ORIGINAL RESEARCH

Mechanism of Action of "Astragali Radix-Cassia Twig-Poria" in Chronic Heart Failure: A Network Pharmacology Approach

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ABSTRACT

This study utilizes network pharmacology analysis to investigate the components, targets, and pathways involved in the treatment of chronic heart failure (CHF) with the combination of "Astragali Radix-Cassia Twig-Poria." The TCMSP, GeneCards, OMIM, PharmGkb, TTD, and DrugBank databases were utilized to identify the active ingredients and targets of this combination for CHF. Protein interactions were derived from the STRING database, and Cytoscape was used to construct the "drug-component-target-disease" network and protein interactions network. The GO function and KEGG signaling pathway were enriched, and molecular docking was performed to verify the stability of the core components and their targets. The study identified 41 active ingredients, 101 targets (including 94 related to CHF), 9 core targets, and 26 core ingredients of "Astragali Radix-Cassia Twig-Poria." Additionally, 1444 GO entries and 140 KEGG pathways (including 36 related to CHF) were found. Molecular docking results confirmed the binding ability of the combination to core targets. Overall, this study provides valuable insights into the key components, targets, and pathways involved in the treatment of CHF with "Astragali Radix-Cassia Twig-Poria," contributing to further research on its pharmacological effects. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Chronic heart failure (CHF) is the end stage of various cardiovascular diseases due to the structural and functional alterations of the heart, resulting in reduced cardiac output that fails to meet the metabolic demands of the body, which consequently causes symptoms such as dyspnea, dizziness, weakness, oliguria, and fluid retention. It is estimated that CHF affects approximately 26 million people worldwide, making it a significant global health concern. In developed countries, CHF is a leading cause of hospitalization among individuals aged 65 and older. The prevalence of CHF increases with age, and it is estimated that over 10% of individuals over the age of 70 are affected by the condition. The current treatment of CHF is mainly based on neuroendocrine antagonists such as angiotensin-converting enzyme inhibitors (ACEI), β-blockers, and aldosterone receptor antagonists, also known as mineralocorticoid receptor antagonists (MRA). Despite the continuous improvement of guidelines and medications, morbidity, rehospitalization, and mortality rates in CHF patients remain high with poor overall prognosis. Traditional Chinese Medicine (TCM) has been shown to inhibit ventricular remodeling to improve symptoms through multiple pathways during the early intervention and prognosis of CHF,[1] which may reinforce its treatment efficiency.

The "Astragali Radix-Poria-Cassia Twig" combination is derived from several classical formulas for the treatment of heart failure diseases, such as Huangqi Guizhi Wuwu Decoction, Zhenwu Decoction, Wuling Powder, Linggui Shugan Decoction, and Shenqi Lixin Decoction. In our previous study, Shenqi Lixin Decoction was found to inhibit apoptosis of cardiomyocytes (CMs) by balancing mitochondrial division and fusion,[2] regulate energy metabolism of damaged cardiomyocytes,[3] and improve cardiac function in CHF

patients.[4] "Astragali Radix-Poria-Cassia Twig" is a common combination used by TCM practitioners in the clinical treatment of heart failure and is also the monarch and minister drugs of the above formula, which is effective in tonifying Qi, warming Yang, invigorating blood, and inducing diuresis. Astragali Radix is the dried root of Mongolian Astragali Radix or membranous pod Astragali Radix of the legume family, which promotes hematopoiesis, exerts positive muscle power, and treats CHF by protecting the kidneys and diuretic effects.[5] Cassia Twig is the dried shoots of Cinnamomum camphora, with the effect of enhancing coronary blood flow, as evidenced by modern pharmacology.[5] Poria is the dried mycorrhizal nucleus of Poria, a fungus of the family Polyporaceae, which is known to be a diuretic and an anti-swelling agent. Importantly, the pachymic acid in Poria displays anti-inflammatory, antioxidant, and anti-apoptotic effects.[5] Although clinical studies have shown that the combination of Astragali Radix-Poria-Cassia Twig improves cardiac function and alleviates clinical symptoms in patients with CHF, the absence of modern pharmacological studies on the mechanism of action of Astragali Radix-Poria-Cassia Twig has greatly hindered its clinical application and promotion.

Network pharmacology aims to analyze the molecular mechanism of action between drugs and diseases by analyzing the interrelationship between drug molecules, targets, and diseases, and using computer algorithms to simulate and predict the mechanism of drug action. TCM compound features multi-component, multi-target, and multi-pathway. Accordingly, network pharmacology contributes to providing new ideas for scientific research, new drug development, and rational clinical use of TCM compounds. Therefore, based on the network pharmacology, the present study constructed a regulatory network to investigate the main active ingredients of the core combination of "Astragali Radix-Cassia Twig-Poria" of Shenqi Lixin Decoction, their targets, and their mechanisms of action in mitigating myocardial cell damage, to provide a reference for the key targets and pathways of Shenqi Lixin Decoction in regulating ischemic myocardial revascularization.

MATERIALS AND METHODS

TCMSP (TCM Systematic Pharmacology Database, https://tcmsp-e.com), GeneCards (Human Gene Database, https://genecards.org), OMIM (online human Mendelian genetic database, https://omim.org), PharmGkb (pharmacogenomics database, https://pharmgkb.org), TTD (target database, http://db.idrblab.net/ttd/), DrugBank (Drug and Drug Target Database, http://drugbank.ca), STRING (Protein Interaction Relationships Database, https://string-db.org), UniProt (Protein Data Bank, https://uniprot.org), PDB (Protein Structure Database, https://rcsb.org), PubChem (Organic Small Molecule Bioactivity Database, https://pubchem.ncbi.nlm.nih.gov), GO (Gene Ontology Repository, http://geneontology.org), KEGG (Tokyo Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/), R software (v4.1.0), Cytoscape software (v3.8.2), and iGEMDOCK software (v2.1) were referred to and used in the study.

Table 1. Some Active Components of "Astragali Radix Cassia Twig - Poria" Combination

Mol ID	Chemical component	OB/%	DL	Herb
MOL000378	7-O-methylisomucronulatol	74.69	0.30	Astragali Radix
MOL000392	Formononetin	69.67	0.21	Astragali Radix
MOL000433	FA	68.96	0.71	Astragali Radix
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl) chroman-7-ol	67.67	0.26	Astragali Radix
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42	Astragali Radix
MOL000211	Mairin	55.38	0.78	Astragali Radix
MOL000371	3,9-di-O-methylnissolin	53.74	0.48	Astragali Radix
MOL000239	Jaranol	50.83	0.29	Astragali Radix
MOL000098	quercetin	46.43	0.28	Astragali Radix
MOL000422	kaempferol	41.88	0.24	Astragali Radix
MOL001736	(-)-taxifolin	60.51	0.27	Cassia Twig
MOL004576	taxifolin	57.84	0.27	Cassia Twig
MOL000492	(+)-catechin	54.83	0.24	Cassia Twig
MOL000073	ent-Epicatechin	48.96	0.24	Cassia Twig
MOL011169	Peroxyergosterol	44.39	0.82	Cassia Twig
MOL000300	dehydroeburicoic acid	44.17	0.83	Poria
MOL000285	(2R)-2-[(5R,10S,13R,14R,16R,17R)-16-hydroxy-3-keto-4,4,10,13,14-pentamethyl-1,2,5,6,12,15,16,17-octahydrocyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	38.26	0.82	Poria
MOL000280	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	31.07	0.82	Poria
MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93	0.81	Poria
MOL000283	Ergosterol peroxide	40.36	0.81	Poria
MOL000296	hederageni	36.91	0.75	Astragali Radix,Poria

Drug active ingredient and target acquisition

The active ingredients contained in Astragali Radix, Poria, and Cassia Twig and their targets were screened by the TCMSP database (oral bioavailability OB ≥ 30%; drug-like DL ≥ 0.18), and the obtained target information was transformed into gene identifiers in the UniProt database (set species as human) for subsequent use.

Acquisition of disease targets

Using "Heart failure" as the keyword, we searched GeneCards, OMIM, PharmGkb, TTD, and DrugBank databases to obtain all known relevant targets of CHF.

Acquisition of common targets for drugs and diseases

The common targets of drugs and diseases after merging and de-duplication were extracted using the Venn plugin in R language, and Venn diagrams were drawn.

Construction of "drug-component-target-disease" network model

The active ingredients obtained in "1.1", the drug-disease common targets obtained in "1.3" and the network information between them were imported into Cytoscape to construct the "drug-ingredient-target-disease" network. Each node is a component and a target, and each edge represents the interaction relationship between the nodes. The core components and core targets of the "Astragali Radix-Cassia Twig-Poria" combination were obtained by CytoNCA analysis and derivation of the results.

Construction of protein-protein interaction (PPI) network

The PPI information was obtained by uploading the intersection targets obtained in "1.3" to the STRING database,

Figure 1. The Number of Genes in CHF in Different Databases

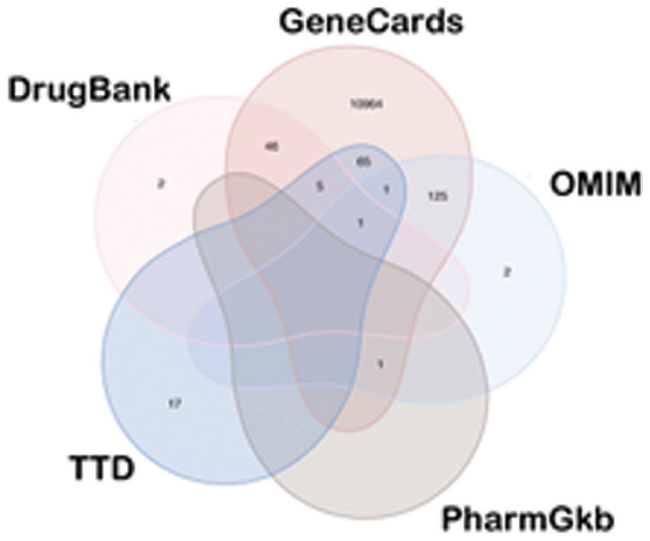
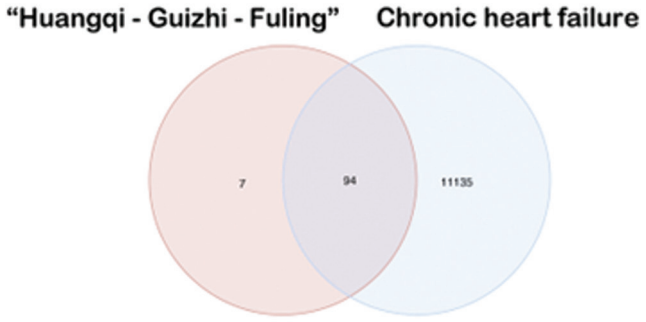


Figure 2. Drug-Disease Common Target



with the species limited to humans and the maximum confidence protein parameter score > 0.99. The PPI information was then imported into Cytoscape to draw the PPI network map, and finally, CytoNCA was used to analyze the degree, meso, and tightness of this network, to filter out the targets with each parameter greater than twice its median to get the core targets and draw the PPI core network.

Gene enrichment analysis

The human gene annotation package R-org.Hs.eg.db was used to annotate the "1.3" intersecting genes as entrezIDs in the NCBI database, with the species restricted to humans. Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using "R-clusterProfiler" at $P < .05$, $Q < 0.05$ for key target genes. The GO functions were selected for biological processes, cellular components, and molecular functions, bubble maps were created by R-ggplot2 for the first 10 entries in each GO category, and for the KEGG pathway, the first 30 entries were selected for bubble mapping.

Table 2. 26 Active Components and Degree of "Huangqi-Guizhi-Fuling" Combination

Active ingredients	Connectivity	Active ingredients	Connectivity
kaempferol	52	Bifendate	6
quercetin	51	ent-Epicatechin	6
7-O-methyliso-mucronulatol	37	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	4
formononetin	32	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	3
isorhamnetin	30	(-)-taxifolin	3
beta-sitosterol	23	FA	3
Calycosin	20	sitosterol	2
3,9-di-O-methylnissolin	19	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	1
(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	18	Ergosterol peroxide	1
hederagenin	14	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	1
Jaranol	12	isomucronulatol-7,2'-di-O-glucosiole	1
(+)-catechin	9	Mairin	1
taxifolin	7	ergosta-7,22E-dien-3beta-ol	1

Molecular docking

The core protein receptor and the molecular ligand with high connectivity in the "Astragali Radix-Cassia Twig-Poria" combination were downloaded from the PDB and PubChem databases, and the mol2 files of the protein receptor and molecular ligand were simulated by iGEMDOCK for docking. The docking scores were used to verify the stability of the binding.

RESULTS

Drug active ingredients and target acquisition

A total of 41 active ingredients of the combination "Astragali Radix-Cassia Twig-Poria" were screened in the TCMSP database by qualifying OB and DL, including 20 of Astragali Radix, 15 of Poria, 7 of Cassia Twig, and 7 of Hederageni. The active ingredients screened included kaempferol, quercetin, taxifolin, calycosin, etc. The 493 obtained targets were de-duplicated, and a total of 101 target information such as PGR, NOS2, and PTGS1 were obtained.

Acquisition of disease targets

A total of 11229 target genes were obtained after de-duplication and merging of the same database with "Heart failure" as the keyword, and 11208 target genes were obtained in GeneCards, 130 in OMIM, 1 in PharmGkb, 89 in TTD, and 54 in DrugBank. 54, as shown in Figure 1.

Acquisition of common drug-disease targets

The 101 drug targets obtained in "2.1" and the 11229 disease targets obtained in "2.2" were used to obtain "drug-disease" by R-Venn. The 94 common targets are shown in Figure 2.

Construction of "drug-component-target-disease" network model

The 41 active ingredients of "Astragali Radix-Cassia Twig-Poria" and 94 "drug-disease" common targets were

Figure 3. Network of "Drug-Component-Target-Disease" of "Huangqi-Guizhi-Fuling" Combination in the Treatment of CHF

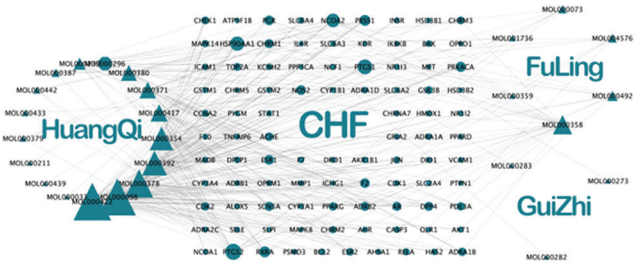


Figure 4. "Astragali Radix Cassia Twig - Poria" Combination of PPI Network (left) and Core Network (Right)

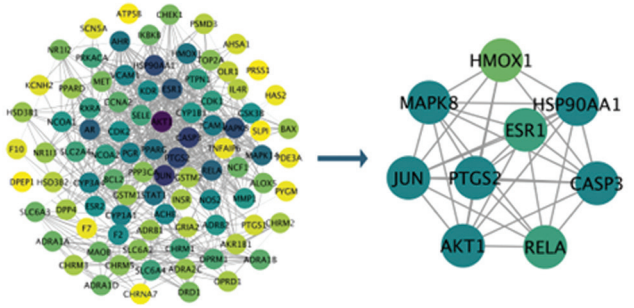
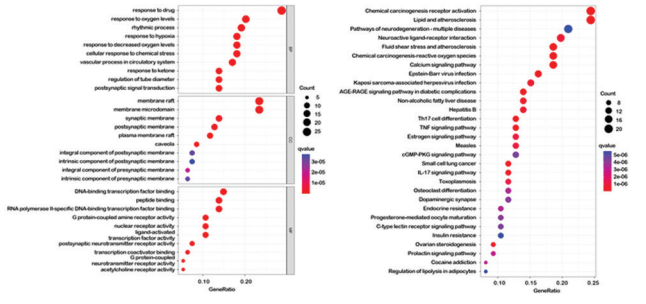


Figure 5. GO Functional Analysis (Left) and KEGG Enrichment Analysis (Right) of Potential Targets of "Astragali Radix Cassia Twig - Poria" Combination in the Treatment of CHF



imported into Cytoscape, and the "drug-ingredient-target-disease" interaction network was drawn (Figure 3), which included 120 nodes (26 active ingredients and 94 targets) and 357 edges. Of the 41 active ingredients, 15 active ingredients were deleted due to their lack of relevant targets, and the remaining 26 active ingredients and their degree of connectivity are shown in Table 2, among which kaempferol and quercetin have a greater degree of connectivity, representing their wide range of action.

Construction of protein interaction network (PPI)

The 94 common targets obtained in "2.3" were imported into STRING, with the species limited to humans and the medium confidence level set to >0.4. A total of 92 direct or indirect targets were obtained, with 619 interactions, as shown in Figure 4. Given the large size of the obtained target network, its connectivity was restricted to grasp its mechanism of action more precisely, and the core network was, therefore, screened out. The targets with large connectivity are closely related to each other, suggesting their potential role as key targets. Therefore, 94 nodes of this network were input to Cytoscape, and CytoNCA was used to analyze the degree, meso, and tightness of this network to find the genes with each parameter greater than twice its median to obtain the core network, as shown in Figure 4. The core network included 9 nodes (MAPK8, CASP3, JUN, HSP90AA1, PTGS2, AKT1, RELA, ESR1, and HMOX1) and 34 interactions, suggesting a key role of these targets in the overall network for drug efficacy.

GO enrichment analysis

The 94 common targets of entrezID obtained in "2.3" were enriched by "R-clusterProfiler" for GO functions, and a total of 1444 GO entries with $P < .05$ were obtained. The top 10 pathways in terms of biological processes, cellular components, and molecular functions were selected for bubble mapping, as shown in Figure 5 (left). The results suggest that the combination of Astragali Radix-Cassia Twig-Poria is mainly involved in the biological process of CHF treatment, such as response to drugs, response to oxygen level, rhythmical process, and response to hypoxia. Cellular composition mainly involves membrane rafts, membrane micro-regions, synaptic membranes, and postsynaptic membranes. Molecular functions are mainly involved in DNA-binding transcription factor binding, peptide binding, and RNA polymerase II-specific DNA-binding transcription factor binding.

KEGG enrichment analysis

A total of 140 KEGG entries with $P < .05$ were obtained by "R-clusterProfiler", and the top 30 pathways were selected for mapping and analysis, as shown in Figure 5 (right). The main types of signaling pathways involved were viral infection (EBV, Kaposi's sarcoma virus, herpes virus, hepatitis B virus, measles virus, etc.), signaling (calcium signaling, AGE-RAGE signaling in diabetic complications, TNF signaling, estrogen signaling, cGMP-PKG signaling, IL-17 signaling, etc.), and age-related changes (lipids and atherosclerosis, pathway-receptor interactions in neurodegenerative diseases, fluid shear stress and atherosclerosis, Alzheimer's disease, osteoclast differentiation, insulin resistance, etc.). A total of 36 pathways related to CHF were screened according to the cross-matching of core genes of "2.5" with genes of the KEGG pathway, which are shown in Table 3, mainly including 6 types of pathways of lipid metabolism, immune inflammation, apoptosis, other diseases, hormone regulation, and biological processes, suggesting that " Astragali Radix-Poria-Cassia Twig" intervenes in heart failure through these pathways.

Molecular docking

Using iGEMDOCK, kaempferol and quercetin, the active ingredients of "Astragali Radix-Cassia Twig-Poria" with Degree >50, were docked with the core targets MAPK8, CASP3, JUN, HSP90AA1, PTGS2, AKT1, RELA, ESR1, and

Table 3. Pathways Associated With "Astragali Radix Cassia Twig - Poria" Combination in the Treatment of CHF

Pathways No.	Pathways	Participating genes
hsa05417	Lipid and atherosclerosis	**HSP90AA1**/RXRA/PPARG/MAPK14/GSK3B/**RELA**/NCF1/OLR1/**JUN**/IKBKB/**AKT1**/BCL2/BAX/**CASP3**/**MAPK8**/MMP1/CYP1A1/ICAM1/SELE/VCAM1/PPP3CA
hsa05418	Fluid shear stress and atherosclerosis	**HSP90AA1**/MAPK14/**RELA**/NCF1/KDR/**JUN**/IKBKB/**AKT1**/BCL2/**MAPK8**/**HMOX1**/ICAM1/SELE/VCAM1/GSTM1/GSTM2
hsa04923	Regulation of lipolysis in adipocytes	PTGS1/**PTGS2**/PRKACA/ADRB1/ADRB2/**AKT1**/INSR
hsa04910	Insulin signaling pathway	PTPN1/GSK3B/PRKACA/PYGM/IKBKB/**AKT1**/**MAPK8**/SLC2A4/INSR
hsa04920	Adipocytokine signaling pathway	RXRA/**RELA**/IKBKB/**AKT1**/**MAPK8**/SLC2A4
hsa03320	PPAR signaling pathway	RXRA/PPARG/PPARD/OLR1/MMP1
hsa04620	Toll-like receptor signaling pathway	MAPK14/**RELA**/**JUN**/IKBKB/**AKT1**/**MAPK8**/STAT1
hsa04625	C-type lectin receptor signaling pathway	**PTGS2**/MAPK14/**RELA**/**JUN**/IKBKB/**AKT1**/**MAPK8**/STAT1/PPP3CA
hsa04668	TNF signaling pathway	**PTGS2**/MAPK14/**RELA**/**JUN**/IKBKB/**AKT1**/**CASP3**/**MAPK8**/ICAM1/SELE/VCAM1
hsa04659	Th17 cell differentiation	**HSP90AA1**/RXRA/MAPK14/**RELA**/**JUN**/IL4R/IKBKB/**MAPK8**/STAT1/AHR/PPP3CA
hsa04657	IL-17 signaling pathway	**PTGS2**/**HSP90AA1**/MAPK14/GSK3B/**RELA**/**JUN**/IKBKB/**CASP3**/**MAPK8**/MMP1
hsa04658	Th1 and Th2 cell differentiation	MAPK14/**RELA**/**JUN**/IL4R/IKBKB/**MAPK8**/STAT1/PPP3CA
hsa04064	NF-kappa B signaling pathway	**PTGS2**/**RELA**/IKBKB/BCL2/ICAM1/VCAM1
hsa04066	HIF-1 signaling pathway	NOS2/**RELA**/**AKT1**/BCL2/**HMOX1**/INSR
hsa04210	Apoptosis	**RELA**/**JUN**/IKBKB/**AKT1**/BCL2/BAX/**CASP3**/**MAPK8**
hsa04218	Cellular senescence	CDK2/CHEK1/MAPK14/CCNA2/**RELA**/**AKT1**/CDK1/PPP3CA
hsa04071	Sphingolipid signaling pathway	MAPK14/**RELA**/OPRD1/**AKT1**/BCL2/BAX/**MAPK8**
hsa04370	VEGF signaling pathway	**PTGS2**/MAPK14/KDR/**AKT1**/PPP3CA
hsa04930	Type II diabetes mellitus	IKBKB/**MAPK8**/SLC2A4/INSR
hsa05415	Diabetic cardiomyopathy	MAPK14/GSK3B/**RELA**/NCF1/ATP5F1B/**AKT1**/**MAPK8**/SLC2A4/INSR
hsa04931	Insulin resistance	PTPN1/GSK3B/PYGM/**RELA**/IKBKB/**AKT1**/**MAPK8**/SLC2A4/INSR
hsa04933	AGE-RAGE signaling pathway in diabetic complications	MAPK14/**RELA**/**JUN**/**AKT1**/BCL2/BAX/**CASP3**/**MAPK8**/STAT1/ICAM1/SELE/VCAM1
hsa05010	Alzheimer disease	NOS2/**PTGS2**/CHRM3/CHRM1/GSK3B/**RELA**/CHRM5/CHRNA7/ATP5F1B/IKBKB/**AKT1**/**CASP3**/**MAPK8**/PSMD3/INSR/PPP3CA
hsa05012	Parkinson disease	PRKACA/MAOB/DRD1/SLC6A3/ATP5F1B/BAX/**CASP3**/**MAPK8**/PSMD3
hsa04913	Ovarian steroidogenesis	**PTGS2**/PRKACA/HSD3B2/HSD3B1/CYP1A1/CYP1B1/ALOX5/INSR
hsa00140	Steroid hormone biosynthesis	HSD3B2/HSD3B1/CYP3A4/CYP1A1/CYP1B1
hsa04915	Estrogen signaling pathway	PGR/ESR2/**HSP90AA1**/NCOA2/**ESR1**/PRKACA/NCOA1/OPRM1/**JUN**/**AKT1**/BCL2
hsa04919	Thyroid hormone signaling pathway	NCOA2/RXRA/**ESR1**/GSK3B/PRKACA/NCOA1/**AKT1**/STAT1/DIO1
hsa04926	Relaxin signaling pathway	NOS2/MAPK14/PRKACA/**RELA**/**JUN**/**AKT1**/**MAPK8**/MMP1
hsa04924	Renin secretion	PDE3A/PRKACA/ADRB1/ADRB2/PPP3CA
hsa04935	Growth hormone synthesis, secretion, and action	MAPK14/GSK3B/PRKACA/**AKT1**/**MAPK8**/STAT1
hsa04151	PI3K-Akt signaling pathway	**HSP90AA1**/CDK2/CHRM1/CHRM2/RXRA/GSK3B/**RELA**/KDR/MET/IL4R/IKBKB/**AKT1**/BCL2/INSR
hsa04010	MAPK signaling pathway	MAPK14/PRKACA/**RELA**/KDR/MET/**JUN**/IKBKB/**AKT1**/**CASP3**/**MAPK8**/INSR/PPP3CA
hsa04020	Calcium signaling pathway	NOS2/CHRM3/CHRM1/CHRM2/ADRA1B/PRKACA/ADRB1/ADRB2/ADRA1D/DRD1/CHRM5/ADRA1A/CHRNA7/KDR/MET/PPP3CA
hsa04726	Serotonergic synapse	PTGS1/**PTGS2**/PRKACA/MAOB/SLC6A4/**CASP3**/ALOX5
hsa04152	AMPK signaling pathway	PPARG/CCNA2/ADRA1A/**AKT1**/SLC2A4/INSR

Note: The core targets of the "Astragali Radix-Cassia Twig-Poria" combination are in bold.

HMOX1 to verify their interaction activity, with smaller docking scores indicating higher binding energy and easier binding to produce effects. The results showed that the core components were able to form stable structures with the core proteins, and the docking scores are shown in Table 4, with quercetin showing the strongest binding to the ligand-protein RELA.

Table 4. Performance of Docking Between Core Target and Compound Molecule

Target name	PDBID	Compound codes	Compound names	Docking scores
MAPK8	4L7F	MOL000422	kaempferol	-91.79
CASP3	3KJF	MOL000422	kaempferol	-107.28
JUN	5T01	MOL000422	kaempferol	-77.87
HSP90AA1	4L8Z	MOL000422	kaempferol	-111.23
PTGS2	5IKR	MOL000422	kaempferol	-114.13
AKT1	5WBL	MOL000422	kaempferol	-109.40
RELA	4KV1	MOL000422	kaempferol	-118.28
ESR1	3OS8	MOL000422	kaempferol	-98.78
HMOX1	1N3U	MOL000422	kaempferol	-100.26
MAPK8	4L7F	MOL000098	quercetin	-96.28
CASP3	3KJF	MOL000098	quercetin	-105.84
JUN	5T01	MOL000098	quercetin	-74.06
HSP90AA1	4L8Z	MOL000098	quercetin	-112.54
PTGS2	5IKR	MOL000098	quercetin	-118.75
AKT1	5WBL	MOL000098	quercetin	-119.18
RELA	4KV1	MOL000098	quercetin	-123.84
ESR1	3OS8	MOL000098	quercetin	-98.79
HMOX1	1N3U	MOL000098	quercetin	-105.09

DISCUSSION

According to TCM, CHF is caused by the dysfunction of the internal organs due to the deficiency of the heart's Qi, blood, Yin, and Yang, with palpitations, shortness of breath, and edema of the limbs as its main manifestations. Cardiac enhancement, diuresis, and vasodilation are the main clinical treatments for heart failure, which are similar to the "Astragali Radix-Cassia Twig-Poria" combination studied in this paper. The joint use of "Astragali Radix-Cassia Twig-Poria" can be found in various TCM formulas, which primarily alleviate CHF symptoms by regulating heart rhythm and blood pressure, improving myocardial cell metabolism, and ameliorating renal function.[6-9] In recent years, there has been an increase in research on the combined treatment of heart failure with modern medicine and TCM, such as acupuncture and TCM rehabilitation training, which have shown promising performance in mitigating CHF symptoms.[9-14] Apoptosis of cardiomyocytes is the most crucial aspect of the pathogenesis of CHF, and the mechanism of action of TCM on cardiomyocytes has been insufficiently studied. To this end, the present study was conducted to analyze the mechanism of CHF cardiomyocyte improvement with the combination of Astragali Radix-Cassia Twig-Poria by establishing a "drug-component-target-disease" network through network pharmacology, and it was found herein that kaempferol and quercetin were the top constituents. It has been shown that kaempferol exerts anti-apoptotic effects by inhibiting the activation of Toll-like receptors, NF-κB, PI3K-Akt, and Wnt signaling pathways.[15-17] In addition, quercetin inhibits cardiomyocyte apoptosis by downregulating the levels of miR-199a and Bmi-1,[18-19] and protects cardiomyocytes by reducing propylene glycol, promoting superoxide dismutase (SOD) production, and inhibiting reactive oxygen species (ROS)-mediated MAPK pathway to inhibit cardiomyocyte fibrosis and oxidative stress.[20,21] Thus, the

"Astragali Radix-Cassia Twig-Poria" combination may ameliorate cardiomyocyte injury in CHF mainly by inhibiting cardiomyocyte apoptosis and protecting against peroxide-induced cardiomyocyte injury.

In this study, 92 targets of action for the treatment of CHF were predicted, which together participate in various biological processes, such as the MAPK8-mediated MAPK signaling pathway among the core targets that drive apoptosis in CHF through the endoplasmic reticulum stress response,[22] while JUN, one of the important inflammatory and apoptotic factors that belongs to the MAPK system along with MAPK8, regulates cell survival alone when the JUN subclass c-Jun is activated by phosphorylation.[23] Cysteine protease 3 (CASP3) is a key enzyme in the apoptotic protease cascade reaction that causes apoptosis in cardiomyocytes by altering the spatial structure of the protein.[24] Heat shock protein 90α (HSP90AA1) promotes angiogenesis by upregulating vascular endothelial growth factor (VEGF) expression. It was shown that the knockdown of HSP90AA1 significantly improved the degree of cardiac fibrosis in CHF mice.[25] Prostaglandin endoperoxide synthase 2 (PTGS2), a key enzyme in prostaglandin synthesis, inhibits the inflammatory response as well as apoptosis. Protein kinase (AKT1) regulates myocardial fibrosis and apoptosis by regulating the expression of transforming growth factor (TGF)-β1. The NF-κB signaling pathway is an important anti-aging and anti-inflammatory pathway, and reticuloendothelial proliferation virus oncogene homolog A (RELA), a subunit of the NF-κB transcription factor protein family, is closely associated with apoptosis of neutrophils.[26] Estrogen receptor 1 (ESR1) only exists co-regionally on myocardial intercalated discs in normal hearts, but cardiomyocytes with CHF were found to be devoid of this co-regionality and associated proteins, suggesting a key role of estrogen in stabilizing intercalated discs.[27] The experimental results showed that all clinical and biochemical indices of CHF deteriorated in female mice with ESR2-knocked out after myocardial infarction,[28] in addition to the role of ESR1 in mediating the prevention of left ventricular hypertrophy.[29] In hypoxia, the hypoxia-inducible factor signaling pathway (HIF-1) increases the expression of its downstream target genes such as VEGF and heme oxygenase (HMOX1) to achieve the protection of cardiomyocytes. The biological processes predicted in this study suggest that the mechanism of action of the "Astragali Radix-Cassia Twig-Poria" combination in the treatment of CHF is related to anti-inflammation, inhibition of apoptosis, inhibition of myocardial fibrosis, and left ventricular hypertrophy, stabilization of myocardial structure, and promotion of revascularization.

A total of 36 pathways related to CHF were screened in the KEGG pathway, including lipid metabolism (Lipid and atherosclerosis, Fluid shear stress and atherosclerosis, Regulation of lipolysis in adipocytes, Insulin signaling pathway, Adipocytokine signaling pathway, and PPAR signaling pathway) immunoinflammatory pathways (Toll-like receptor signaling pathway, C-type lectin receptor signaling pathway, TNF signaling pathway, Th17 cell differentiation, IL-17 signaling pathway, Th1 and Th2 cell differentiation, NF-kappa B signaling pathway, and HIF-1 signaling pathway), apoptotic pathways (Apoptosis, Cellular senescence, Sphingolipid signaling pathway, and VEGF signaling pathway), disease-related pathways (Type II diabetes mellitus, Diabetic cardiomyopathy, Insulin resistance, AGE-RAGE signaling pathway in diabetic complications, Alzheimer disease, and Parkinson disease), hormonal regulatory pathways (Ovarian steroidogenesis, Steroid hormone biosynthesis, Estrogen signaling pathway, Thyroid hormone signaling pathway, Relaxin signaling pathway, Renin secretion, Growth hormone synthesis, and Secretion and action), and biological process pathways (PI3K-Akt signaling pathway, MAPK signaling pathway, Calcium signaling pathway, Serotonergic synapse, and AMPK signaling pathway). It has been shown that treatment with anti-IL-17A monoclonal antibody after an episode of myocarditis in mice can eliminate myocardial fibrosis due to myocarditis, and thus protect their cardiac function.[29] TLR4 is a key receptor of the Toll-like receptor signaling pathway that promotes myocardial injury through inflammatory factor pathways such as TNF and NF-κB.[30] The effect of the relaxin signaling pathway is superior to that of classical vasodilators in improving the long-term prognosis of CHF patients in terms of vascular inflammation, oxidative stress, tissue fibrosis, and cell death.[31] The HIF-1 signaling pathway is the most important regulatory pathway for cells to maintain oxygen homeostasis, and functions in aggregation within hypoxic myocardium to enhance myocardial resistance to ischemia and hypoxia through pro-angiogenesis and regulation of energy metabolism.

Atherosclerosis (AS) is a chronic inflammatory disease whose intraplaque lipid enrichment is the underlying cause of most cardiovascular diseases and their complications, including CHF. Herein, six signaling pathways related to lipid metabolism and eight related to inflammatory immunity were identified, and the pathways interact with each other to promote the development of AS. The adipocyte factor signaling pathway exerts anorexigenic effects mainly through leptin-regulated neuropeptides and regulates adipocyte volume as well as number through the lipocalin-induced AMPK pathway stimulating skeletal muscle fatty acid oxidation and glucose uptake.[32] Adipocytes enter the regulatory pathway of adipocyte lipolysis by insulin activation of phosphodiesterase-3B (PDE-3B) to degrade cAMP to inhibit catecholamine-induced lipolysis,[33] whereas in the insulin signaling pathway, the serine kinase Akt is activated by insulin, which activates glycogen synthase (GYS) synthesis by inactivating glycogen synthase kinase 3 (GSK-3) on one hand, and on the other hand, the activation of Akt leads to the transfer of glucose transporter protein 4 (GLUT4) vesicles from the intracellular to the plasma membrane that allows for cellular uptake of glucose and reduces blood glucose concentration.[34-36] Obesity and insulin resistance are commonly seen in obese patients with clinical heart failure

combined with diabetes mellitus. TNF-α is a linking factor between the adipocytokine pathway and the insulin resistance pathway that contributes to cellular uptake of glucose and glycogen synthesis by promoting serine phosphorylation of insulin receptor substrate 1 (IRS1) in insulin resistance leading to the decreased displacement of GLUT4, which results in increased glucose uptake and glucose synthesis in skeletal muscle and increased hepatic gluconeogenesis and decreased hepatic glycogen synthesis.[37] Similar to insulin resistance, in the PPAR pathway, the peroxisome proliferator-activated receptor (PPAR) exerts its role in scavenging cellular lipids, promoting adipocyte differentiation, and enhancing glucose uptake by regulating the expression of genes related to lipid metabolism in the liver and skeletal muscle.[38] Elevated LDL levels are a major risk factor for AS. The accumulation of LDL in the vessel wall and its oxidative modification leads to endothelial dysfunction, inducing increased differentiation of monocytes in this region into macrophages that absorb lipoproteins and form cholesterol-filled "foam cells", and smooth muscle cells proliferate and migrate to this region after cell death,[33] resulting in a decrease in the internal diameter of the vessel and a change in the shear stress. It has been demonstrated that reduced shear stress leads to activation of the NF-kB signaling pathway and leukocyte recruitment simultaneously with endothelial cells non-aligned in the direction of blood flow.[39] Moreover, in addition to lipid metabolism, a growing body of research has found that inflammatory and immune responses are involved in all stages of plaque formation. LDL is oxidatively modified by endothelial cells, smooth muscle cells, macrophages, and oxygen free radicals to form oxidized LDL, OX-LDL, which activates Toll-like receptor 4 (TLR4) present on the surface of immune cells, cardiomyocytes, vascular endothelium, and other cells, causing NF-kB translocation to the nucleus while initiating tumor necrosis factor, Th17 cell differentiation and IL-17(OX-LDL can enhance the differentiation of CD4+ T cells into Th17 cells, which are a major source of IL-17. This effect is mediated by various signaling pathways, including the activation of nuclear factor kappa B (NF-κB) and the production of inflammatory cytokines, such as interleukin-6 (IL-6) and transforming growth factor-beta (TGF-β)), and other signaling pathways,[40] thereby accelerating leukocyte aggregation and plaque formation.[41-43] Therefore, regulation of lipid metabolism and control of inflammatory response may be one of the main mechanisms of the "Astragali Radix-Poria-Cassia Twig" combination in the treatment of heart failure, which is consistent with the clinical use of statins and aspirin to reduce the risk of cardiovascular disease.

In this study, four apoptosis-related pathways were identified, all of which were associated with oxidative stress and inflammatory responses. Ceramide (Cer) directly or indirectly activates CASP3 through the MAPK pathway and promotes apoptosis by upregulating the amount of TNFR1 in the tumor necrosis factor signaling pathway.[44] Sphingosine (Sph) is one of the components of cell membranes and promotes apoptosis by inhibiting protein kinase C (PKC) and cell cycle regulatory proteins (CDKs)-dependent protein kinases promoting the activity of Caspases, TNF-α, and other factors.[45] It was found that sphingosine-1-phosphate receptor 2 (S1P2) plays a key role in the development of AS. S1P2 receptor activation in monocytes/macrophages promotes the development of AS by regulating LDL, cytokine production, and cell migration, while endothelial S1P2 receptor activation leads to nitric oxide synthase (eNOS) activation and promotes the production of pro-inflammatory cytokines. Smooth muscle cell S1P2 receptor activation, in contrast, may destabilize the platelet by inhibiting cell proliferation and migration;[46] there is evidence that S1P is involved in the inflammatory and formation process of myocardial fibrosis through altered vascular permeability, leukocyte infiltration proliferation and migration, and fibroblast differentiation.[47] Furthermore, hypoxia-inducible factor 1α (HIF-1α) activates the MAPK pathway to initiate DNA synthesis and cell growth through VEGF dimerization, while (PI3K)-Akt pathway activation leads to increased endothelial cell survival.[46] Therefore, it is hypothesized that the core active ingredient of the "Astragali Radix-Poria-Cassia Twig" combination for the treatment of CHF may be used to counteract cardiomyocyte apoptosis and fibrosis by selectively antagonizing S1P receptors, inhibiting inflammatory factors such as TNF-α and CASP3, and promoting angiogenesis. In addition, this study identified six disease-related pathways that assist in improving cognitive dysfunction in diabetes. Some improve glucolipid metabolism by regulating blood glucose concentration, while others act directly on the Alzheimer's pathway or the 5-hydroxytryptaminergic synaptic pathway to inhibit the formation of intracerebral Aβ plaques to regulate intracerebral glucose metabolic pathways and exert anti-glucose cognitive impairment. Research has suggested that Aβ plaques in the brain upregulate the expression of JAK-STAT, NF-kB, PPAR, and MAPK, causing neuroinflammation,[48] which plays an important role in Alzheimer's disease and Parkinson's disease. In addition to cognitive impairment, the combination mitigates diabetes and its complications. Free radical generation and scavenging mechanisms are impaired in diabetic patients, and the accumulation of free radicals leads to vascular endothelial damage and increased vascular permeability, resulting in a cascade of cardiovascular diseases,[49,50] while kaempferol, which displays the strongest molecular docking capacity in the combination, exhibits excellent scavenging effects on free radicals and helps to maintain the activity of various peroxidases. In addition, animal tests have shown that kaempferol protects the heart against heart failure in rats by reducing hemodynamic, biochemical, and histological changes, aside from attenuating hyperglycemia and serum markers of myocardial injury. Kaempferol also inhibits oxidative stress, inflammatory response, and apoptosis in cardiomyocytes by reducing changes in signaling pathways such as Nrf-2, NF-kβ, PI3K-Akt, and MAPK.[17] Hence, it is inferred that the combination of "Astragali Radix-Poria-Cassia Twig" may improve diabetic cognitive impairment by regulating glucolipid metabolism,

inhibiting Aβ plaque formation in the brain, suppressing inflammatory factors, resisting oxidative stress damage in cardiac myocytes by reducing free radicals in the blood, and maintaining peroxidase activity.

Furthermore, seven pathways related to hormone regulation were identified in this study, three of which were related to steroid hormones. Estrogen is an important ovarian-secreted steroid hormone that mediates cardiovascular changes in both "genomic" and "non-genomic" ways through the classical estrogen receptor (ER)-α, ER-β, and G protein-coupled estrogen receptor (GPER). While the "genomic pathway" relies on genetic changes to act on vascular tissues,[51] "non-genomic pathway" acts by mediating an increase in nitric oxide synthase (eNOS)[52] and increasing the activity of antioxidant and catalase enzymes.[53] These two mechanisms induce an inhibitory effect on the vascular damage response and atherosclerosis prevention, which are important guidelines for the clinical management of cardiovascular diseases in women during menopause.

Thyroid hormones include levothyroxine (T4) and triiodothyronine (T3). It has been demonstrated that T3 enhances the activity of vascular endothelial macrophages, promotes macrophage foaminess and migration, and induces a more severe inflammatory response by accelerating the polarization of macrophages toward the M1 type that expresses various inflammatory factors and chemokines.[54] Relaxin has vasodilatory, antifibrotic, and angiogenic effects. Recent research has found that the treatment of heart failure patients with relaxin is effective in improving hemodynamics, lowering NT-proBNP levels, and improving renal function without causing hypotension.[55] An animal study showed that treatment with relaxin reversed insulin resistance in mice on a high-fat diet,[56] thereby indirectly delaying the course of heart failure. Growth hormone deficiency (GHD) and obesity both cause vascular endothelial dysfunction and are among the important risks for AS. In a clinical trial of children with GHD, improvements in vascular endothelial dysfunction were observed in both groups of patients after GH treatment.[57,58] Another study in patients with adult growth hormone deficiency (AGHD) showed a significant reduction in plasma apolipoprotein B (ApoB) and C-reactive protein (CRP) levels and an increase in endothelial nitric oxide synthase (ENOS) expression after GH treatment.[59] The pathway also includes the renin secretory pathway, which is a key regulator of the renin-angiotensin system (RAS). The RAS regulates vasoconstriction and maintains homeostasis of the internal environment. During heart failure, the neurological, humoral, and endocrine systems undergo an array of changes, which are involved in the formation of cardiac anterior and posterior loads and this leads to further deterioration of heart failure. So, the assumption of using RAS as a drug target to reduce the risk of cardiovascular disease is feasible. Thus, the predicted results of this study are reasonably accurate. Therefore, it is inferred that the combination of "Astragali Radix-Poria-Cassia Twig" may improve the hemodynamic index of heart failure patients by modulating eNOS and RAS at the hormonal level and resisting the deterioration of the vascular endothelial environment by increasing the activity of antioxidant enzymes, regulating lipid metabolism, and inhibiting inflammatory factors.

The findings of this study have significant clinical implications for the management and treatment of CHF. The identification of core targets and core ingredients associated with the "Astragali Radix-Cassia Twig-Poria" combination offers a targeted approach to CHF treatment. By focusing on these key components, future drug development efforts can be directed toward developing novel therapies that specifically modulate these targets, potentially leading to more effective and personalized treatment options for CHF patients. Moreover, the study supports the use of TCM, specifically the "Astragali Radix-Cassia Twig-Poria" combination, as a potential adjunct or alternative treatment option for CHF. TCM provides a complementary approach that can potentially address the multifactorial nature of CHF by targeting various pathways and biological processes implicated in the disease. These findings provide a scientific basis for integrating TCM into mainstream treatment protocols for CHF patients.

While our study provides valuable insights into the potential therapeutic effects of the "Astragali Radix-Cassia Twig-Poria" combination in CHF, it is important to acknowledge the limitations of the study and consider potential factors that could impact the interpretation of the results. 1) The accuracy and reliability of the study heavily rely on the quality and availability of the data used. The computational methods utilized in this study depend on existing databases, literature, and bioinformatics tools. Limitations in the completeness and accuracy of these resources may introduce biases or missing information, which could impact the identification of active ingredients, targets, and pathways associated with the combination. 2) While the computational predictions and network analyses provide a foundation for further investigation, *in vitro* and *in vivo* experiments are essential to validate the predicted mechanisms and confirm the therapeutic potential of the "Astragali Radix-Cassia Twig-Poria" combination. The absence of experimental data limits the robustness and clinical application of the findings. By conducting these proposed *in vitro* and *in vivo* experiments, the predicted mechanisms of action of the "Astragali Radix-Cassia Twig-Poria" combination can be further validated, providing more robust evidence for its therapeutic potential in CHF. These experimental validations will bridge the gap between the network pharmacology predictions and the actual effects observed in cellular and animal models, thus supporting the need for future clinical trials.

Our study makes several novel contributions to the field of cardiovascular medicine and network pharmacology, offering a unique and valuable approach. 1) Our study bridges the gap between traditional Chinese medicine and network pharmacology by investigating the "Astragali Radix-Cassia Twig-Poria" combination in the context of chronic

heart failure (CHF). Traditional Chinese medicine has a long history of use in the treatment of cardiovascular diseases, and our study utilizes network pharmacology to unravel the underlying mechanisms of this combination. This integration allows for a comprehensive understanding of the potential therapeutic effects and molecular targets associated with the combination. 2) Our findings have implications for personalized medicine and combination therapies in CHF. The identification of core ingredients, targets, and pathways associated with the combination offers opportunities for personalized treatment approaches. By considering individual patients' genetic and molecular profiles, it may be possible to tailor interventions and optimize treatment outcomes. Additionally, our study supports the potential benefits of combining traditional Chinese medicine with conventional treatments, providing a rationale for exploring synergistic effects and improving therapeutic strategies.

CONCLUSION

The present study analyzed the molecular biological mechanism of the "Astragali Radix-Cassia Twig-Poria" combination in the treatment of CHF through network pharmacology and concluded that the treatment of CHF with "Astragali Radix-Cassia Twig-Poria" is a multi-component, multi-target, and multi-pathway process. The results of the study initially validated and predicted the molecular mechanism of the "Astragali Radix-Poria-Cassia Twig" combination for the treatment of heart failure, which provides the pioneer information and basis for further investigation of its mechanism of action, and also provides a reference for the study of the mechanism of action of TCM compound with more complex composition.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR'S CONTRIBUTION

All authors completed the analysis and discussion, and all authors read and approved the final manuscript.

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CONSENT FOR PUBLICATION

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