK1, TP53, EGFR, PIK3CG	9.20E-05
hsa05164	Influenza A	11	GSK3B, IL-6, PRSS1, JUN, IFNG, IL1B, MAPK1, CCL2, MAPK14, TNF, PIK3CG	9.94E-05

KEGG = Kyoto Encyclopedia of Genes and Genomes, NOS = nitric oxide synthase, VEGFA = vascular endothelial growth factor A.

crucial role in regulating endocardial formation through epithelial-mesenchymal transition. Therefore, precise regulation of VEGFA expression may reduce the occurrence of cardiovascular diseases, including CHD.[27] This further confirms the characteristics of GLXBGZD to treat CHD through multiple components and targets.

The GO biological process of GLXBGZD in the treatment of CHD involves the reaction of the drug, positive regulation of nitric oxide biosynthesis process, response to hypoxia, extracellular space, plasma membrane, enzyme binding, protein homopolymerization activity, steroid binding, and other biological processes. This indicates that all of the above biological processes are essential in treating CHD by GLXBGZD. The enrichment analysis of the KEGG pathway revealed that GLXBGZD was mainly involved in the treatment of CHD through the HIF-1, TNF, cancer, PI3K-Akt, calcium, and estrogen signaling pathway, etc. HIF-1 can regulate the occurrence and development of atherosclerosis. Under hypoxic conditions, factors in the HIF-1 signaling pathway induce VEGF gene expression and can promote neovascularization.[28] Activation of the PI3K-Akt signaling pathway promotes atherosclerotic vascularization and the expression of inflammatory factors, and the PI3K/Akt signaling pathway enables Nuclear factor kappa-B (NF-κB) transcription factors to enhance the activity of inflammatory mediator genes, thus promoting the production of many cytokines.[29] Thus, inhibition of the PI3K/Akt signaling pathway allows inhibiting the progression of coronary atherosclerosis. Coronary artery calcification is closely related to the diagnosis and treatment of CHD. Factors related to the estrogen signaling pathway can regulate the expression of proteins related to bone-associated protein Bone morphogenetic protein 2 (BMP-2) and Phosphorylated drosophila mothers against decapentaplegic protein gene 1/5/8 (p-Smad1/5/8) signaling pathway to inhibit vascular calcification and thus the progression of CHD.[30] All of the above studies suggest that GLXBGZD can be involved in the treatment of CHD through multi-component, multi-target, and multi-pathway.

5. Conclusions

In summary, this study used network pharmacology to analyze that the treatment of CHD by GLXBGZD is associated with 27 active ingredients, which are involved in the therapeutic process of CHD through 100 intersecting targets. By constructing a "drug-component-target-disease" network diagram, this article also illustrates the treatment of CHD by GLXBGZD at the molecular level and indirectly proves the rationality of the drug formulation. This study provides a new basis for the promotion and use in the clinical treatment of CHD and provides a new method and strategy for the scientific research of Chinese medicine.[31] Due to the limited number of database articles, the data collection process has limitations. Therefore, the conclusions obtained in this article still need to be validated by subsequent clinical trials.

Author contributions

TC and LXZ conceived and designed this study; WY, PS, and WX performed the acquisition and analysis of data, and all authors reviewed the article.

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