Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
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RESEARCH ARTICLE

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Study on the molecular mechanism of Guizhi Jia Shaoyao decoction for the treatment of knee osteoarthritis by utilizing network pharmacology and molecular docking technology

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Received 30 July 2021; Accepted 23 August 2021
Available online 1 November 2021

KEYWORDS
Knee Osteoarthritis;
Guizhi Jia Shaoyao Decoction;
Network Pharmacology;
Molecular Docking;
Targets

Abstract
Background and objective: Guizhi Jia Shaoyao decoction (GSD) is widely used in the clinical treatment of knee osteoarthritis (KOA). However, the underlying molecular mechanisms remain unclear. The aim of this study was to explore functional mechanisms of GSD in treating KOA by utilizing network pharmacology-based approaches.
Methods: Candidate components and targets of GSD were retrieved from the Traditional Chinese Medicine Systems Pharmacology database. NCBI, Genecards, Drugbank, and Therapeutic Target Database (TTD) were used to establish a target database for KOA. Then, an interactive network diagram of "drugs-active components-targets" was plotted with Cytoscape open source bioinformatics software. A protein-protein interaction network was constructed and related protein interaction relationships were analyzed based on the STRING database. Gene ontology analysis and Kyoto Encyclopedia of Genes and Genomes pathway-enrichment analysis were conducted based on intersected targets. Molecular docking provided an assessment tool for verifying binding of components and targets. It was performed by AutoDock molecular modeling simulation software.
Results: In all, 103 active components were successfully identified, and corresponding 133 targets were searched for treating KOA. Functional enrichment analysis suggested that GSD exerts its pharmacological effect in treating KOA by regulating multiple pathways, such as PI3K-Akt, tumor necrosis factor, Toll-like receptor (TLR), and nuclear factor kappa B signaling pathways. Molecular docking analysis depicted that representative components bound firmly to key targets.

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https://doi.org/10.15586/aei.v49i6.484

Conclusion: This study revealed the synergistic effects of multiple components, targets, and pathways of GSD for treating KOA. This would enhance the understanding of potential molecular mechanisms of GSD for treating KOA and lay a foundation for further experimental research.

Introduction

Knee osteoarthritis (KOA) is a degenerative disease mainly characterized by articular cartilage degeneration and subchondral bone hyperplasia and degeneration. It is a common and frequently occurring disease in clinical orthopedics and traumatology. The main symptoms of KOA are pain, swelling, and stiffness, thereby seriously affecting the quality of life of middle-aged and elderly individuals.[1] According to the China Health and Elderly Care Follow-up Survey data, the prevalence of symptomatic KOA in China is about 8.1%, which is relatively high.[2] Inflammation is involved in the pathogenesis of KOA.[3] A variety of treatment strategies are available for KOA,[4] and early-stage therapies encompass a wide range of pain relief drugs. The current mainstream therapeutic drugs for KOA are analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), and glucosamine.[5] However, the latest studies have shown that long-term use of these drugs may trigger a series of side effects, such as gastrointestinal bleeding, impairment in renal functioning, etc.[6] Therefore, it is urgent to seek safe and effective complementary therapies. Traditional Chinese medicine (TCM), used empirically for thousands of years for treating KOA[7] has attracted widespread attention due to its satisfactory efficacy and minimum side effects.[8]

Guizhi Jia Shaoyao decoction is a TCM compound that originated from Treatise on Cold Damage and Miscellaneous Diseases (200–210 AD). It consists of the following five herbs: *Cinnamomi ramulus* (Guizhi), *Paeoniae radix alba* (Bai Shao), licorice (Gancao), *Jujubae fructus* (Dazao), and *Zingiber officinale* roscoe (Shengjiang). We have found that GSD can significantly relieve the symptoms of KOA, such as pain, swelling, stiffness in clinical application, and hence is widely applied clinically for treating tendons and joints pain.[9] However, the molecular mechanisms of GSD in treating KOA has remained obscure, thereby limiting its clinical application. It is therefore necessary to explore the underlying mechanism of GSD for treating KOA in order to provide scientific guidance and a theoretical basis for the clinical application of GSD.

With rapid development of bioinformatics, systems biology, and poly-pharmacology, network-based drug discovery has been considered to be a promising approach to develop effective drugs. In 2007, Hopkins first proposed the concept of "network pharmacology."[10] It is an approach that analyzes the intervention of drugs and potential therapeutic targets of illness. The network pharmacology highlights a model shift from the existing "one target, one drug" strategy to a novel "network target, multi-component" strategy. The systematism of the strategy resonates well with the holistic view of TCM as well as the mechanism of multi-component, multi-pathway, and multi-target synergy of Chinese herbal formulations.[11,12] Therefore, the present study aimed to reveal the active components of GSD to treat KOA, and predict the potential mechanism of GSD by using the network pharmacology method. The flow diagram of this study is depicted in Figure 1.

Methods

Active components and targets of GSD

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) is a unique systematic pharmacology platform designed for studying the mechanism of Chinese herbal medicines (CHMs).[13] The components of GSD were searched in TCMSP, including Cinnamomi ramulus, Paeoniae radix alba, licorice, Jujubae fructus, and Zingiber officinale roscoe. We screened active components under the standards of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 and obtained the related targets of each component.[14]

Target genes of KOA

Genes associated with KOA were obtained from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/),[15] Genecards (https://www.genecards.org/),[16] Drugbank (https://www.drubank.com/),[17] and Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/)[18] by searching the terms "osteoarthritis of knee" and "homo sapiens." A total of 158 genes were obtained in NCBI, 2040 in Genecards (score ≥ 1), 62 in Drugbank, and 1 gene in TTD. Finally, 2075 genes were obtained after excluding duplicates.

Potential targets of GSD in the treatment of KOA

The targets of GSD and those associated with KOA were intersected with the Venn map (http://bioinfogp.cnb.csic.es/tools/venny/). The common targets were the target genes of GSD for treating KOA.

Construction of "drugs-active components-targets" network

We imported chemical components and targets relationships into the Cytoscape 3.8.1 software, and the regulatory network diagram of "drugs-active components-targets" was constructed.

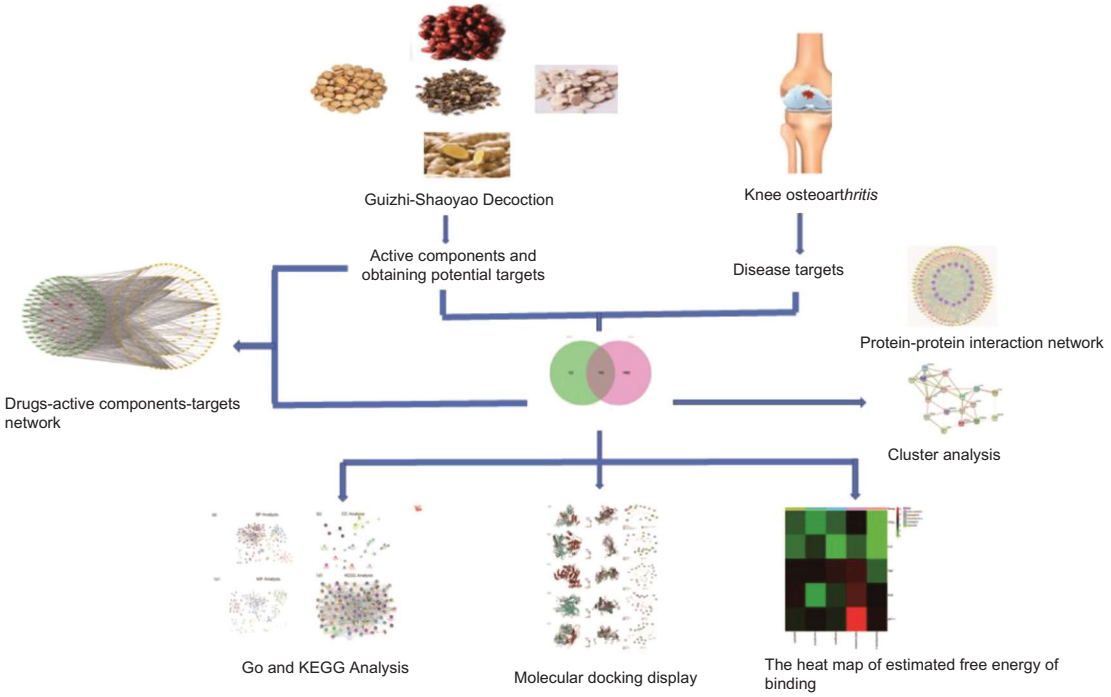


Figure 1 Whole framework based on network pharmacology and molecular docking.

Protein-protein interaction (PPI) data

We imported intersected genes into the STRING database (http://string-db.org/cgi/input.pl),[19] and defined the species as "Homo sapiens," to construct a PPI network. The data were saved in a tab-separated value (TSV) format. Using Cytoscape 3.8.1, the interaction network was drawn and analyzed.

Cluster analysis

Molecular Complex Detection (MCODE) is an application program (app) for clustering in Cytoscape. Cluster analysis of PPI network was performed using MCODE.[20]

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genome (KEGG) pathway-enrichment analysis

The Clue GO in Cytoscape was applied to carry out GO and KEGG pathway-enrichment analysis of common targets. The species was limited to "Homo sapiens." Items with $P < 0.05$ were selected for further study.[21]

Molecular docking

Molecular docking was executed to further investigate the possible interaction between hub target proteins and main active components. The crystal structures of proteins were downloaded from the RCSB Protein Data Bank (http://www.pdb.org/). Using Pymol and AutoDock 1.5.6 software, conformations of proteins were modified, including ligand and water removal, hydrogen addition, amino acid optimization, and computation of charge. The structure files of important components were downloaded from TCMSP, and the components' energy was minimized by using Chem3D software. The native formats of proteins and components were converted into PDBQT formats. Molecular docking was performed by running autogrid 4 and autodock 4 software, and the docking results were visualized with the help of Discovery Studio 2019 software.

Results

Main active components of GSD

A total of 983 active components of GSD were obtained from the TCMSP database, including 146 components with OB ≥ 30% and DL ≥ 0.18. There were 7 components of Cinnamomi ramulus, 13 of Paeoniae radix alba, 5 of Zingiber officinale roscoe, 29 of Jujubae fructus, and 92 of Licorice. Finally, 134 active components remained after removing duplicate ones. Based on the screening results, the active components with common source and the top 5 active components with OB values of each drug are listed in Table 1.

Targets of GSD in the treatment of KOA

In all, 255 targets of GSD were obtained based on the TCMSP database, and 2075 targets of KOA were obtained from NCBI, Genecards, Drugbank, and TTD. Finally, 133 targets of GSD in treating KOA were obtained as shown in Figure 2.

Table 1 Basic information of GSD components.

ID	Component	OB (%)	DL	Source
MOL000492	(+)-Catechin	54.83	0.75	Cinnamomi ramulus, Paeoniae radix alba, Jujubae fructus
MOL000358	ß-sitosterol	36.91	0.27	Cinnamomi ramulus, Paeoniae radixalba, licorice, Zingiber officinale roscoe
MOL000359	Sitosterol	36.91	0.82	Cinnamomi ramulus, Paeoniae radix alba, licorice
MOL000211	Mairin	55.38	0.78	Paeoniae radix alba, licorice, Jujubae fructus
MOL000422	Kaempferol	41.88	0.24	Paeoniae radix alba, licorice
MOL000098	Quercetin	46.43	0.28	Licorice, Jujubae fructus
MOL000449	Stigmasterol	43.83	0.76	Zingiber officinale roscoe, Jujubae fructus
MOL012940	Spiradine A	113.52	0.61	Jujubae fructus
MOL012992	Mauritine D	89.13	0.45	Jujubae fructus
MOL008647	Moupinamide	86.71	0.26	Jujubae fructus
MOL008034	21302-79-4	73.52	0.77	Jujubae fructus
MOL003410	Ziziphin_qt	66.95	0.62	Jujubae fructus
MOL001736	(-)-Taxifolin	60.51	0.27	Cinnamomi ramulus
MOL004576	Taxifolin	57.84	0.75	Cinnamomi ramulus
MOL000073	ent-Epicatechin	48.96	0.24	Cinnamomi ramulus
MOL011169	Peroxyergosterol	44.39	0.24	Cinnamomi ramulus
MOL001918	Paeoniflorgenone	87.59	0.37	Paeoniae radix alba
MOL001925	Paeoniflorin_qt	68.18	0.4	Paeoniae radix alba
MOL001928	Albiflorin_qt	66.64	0.33	Paeoniae radix alba
MOL001910	11α,12α-epoxy-3β-23-dihydroxy-30-norolean-20-en-28,12β-olide	64.77	0.38	Paeoniae radix alba
MOL001924	Paeoniflorin	53.87	0.79	Paeoniae radix alba
MOL002311	Glycyrol	90.78	0.67	Licorice
MOL004990	7,2′,4′-trihydroxy-5-methoxy-3-arylcoumarin	83.71	0.27	Licorice
MOL004904	Licopyranocoumarin	80.36	0.65	Licorice
MOL004891	Shinpterocarpin	80.3	0.73	Licorice
MOL005017	Phaseol	78.77	0.58	Licorice
MOL006129	6-Methylgingediacetate2	48.73	0.32	Zingiber officinale roscoe
MOL008698	Dihydrocapsaicin	47.07	0.19	Zingiber officinale roscoe
MOL001771	Poriferast-5-en-3β-ol	36.91	0.75	Zingiber officinale roscoe

DL: Drug-likeness

Analysis of drugs-active components-targets network

Cytoscape software was used to construct a "drugs-active components-targets" network. In this study, a total of 146 components of GSD were obtained from TCMSP; 134 active components of GSD were screened, of which 31 components had no targets. Finally, 103 components were collected from the TCMSP database—Red nodes represented drugs, green nodes represented active components, and potential targets were represented by yellow nodes. The edges represented correlation between active components and targets, which fully reflected the multi-component and multi-target characteristics of GSD (Figure 3).

Analysis of PPI network

Using the STRING database, an initial PPI network was established based on 133 candidate genes. There were 133 nodes and 3029 edges in Figure 4, of which the average node degree was 45.5 and the local clustering coefficient was 0.679. The size of node represented the degree value of targets: larger the node, greater the degree value. The thickness of edge indicated the combination score. The coarser the edge, the greater the combination score value. The nodes represented the proteins and the edges represented the interaction relationships of proteins. Proteins with a large degree played a crucial role in the whole network, and the degree-wise top five proteins were

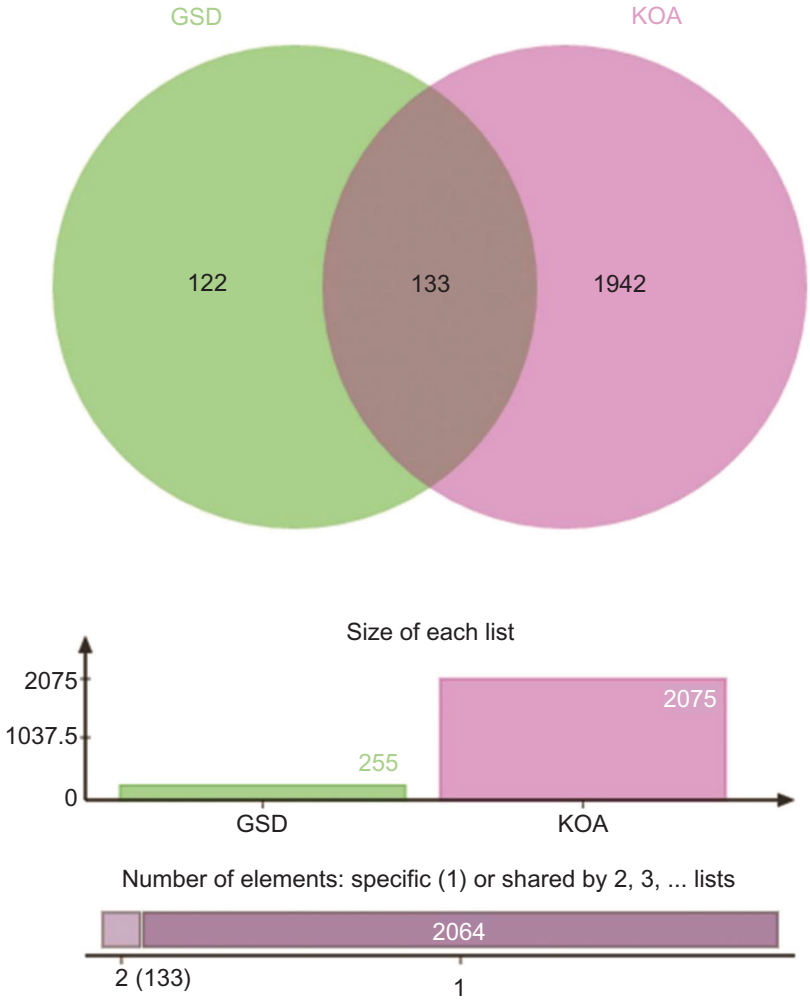


Figure 2 Venn diagram of GSD- and KOA-related targets; 133 common targets were obtained.

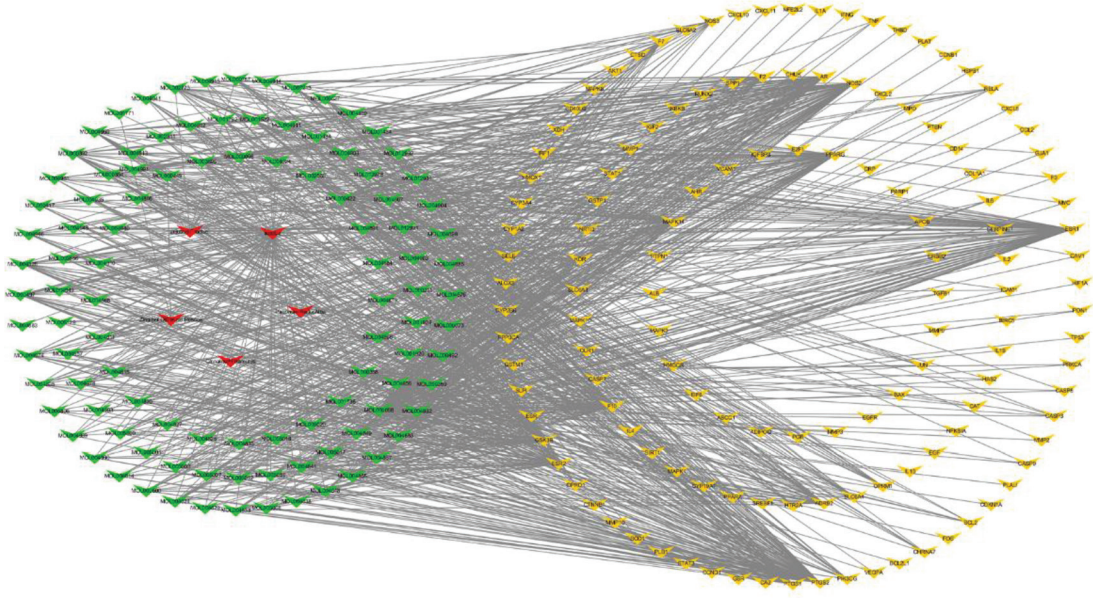


Figure 3 Drugs-active components-targets network. Note. Red nodes represent drugs, green nodes represent active components, and yellow nodes represent targets.

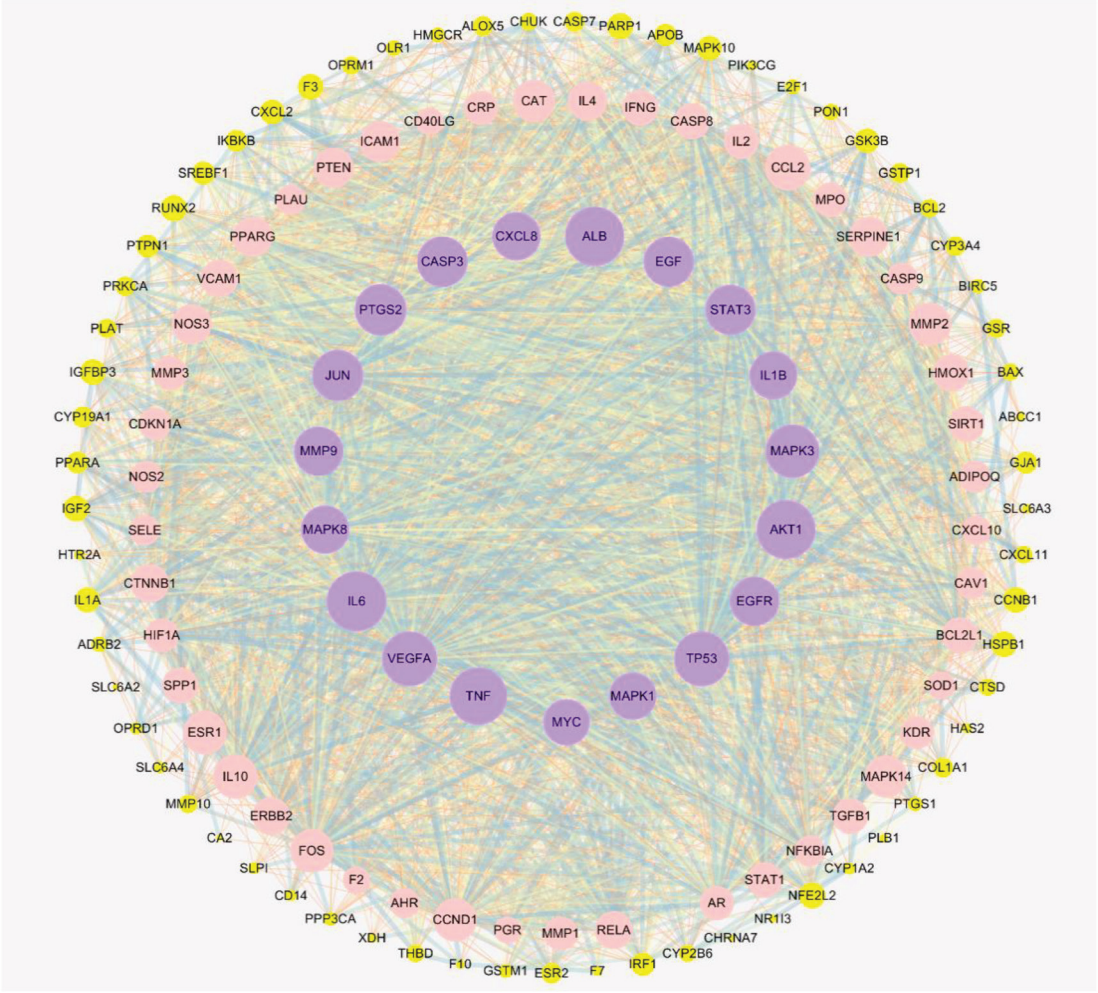


Figure 4 Protein–protein interaction network. Note. Interaction network of proteins related to treating KOA by GSD. The nodes represent proteins and the edges represent interaction relationships of proteins. Purple nodes represent proteins with high degrees, and the node size is proportional to the degree of proteins in the network.

as follows: Interleukin-6 (IL-6), albumin (ALB), AKT serine/threonine kinase 1 (AKT-1), tumor necrosis factor (TNF), and tumor protein P53 (TP53) (Table 2).

Table 2 Basic information of key targets.

GC ID	Symbol	Degree	Description
GC07P022765	IL-6	108	Interleukin 6
GC04P073397	ALB	107	Albumin
GC14M104769	AKT1	106	AKT Serine/threonine Kinase 1
GC06P047305	TNF	103	Tumor necrosis factor
GC17M007661	TP53	98	Tumor protein P53

Cluster analysis

The MCODE network analysis revealed four clusters. The scores were 48.9, 4.6, 3.0, and 2.5 respectively. The highest scoring cluster, cluster 1, contained 59 nodes (*PLAU, PPARG, MYC, PTEN. RELA, EGF, ICAM1, STAT3, CD40LG, IL1B, MAPK3, CRP, MMP1, CAT, IL4, IFNG, CASP8, IL2, CCL2, MPO, SERPINE1, CASP9, CCND1, MMP2, HMOX1, SIRT1, ADIPOQ, FOS, AKT1, BCL2L1, EGFR, TP53, ERBB2, TNF, IL10-KDR, VEGFA, CCNB1, ESR1, IL-6, MAPK14, MAPK8, SPP1, MMP9, TGFB1, JUN, HIF1A, PTGS2, MAPK1, CTNNB1, SELE, STAT1, CASP3, CXCL8, MMP3, AR, NOS3, ALB*, and *VCAM1*) and 1419 edges. Cluster 2 contained 17 nodes (*RUNX2, NFKBIA, AHR, IL1A, IRF1, CXCL2, NFE2L2, CXCL10, HSPB1, NOS2, CAV1, PARP1, CDKN1A, MAPK10, PGR, GJA1*, and *SOD1*) and 37 edges. Cluster 3 contained three nodes (*BCL2, IKBKB*, and *CHUK*) and three edges. The details of other clusters are provided in Figure 5 and Table 3.

GO function and KEGG pathway-enrichment analysis

A total of 10952 GO terms were obtained from GO-enrichment analysis, including 10737 biological process (BP) terms (Figure 6a), 31 cellular component (CC) terms (Figure 6b), and 184 molecular function (MF) terms (Figure 6c). The results showed that the biological process of GSD for the treatment of KOA mainly involved response

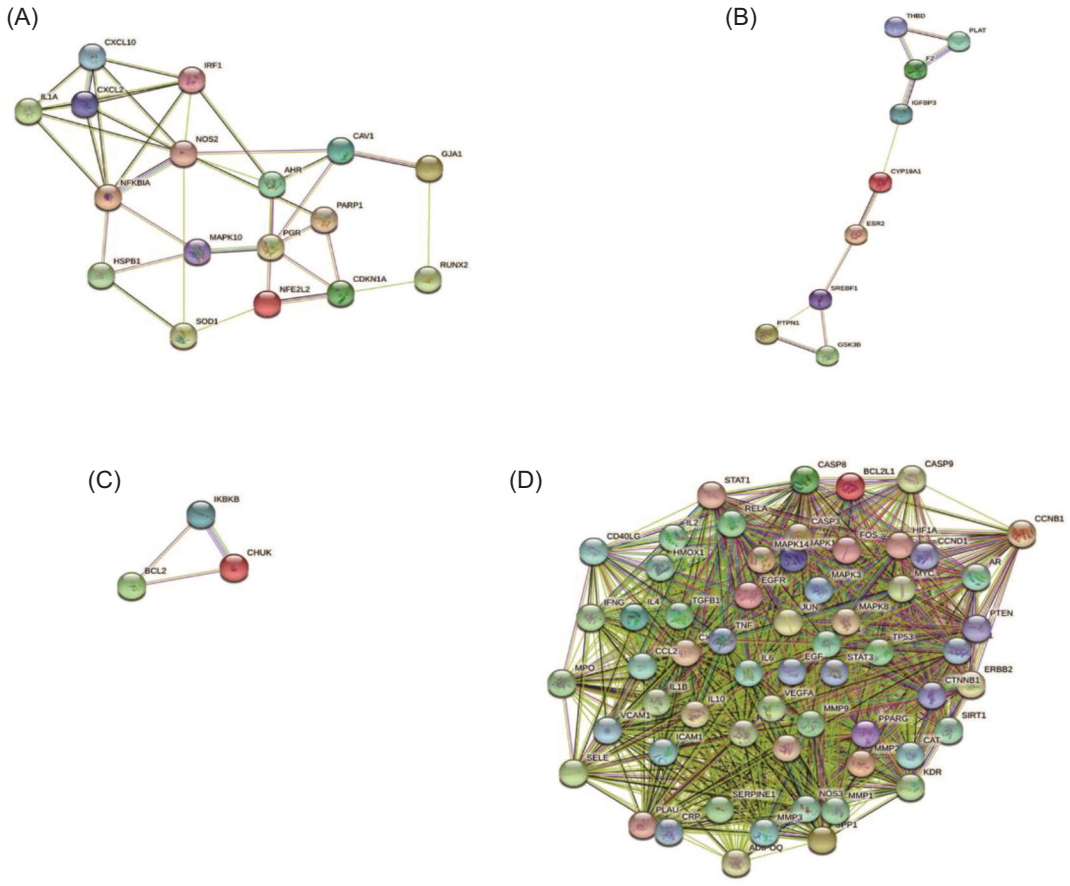


Figure 5 Classification of PPI network group in treating KOA with GSD.

Table 3 Clusters of PPI network.

Cluster	Score	Node	Edge	Genes
1	48.9	59	1419	*PLAU, PPARG, MYC, PTEN. RELA, EGF, ICAM1,STAT3, CD40LG, IL1B, MAPK3, CRP, MMP1, CAT, IL4, IFNG, CASP8, IL2, CCL2, MPO, SERPINE1, CASP9, CCND1, MMP2, HMOX1, SIRT1, ADIPOQ, FOS, AKT1, BCL2L1, EGFR, TP53, ERBB2, TNF, IL10, KDR, VEGFA, CCNB1, ESR1, IL-6, MAPK14, MAPK8, SPP1, MMP9, TGFB1, JUN, HIF1A, PTGS2,MAPK1,CTNNB1, SELE, STAT1, CASP3, CXCL8, MMP3, AR, NOS3, ALB, VCAM1*
2	4.6	17	37	*RUNX2, NFKBIA, AHR, IL1A, IRF1, CXCL2, NFE2L2, CXCL10, HSPB1, NOS2, CAV1, PARP1, CDKN1A, MAPK10, PGR, GJA1, SOD1*
3	3.0	3	3	*BCL2, IKBKB, CHUK*
4	2.5	9	10	*THBD, GSK3B, CYP19A1, F2, SREBF1, IGFBP3, PLAT, ESR2, PTPN1*

to lipopolysaccharide, positive regulation of leukocyte-mediated immunity, response to hypoxia, regulation of inflammation signaling pathway, regulation of endothelial cell proliferation, and positive regulation of neuron apoptotic process and so on. The cellular component was mainly involved in membrane rafts, plasma membrane rafts, and membrane microdomains. The molecular function was mainly involved in nuclear factor kappa B (NF-κB) binding, p53 binding, negative regulation of NF-κB transcription factor activity, and protease binding. The rich biological functions, to some extent, explain the reason why the same prescription could treat multiple diseases, and these biological functions also lay the foundation for exploring effective components and searching for signaling pathways.

A total of 132 pathways were obtained from KEGG pathway-enrichment analysis. Important selected items according to P-values are displayed in Figure 6d. The signaling pathway was mainly involved in the PI3K-Akt signaling pathway (P = 7.27E-14, molecular amount: 29), TNF signaling pathway (P = 7.12E-27, molecular amount: 28), Toll-like receptor (TLR) signaling pathway (P = 1.73E-19, molecular amount: 22), mTOR signaling pathway (P = 6.71E-04, molecular amount: 9), and NF-κB signaling pathway (P = 2.73E-13, molecular amount: 17).

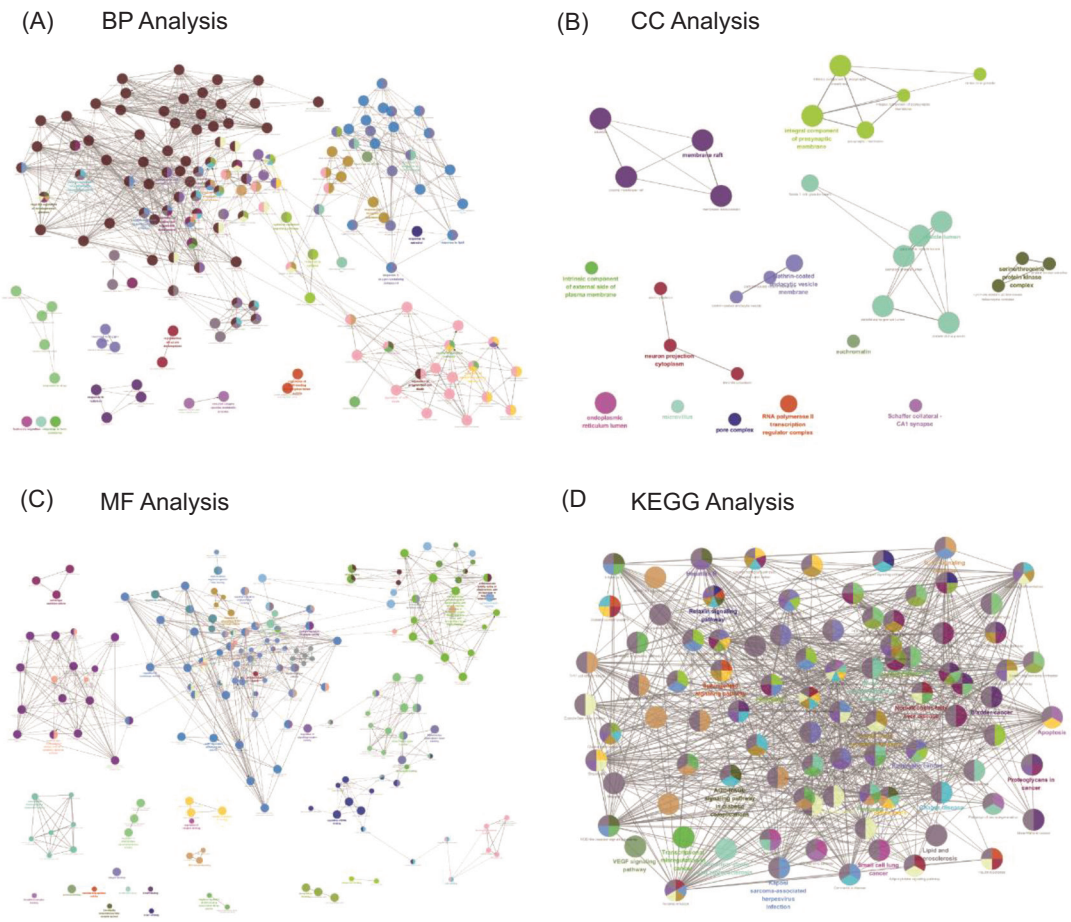


Figure 6 Enrichment of GO and KEGG pathway of GSD in treating KOA. Enriched GO terms are for (A) BP analysis; (B) CC analysis; (C) MF analysis; and (D) KEGG pathway analysis.

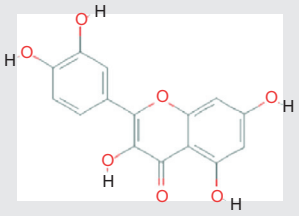
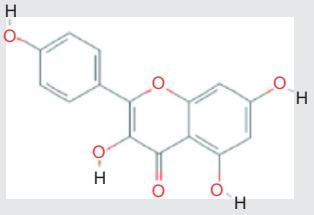
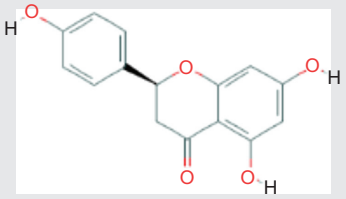
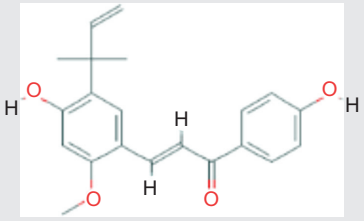
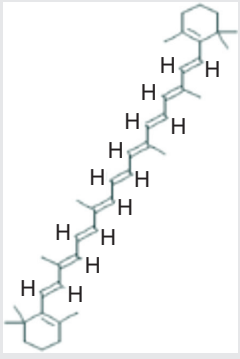
Molecular docking analysis

To further validate the prediction results of network pharmacology and elaborate the mechanism and scientific connotation of GSD as a classical prescription for the treatment of KOA, the following key active components for molecular docking with IL-6, ALB, AKT1, TNF, and TP53 were selected: quercetin, kaempferol, naringenin, β-carotene, and Licochalcone-A (Table 4). The binding energy between ligands and receptors is an important indicator to evaluate binding capacity. It is generally believed that lower the binding energy of ligand and receptor, more stable is conformation. The molecular docking validation was performed for key active components and hub targets. The binding energy ≤−5.0 kcal/mol was used as a criterion.[22] According to docking results, most of the binding free energy of key active components of GSD docked with the hub targets was less than −5.0 kcal/mol (Table 5). It was suggested that the key active components of GSD were well combined with the key targets. It also demonstrated that the prediction of this study was reliable. Furthermore, top five stable conformations are as follows: IL-6 with kaempferol, and AKT-1, ALB, TNF, and TP53 with β-carotene (as shown in Figures 7 and 8). It was also found that hydrogen bonding, Van der Waals force, and Pi-alkyl were the main forms of interactions. For instance, the hydroxy of kaempferol formed hydrogen bonds with proteins, and the methyl of β-carotene also formed Van der Waals force with proteins.

Discussion

Traditional Chinese medicine has accumulated tons of clinical experience for thousands of years, especially within the aspect of Chinese medicine formulas. With a boost of modernization of TCM, many researchers have explored the pharmacological mechanism of TCM and prescriptions through network pharmacology, aiming to provide new thoughts and strategies for the development and innovation of TCM. GSD is a topical CHM compound made from five herbs. Cinnamomi ramulus relieves spasm and pain, promotes yang, and expels qi, thereby warming channel, dredging vein, and dispelling cold. Paeoniae radix alba has the effect of spasmolysis and pain relief and works with Cinnamomi ramulus to gather the essence of water and grain, which does not dissipate with the flow of yang qi.[23] Our clinical experience has shown that GSD has good outcomes for the treatment of KOA. The related clinical research has shown that GSD has obvious effects in treating

Table 4 Basic information of key components.

Component	Number of targets	Source	Structure
Quercetin	92	Licorice, Jujubae fructus	
Kaempferol	37	Paeoniae radix alba, licorice	
Naringenin	23	Licorice	
β-carotene	21	Jujubae fructus	
Licochalcone-A	19	Licorice	

osteoarthritis (OA).[9] To the best of our knowledge, this is the first study to demonstrate the mechanism of GSD in treating KOA by applying network pharmacology analysis. In this study, 103 active components and 225 potential targets of GSD were obtained. There were 2075 targets of KOA, 133 targets of which were shared between GSD and KOA. In addition, 10952 GO functional-enrichment items, and 132 KEGG-related pathways were obtained, which explained that GSD has the hierarchical network characteristics of "multi-component, multi-target, multi-function, and multi-channel" against KOA. Quercetin, kaempferol, naringenin, ß-carotene, licochalcone-A, and other active components play an indispensable role in GSD for treating KOA. The target amount of quercetin is 92, which is much more than other components. The results of molecular docking depicted that quercetin, kaempferol, naringenin, ß-carotene, and licochalcone-A were well combined with IL-6, ALB, AKT1, TNF, and TP53.

Analysis of key targets

Through the PPI network, five hub genes were recognized, including IL-6, ALB, AKT1, TNF, and TP53, which were the key targets for GSD in treating KOA. GSD may exert its therapeutic effects on KOA by modulating these protein

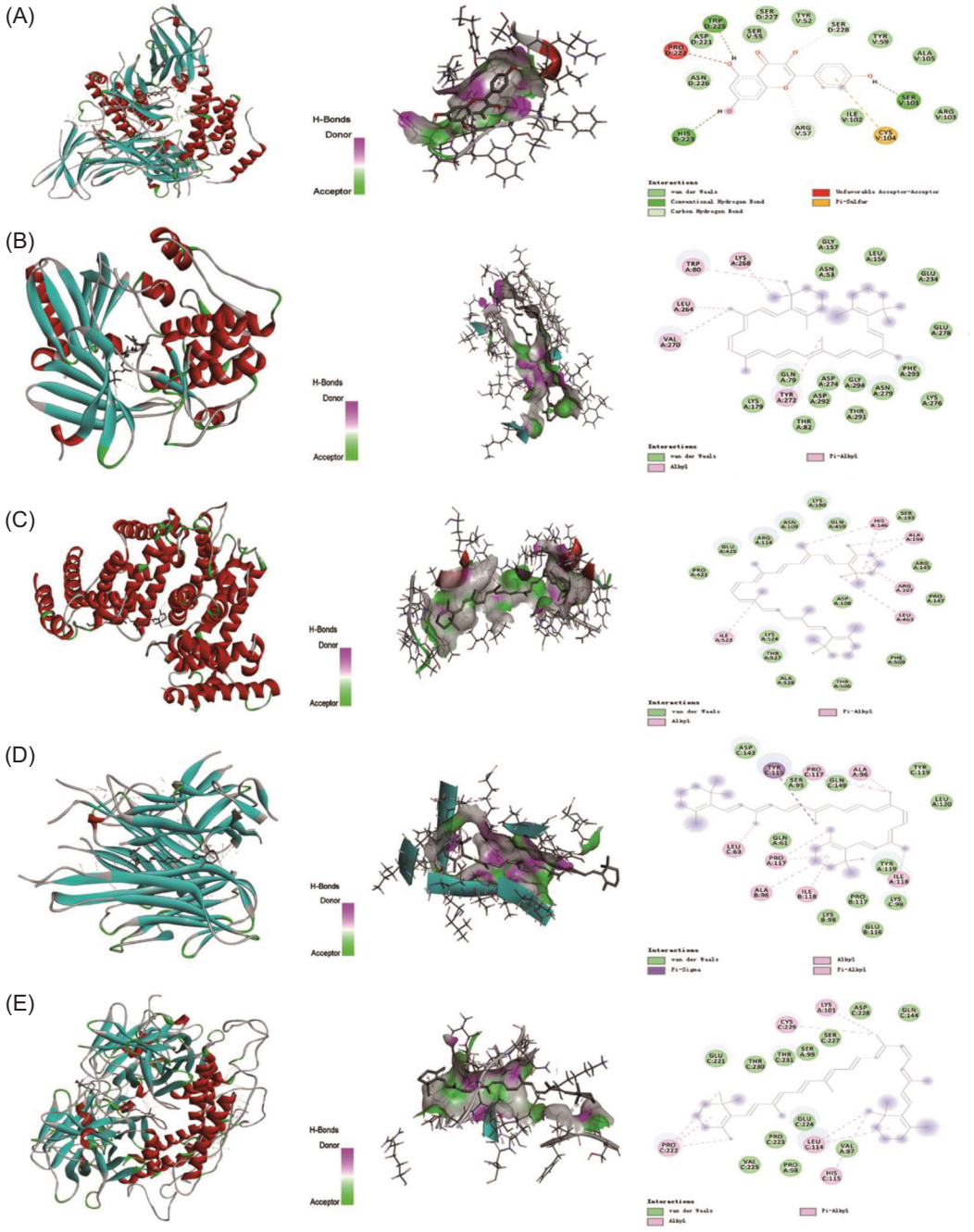


Figure 7 Molecular docking. Binding mode of (A) IL-6 with kaempferol; (B) AKT1 with β-carotene; (C) ALB with β-carotene; (D) TNF with β-carotene; and (E) TP53 with β-carotene.

targets. IL-6 is a mediator of inflammation and immune response and can be detected and expressed, respectively, in synovial fluid and OA cartilage. Hence, it may serve as a potential target for the diagnosis of KOA.[24,25] Besides, a recent study has shown that circulating levels of IL-6 and TNF-α are associated with knee radiographic OA and loss of knee cartilage in older adults.[26] IL-6 plays a crucial role in chronic inflammation, and the expression levels of IL-6 increase in human inflammatory diseases. Li et al.[27] indicated that IL-6 was increased in KOA synovial membranes. TNF refers to an inflammatory mediator in the tumor necrosis factor signaling pathway, which has many biological effects. TNF-α is considered a critical factor in the pathological progress of KOA. TNF-α can combine two membrane receptors (TNF-R1 and TNF-R2).[28] With the comparison of TNF-R2, the damage to articular cartilage caused by TNF-R1 is more serious.[29] Murahashi et al.[30] demonstrated that oral administration of EP4-selective agonist KAG-308 suppresses development of KOA in mouse through reduction of chondrocyte hypertrophy and TNF secretion. Hence, TNF could be a potential therapeutic target to treat KOA. As for ALB, it is reported that increased levels

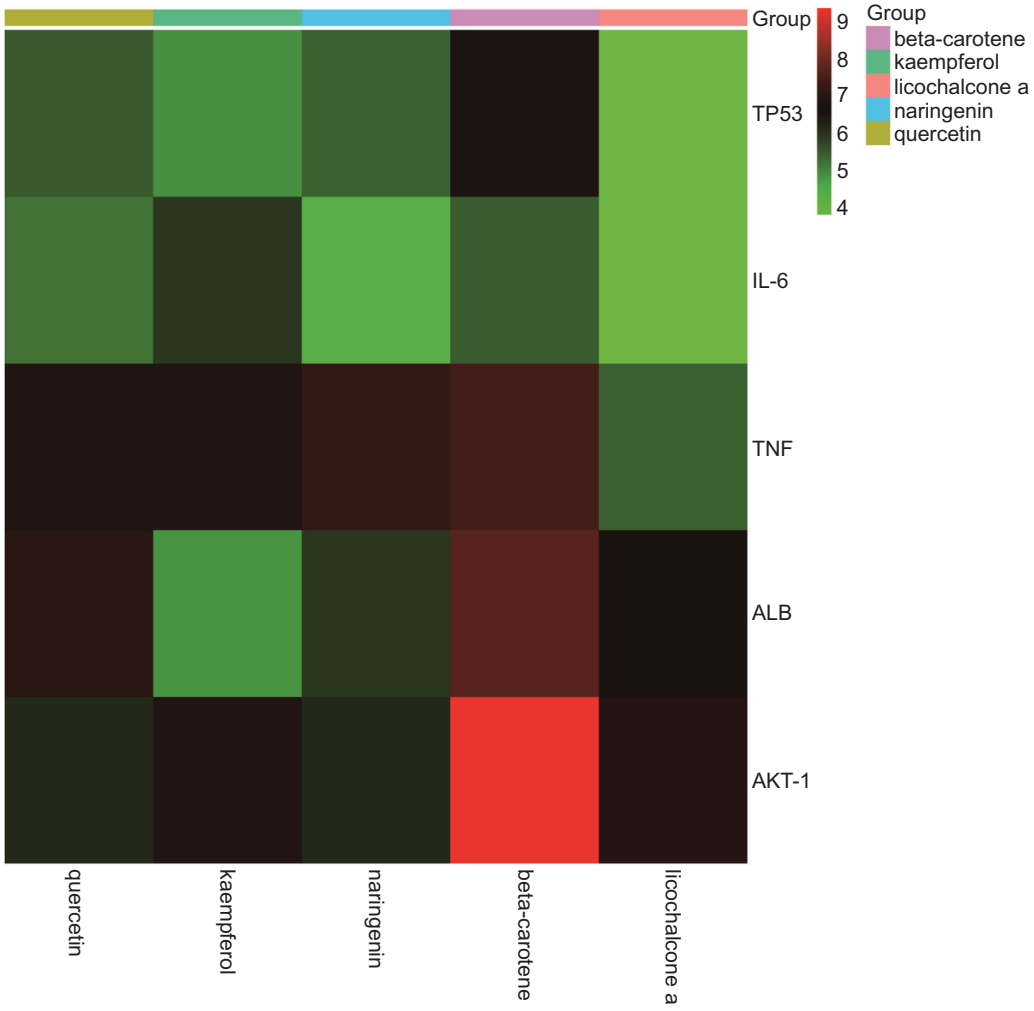


Figure 8 Heat map of estimated free binding energy. Note. Color change from green to red indicates binding energy from high to low.

of chemical-modified ALB are closely related to OA. ALB maybe a prescient risk marker for the progression of OA.[31] AKT1 is one of AKT's subtypes. A previous study[32] identified AKT1 as a unique signaling intermediate in osteoblasts that could control both osteoblast and osteoclast differentiation. Furthermore, the results of molecular docking analysis also showed that AKT1 was well combined with several components of GSD, especially ß-carotene, confirming the role of AKT1 as one of the key targets of GSD to treat KOA. TP53 is considered to curb inflammation to a large extent.[33] Shatz et al.[34] found that combined activation of TP53 and TLR5 pathways synergistically increased the expression of over 2000 genes, which were mostly related to immunity and inflammation. In addition, one study demonstrated that both cyclin D1 and TP53 were related with the severity of KOA and were involved with the progression of OA through the regulation of cell apoptosis.[35]

Potential active components of GSD for treating KOA

According to the "drugs-active components-targets" network, the following five hub components were found: quercetin, kaempferol, naringenin, ß-carotene, and licochalcone-A. Among them, quercetin is a flavonoid compound with multiple biological activities and functions such as anti-oxidation, anti-inflammatory, and anti-hypertensive role. It was reported that quercetin exhibits pain-relieving function caused by arthritis.[36] In addition, quercetin could decrease the generation of proinflammatory cytokines and inhibit the apoptosis of chondrocytes in the knee joint as well as the degradation of extracellular matrix of cartilage, thereby protecting articular cartilage and delaying the progression of OA.[37] Besides, one study demonstrated that quercetin exhibited therapeutic effects in OA rats, which could be related to the inhibition of TNF-α production.[38] Kaempferol alleviates the IL-1ß-induced inflammation in rat OA chondrocytes by suppressing NF-κB.[39] Also, kaempferol could downregulate the expression of IL-1ß and inhibit NF-κB. It then reduced the risk of inflammation of rat OA chondrocytes, which could be beneficial for the protection of OA chondrocytes in rats.[40] It is speculated that kaempferol could alleviate the IL-6-induced inflammation in rat OA chondrocytes by inhibiting the NF-κB signaling pathway. In addition, there is evidence that kaempferol has an inhibitory effect on gene expression and secretion of TNF-α and IL-6.[41] Naringenin has an activity similar to non-steroidal,

Table 5 Free binding energy of the key active components of GSD and key targets.

Component	Chemical formula	Relative molecular mass	Target	Free binding energy (kcal/mol)
Quercetin	$C_{15}H_{10}O_7$	302.2	IL-6	−5.2
			ALB	−6.95
			AKT-1	−6.12
			TNF	−6.8
			TP53	−5.47
Kaempferol	$C_{15}H_{10}O_6$	286.2	IL-6	−5.9
			ALB	−4.8
			AKT-1	−6.82
			TNF	−6.83
			TP53	−4.88
Naringenin	$C_{15}H_{12}O_5$	272.2	IL-6	−4.31
			ALB	−5.92
			AKT-1	−6.17
			TNF	−7.14
			TP53	−5.38
ß-carotene	$C_{40}H_{56}$	536.9	IL-6	−5.45
			ALB	−7.67
			AKT-1	−9.33
			TNF	−7.42
			TP53	−6.76
Licochalcone-A	$C_{21}H_{22}O_4$	338.4	IL-6	−3.79
			ALB	−6.71
			AKT-1	−6.94
			TNF	−5.36
			TP53	−3.81

anti-inflammatory drugs. It plays a crucial role of antipyretic, analgesic as well as anti-inflammatory factor. A few reports have shown that naringenin can also remarkably reduce the inflammatory cytokines TNF-α and IL-6.[42-47] We hypothesize that naringenin may relieve inflammation and pain in KOA by suppressing inflammatory cytokines TNF-α and IL-6. ß-carotene can inhibit inflammatory signaling, tissue damage, and diminish the risk of inflammatory diseases. It prevents the progression of inflammatory diseases such as atherosclerosis and OA.[48] Over the past few decades, ß-carotene has been used to avoid the development of inflammatory illnesses and improve immune system.[49,50] It can also downregulate the expression of proinflammatory genes by prohibiting NF-κB activation in lipopolysaccharide-stimulated macrophages.[51] Therefore, it is speculated that ß-carotene could inhibit inflammatory factors or signaling, thereby reducing the inflammation and treating KOA. Licochalcone-A, a flavonoid isolated from licorice root (*Glycyrrhiza glabra*), has been reported to have an anti-inflammatory effect.[52] In summary, these active components are the material basis of GSD in the treatment of KOA. The representative active components, such as quercetin, kaempferol, and ß-carotene, were well combined with those hub targets. It indicates the multi-component and multi-target characteristics of GSD in treating KOA. Kaempferol, naringenin, quercetin, and ß-carotene have good anti-KOA effects. Quercetin has the lowest administration, which may be the most important factor in performance of being anti-KOA. At the same time, the results provide further support to the hypothesis that quercetin, kaempferol, naringenin, ß-carotene, and licochalcone-A have important research and development values in anti-KOA therapy.

Mechanism analysis of GSD for treating KOA

KEGG pathway and GO function-enrichment analysis showed that the targets of GSD in treating KOA mainly concentrated on PI3K-Akt, TNF, TLR, and NF-κB signaling pathways in the analysis of network pharmacology. This mainly involved response to lipopolysaccharides, positive regulation of leukocyte-mediated immunity, response to hypoxia, and other biological processes.

Among KEGG pathways, the PI3K-Akt signaling pathway is an important signal transduction pathway for regulating cell proliferation, apoptosis, and promoting related tissue regeneration.[53] The result is consistent with that of Feng et al.[22] Akt can also activate specific downstream targets and interact with NF-κB, mTOR, and P53 pathways. Additionally, Wang et al.[54] demonstrated that genetic variation of PI3K-AKT-mTOR is associated with KOA susceptibility in the Chinese population. It is putative that the PI3K-AKT-mTOR pathway is very important in KOA pathogenesis. TNF is an inflammatory mediator with many biological effects in the TNF signaling pathway. TNF-α can induce the production of IL-6 and activate the protease that decomposes cartilage and synovium.[26] There are two forms of TNF: TNF-α and TNF-ß. Min et al.[55] compared the serum levels of DKK1, TNF-α, and OPG in patients with KOA and healthy controls to analyze the interrelationship and the severity of joint destruction. The study result indicated that TNF-α could be a valuable biological marker in predicting the severity of KOA radiology in the clinic. Chen et al.[56] investigated clinical efficacy between moxibustion and acupuncture for KOA to observe effect on serum TNF-α and IL-1ß. Their investigation demonstrated that the levels of serum TNF-α and IL-1ß after treatment were lower when compared with those before treatment. We think the level of TNF-α is associated with KOA. Also, a previous study has confirmed that the serum level of TNF-α is related to loss of knee cartilage in older individuals, which suggest that the serum level of TNF-α played a crucial role in the pathogenesis of KOA.[26] It is noted that suppressing TNF signaling pathway and inhibiting the expression of TNF-α reduce the inflammatory response of KOA in its treatment. Synovial inflammation, a character of both OA and meniscal injury, is thought to be triggered in part via stimulation of TLRs.[57] Barreto et al.[58] found that activation of TLRs led to pro-survival and pro-apoptotic signaling, which might have different effects on healthy and diseased cells. At the same time, the death tendency of osteoarthritic cells was increased. The expression and signal transduction of TLR are associated with the pathogenesis of OA. The NF-κB signaling pathway has been considered a canonical pro-inflammatory signaling pathway, mainly based on the activation of NF-kB by proinflammatory cytokines such as IL-1 and TNF-α.[59] Zhao et al.[3] investigated the role of microRNA-26a (miR-26a) in synovial inflammation and cartilage damage in OA, involving the NF-κB signaling pathway. The research indicated that miR-26a inhibited the

activation of NF-κB signaling pathway, thereby alleviating synovial inflammation and cartilage damage in osteoarthritic rats. Meanwhile, It is noted that some signaling pathways that are remarkably enriched by targets are closely related to other OA, suggesting that GSD may exert potential treatment effects on various OA, namely hip arthritis, rheumatoid arthritis, and gouty arthritis.

Conclusion

To summarize, this study is the first to explore the underlying molecular mechanisms of GSD in treating KOA using network pharmacology-based analysis. The results suggest the synergistic effects of multiple components, targets, and pathways of GSD in treating KOA. Our study also helps to guide the future research on the molecular mechanisms of other Chinese herbs in treating KOA and provides a new perspective for using the network pharmacology approach to investigate the underlying mechanism of TCM to treat other forms of arthritis. However, there are some limitations to this research. The present study provides an outline of the mechanisms of GSD in treating KOA based on the existing database. An advanced exploration confirmation *in vivo* and *in vitro* must be embraced to guarantee the quality and reasonability of results.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgment

This work was supported by grants from the Beijing Municipal Administration of Traditional Chinese Medicine 2019 Clinical Collaborative Capacity Construction Project of Chinese and Western Medicine for Major Difficult Diseases. (No. 201803190106). Sub-project name: Staged stepwise treatment of knee osteoarthrosis with Chinese and Western medicine.

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