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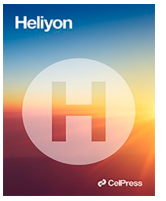

Research article

Mechanism of modified danggui buxue decoction in glucocorticoid-induced osteoporosis: A discussion based on network pharmacology and molecular docking

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ABSTRACT

Objective: Glucocorticoid-induced osteoporosis (GIOP) represents a major complication arising from the long-term use of glucocorticoids, which are widely prescribed for various inflammatory and autoimmune conditions. Despite its prevalence, the current therapeutic options for GIOP are limited in terms of efficacy, safety profiles, and patient compliance. The Modified Danggui Buxue Decoction (DGBXD), a traditional Chinese herbal formulation, has shown promise in preliminary studies for its potential osteoprotective effects. The present study aimed to explore the mechanistic underpinnings of DGBXD's action on GIOP using network pharmacology and molecular docking approaches, bridging traditional medicine with modern pharmacological insights.
Method: Network pharmacology is applied to screen drug-active compounds and potential core target proteins for disease treatment and to explore the drugs' therapeutic mechanisms.
Result: Altogether, 78 DGBXD active compounds and 223 DGBXD-related, 146 component-disease common, and 2168 GIOP-associated target genes were obtained. The PPI network had 43 nodes and 462 edges, and a total of 10 core target genes, including TP53, JUN and MAPK3, were identified. The results of the GO enrichment analysis implied that DGBXD might participate in biological activities, including responses to oxidative stress and nutrient levels. The outcomes of the KEGG pathway enrichment analysis showed that DGBXD may treat GIOP through TNF, IL-17, and phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathways. Based on to the molecular docking results, biologically active compounds (beta-carotene, formononetin, luteolin, and isorhamnetin) exhibited good binding to AKT1 and ESR1.
Conclusion: DGBXD may aid in GIOP treatment by modulating multiple therapeutic targets and signaling pathways.

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1. Introduction

Glucocorticoid is often utilized to treat various inflammatory states and immune diseases owing to its numerous pharmacological actions, including anti-inflammation, antitoxin, antishock, and immune response inhibition [1]. Presently, approximately 1 % of all adults and 3 % of those aged >50 years take glucocorticoids for a prolonged period of time [2,3]. The two basic types of osteoporosis are primary and secondary osteoporosis. Glucocorticoid-induced osteoporosis (GIOP), second to senile o and postmenopausal osteoporosis in terms of incidence, is the most prevalent secondary osteoporosis and the main cause of non-traumatic osteonecrosis [4]. Among the osteoporosis patients receiving long-term glucocorticoid administration, 9%–40 % develop osteonecrosis and 30%–50 % develop osteoporosis-induced pathological fracture [5]. Zhang et al. [6] performed a multi-center study on the incidence of osteopenia and osteoporosis caused by glucocorticoid treatment for rheumatism in 3136 patients. They discovered that as many as 90 % of the patients had osteopenia or osteoporosis, with the incidence of osteoporosis being 41.4 %.

Currently, the general preventive and treatment methods for GIOP include maintaining healthy living habits, smoking and alcohol cessation, balanced diet, engaging in regular weight-bearing or endurance exercise, and maintaining a normal weight [7,8]. Drug therapy comprises intake of oral calcium and vitamins, bisphosphonates (alendronate or risedronate), and parathyroid hormone (teriparatide). However, the long-term use of these drugs can result in severe gastrointestinal reactions [9]. The treatment of GIOP is reportedly unsatisfactory, with the efficacy rate being only 14%–27 % [10,11]. Fortunately, traditional Chinese medicine (TCM) has certain advantages in GIOP prevention and management, exhibiting fewer gastrointestinal reactions [12].

The Modified Danggui Buxue Decoction (DGBXD) is composed of Dipsaci Radix, Drynariae Rhizoma, Cortex Eucommiae, Radix Glycyrrhizae, *Angelicae sinensis* Radix, and Radix Rehmanniae Praeparata. Clinically, DGBXD has remarkable curative effects. For example, it can tonify Qi and kidney, strengthen the bones and muscles, and promote blood circulation [13]. Recent studies have found that DGBXD has estrogen-like activity, which not only can regulate the decline of organ coefficient of the reproductive system and estrogen level abnormalities in SD rats with perimenopausal syndrome, but also can improve the decline in bone mineral density and trabecular thinning [14,15]. In an in vitro study, serum containing DGBXD promoted the differentiation of endothelial progenitor cell into osteoblasts by affecting the phosphatidylinositol 3-kinase (PI3K)-AKT signaling pathway [16]. Nevertheless, the DGBXD mechanism is not clear due to the lack of relevant research.

By virtue of multi-target, multi-component, multi-gene, and multi-pathway characteristics similar to TCM prescriptions, network pharmacology can adapt to the development of modern TCM [17]. In this study, the effective compounds of DGBXD and predicted disease targets were screened, and a "drug-active component-disease target" network was established using network pharmacology and molecular docking technology. Besides, gene ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed to uncover the mechanism and biological process of DGBXD in improving the symptoms of patients with GIOP. Collectively, the objective of the present study was to offer a reference framework for the use of TCM in treating GIOP. The flow chart is displayed in Fig. 1.

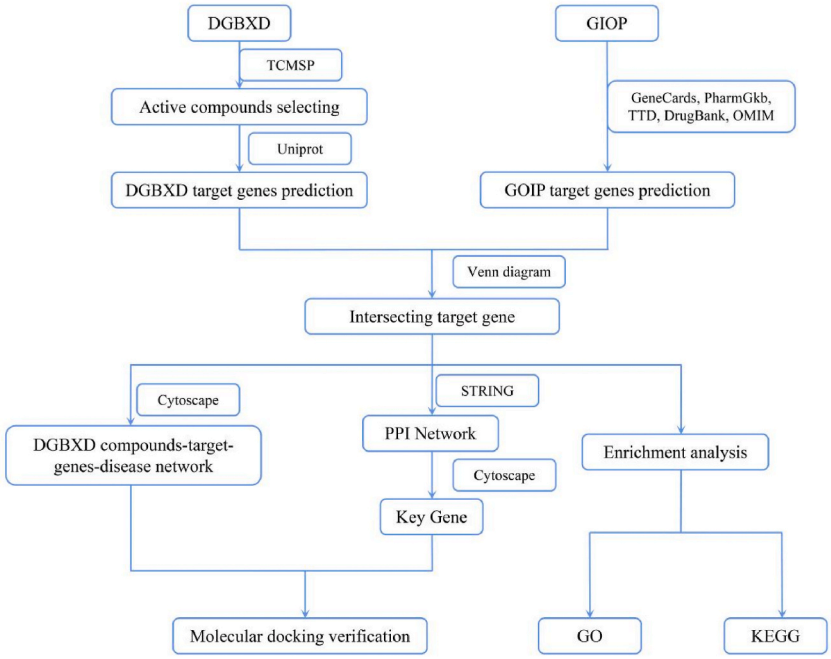

Fig. 1. Overview of the methodological framework for investigating the effects of Modified Danggui Buxue Decoction (DGBXD) on glucocorticoid-induced osteoporosis (GIOP). The schematic diagram outlines the step-by-step approach utilized in our study, including the identification of active compounds in DGBXD, prediction of target genes, network pharmacology analysis, and molecular docking studies.

2. Materials and methods

2.1. Screening of action targets and active compounds of DGBXD

The active components of *Angelicae sinensis* Radix, Dipsaci Radix, Drynariae Rhizoma, *Hedysarum multijugum Maxim,* and Radix Rehmanniae Praeparata were searched from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmsp.php). To find the DGBXD compounds with a higher activity, the search and screening conditions were adjusted to "oral bioavailability (OB) ≥30 % and drug-like property (DL) ≥0.18." Besides, "Organisms" was set as "Human" in the Uniprot database (https://www.uniprot.org/) to perform gene symbol transformation of the target proteins of the different herbs in DGBXD. Next, the related target proteins were named in a standardized manner, and a database for the action targets and active compounds of DGBXD was constructed. The naming of the targeted proteins was aligned with the Human Genome Organisation Gene Nomenclature Committee (HGNC) guidelines. For ambiguous cases, where target proteins were identified by multiple aliases across different databases, we utilized the UniProt gene name conversion tool to reconcile the discrepancies, defaulting to HGNC-approved nomenclature.

2.2. Acquisition of GIOP target genes

The GIOP-related target genes were searched from Online Mendelian Inheritance in Man (OMIM, https://omim.org/), GeneCards (https://www.genecards.org/), PharmGkb (https://www.pharmgkb.org/), DrugBank (https://www.drugbank.ca/), and Therapeutic Target Database (TTD, http://db.idrblab.net/ttd/) after setting "Glucocorticoid osteoporosis" as keywords. Subsequently, the disease gene database related to GIOP was constructed after integrating and deduplicating the potential targets obtained from the above-mentioned databases. Additionally, the Online Venn map platform (http://bioinfogp.cnb.csic.es/tools/venny/index.html) was employed for plotting the Venn diagram.

2.3. Common targets

The Online Venn map platform was utilized to plot the Venn diagram for the GIOP-related targets and active components of DGBXD. Then, these data were acquired for further analysis.

2.4. Establishment of effective compound–target protein interaction network

The common target genes of drug-active component-disease were imported into STRING (https://string-db.org/), with minimum interaction threshold as "Medium Confidence," protein type set as "Homo sapiens," and other parameters as default. After obtaining a comprehensive score, the most reliable data (score >0.9) were used to establish the protein–protein interaction (PPI) network. Upon importing the data from STRING into Cytoscape 3.8.0 software, a network topology analysis was performed. Then, network centrality, local average connectivity-based method, eigenvictor centrality, degree centrality (DC), closeness centrality (CC), and betweenness centrality were calculated. Considering the abovementioned values exceeding the median as screening conditions, the core target genes were identified and collected. Moreover, an interaction network of "drug-active component–target gene–GIOP" was constructed with the Cytoscape 3.8.0 software.

2.5. GO functional annotation and KEGG pathway enrichment analysis

GO functional annotation and KEGG pathway enrichment analysis were conducted on common target genes through the Database for Annotation, Visualization, and Integrated Discovery (DAVID, version 6.8, https://david.ncifcrf.gov/). In the GO enrichment analysis, the top 10 items of molecular functions (MFs), cell components (CCs), and biological processes (BPs) were visually analyzed. As for the KEGG enrichment analysis, we visually analyzed the top 30 related items.

2.6. Molecular docking

The network analysis of "disease-effective compound–target" revealed quercetin, naringenin, luteolin, formononetin and kaempferol as the top 5 effective compounds. For further validating the network predictions and illuminating the mechanisms of DGBXD in GIOP, molecular docking of the abovementioned compounds with the top 5 targets, i.e., JUN, TP53, MAPK1, MAPK3 and AKT1, was carried out in turn. The structure data files of the five effective compounds were downloaded from PubChem (https://www.ncbi.nlm.nih.gov/). The abovementioned small molecular ligands of the target proteins were converted into 3D structures on ChemBio3D, and the 3D structures were optimized by minimize energy. Next, the 3D structure data of the target proteins were downloaded from the Protein Data Bank (http://www.rcsb.org/) database. The protein receptor file was obtained after the removal of water molecules and small molecule ligands by PyMOL Molecular Graphics System 2.1.0. Then, molecular docking and binding free energy calculations of the ligands and receptors were carried out with the AutoDockTools, and the active site was plotted. Finally, the lowest free energy model selected by using AutoDock Vina was utilized to visualize the molecular docking results.

3. Results

3.1. Prediction of GIOP targets and common targets with active compounds in DGBXD for GIOP therapy

Altogether, 78 active compounds of DGBXD were searched from the relevant literature and TCMSP database (Table 1). After searching from the GeneCards, OMIM, TTD, PharmGkb, and DrugBank databases, 2168 targets related to GIOP were acquired (Fig. 2A). Upon comparing the 223 related targets of DGBXD with the targets of GIOP, 146 common target genes were identified, and their Venn diagram was plotted (Fig. 2B). These common target genes were considered potential therapeutic targets for DGBXD in the treatment of GIOP. Under the help of the Cytoscape 3.8.0 system, the "DGBXD-active compound–target gene" network was established.

3.2. Protein–protein interaction network suggesting the multi-target effects of DGBXD

To clarify the correlation of the effective active compounds of DGBXD with the common target genes and GIOP, a PPI network diagram was generated by importing 146 common target genes into STRING. The PPI network diagram had 146 intrinsic nodes and 462 edges. The nodes represented the proteins, and the edges represented the intrinsic relationship between proteins (Fig. 3).

3.3. Key targets and active compounds in DGBXD identified as potential therapeutic agents for GIOP through a network analysis

Subsequently, the top 10 key target genes were screened by Cytoscape software (Fig. 4A). Furthermore, the network analysis tool of Cytoscape software was adopted for the degree analysis, and the Sankey diagram for the 10 main effective active compounds and 10 key target genes was plotted (Fig. 4B). The 10 main active compounds included beta-sitosterol, quercetin, beta-carotene, kaempferol, luteolin, naringenin, formononetin, isorhamnetin, calycosin, and stigmasterol. The key target genes comprised TP53 (degree = 72), JUN (degree = 70), MAPK3 (degree = 68), AKT1 (degree = 66), MAPK1 (degree = 64), RELA (degree = 60), ESR1 (degree = 54), CTNNB1 (degree = 54), MAPK14 (degree = 50), and FOS (degree = 50) (Fig. 4).

3.4. DGBXD potentially influences specific pathways based on the results of the GO and KEGG analyses

To determine the possible mechanism of DGBXD in GIOP, GO functional enrichment analysis was performed on 146 common target genes of DGBXD in the treatment of GIOP using the DAVID database. Altogether, 2691 genes related to MFs, BPs. and CCs were obtained, and the significant value was $p < 0.05$. The top 10 items of MFs, CCs, and BPs were also visualized. Specifically, the common target genes are mainly enriched in BPs, such as responses to nutrient levels, xenobiotic stimulus, and oxidative stress. Moreover, these genes were primarily enriched in CCs, such as the membrane microdomain, membrane raft, vesicle lumen, and caveolae of sarcolemma. Besides, MFs of the common target genes included DNA-binding transcription factor binding, ligand-activated transcription factor activity, and nuclear receptor activity (Fig. 5A).

Regarding the results of the KEGG analysis, the common target genes were primarily enriched in the AGE–RAGE signaling pathway, PI3K–Akt signaling pathway, lipid and atherosclerosis, IL–17 signaling pathway, TNF signaling pathway and other signaling pathways (Fig. 5B). The PI3K–Akt signaling pathway, identified as one of the most significantly enriched pathways, plays a pivotal role in promoting osteoblast differentiation and survival, an essential process in counteracting GIOP. The enrichment of the TNF signaling pathway underscores its involvement in inflammation-induced osteoclastogenesis, suggesting that DGBXD may exert protective effects by modulating this pathway. Similarly, the IL-17 signaling pathway's enrichment suggests a mechanism by which DGBXD could potentially influence the inflammatory responses within the bone microenvironment. To conclude, the results of the KEGG analysis provide a comprehensive overview of the molecular mechanisms through which DGBXD exerts its therapeutic effects on GIOP.

3.5. Molecular docking validates the high affinity binding of DGBXD's key compounds to target proteins, supporting its mechanistic action in GIOP

To corroborate the prediction results and mechanism of DGBXD in GIOP, the top 10 active compounds and top 10 target proteins in the drug-active compound-disease-target network were analyzed using molecular docking. The top 10 compounds included quercetin, luteolin, kaempferol, naringenin, formononetin, beta-carotene, isorhamnetin, beta-sitosterol, calycosin, and stigmasterol. The top 10 target proteins were MAPK3, JUN, TP53, MAPK1, AKT1, RELA, ESR1, CTNNB1, MAPK14, and FOS. Generally, the molecular docking binding energy of < -4.25 kcal/mol indicates a certain binding activity between the ligand and receptor, <-5.0 kcal/mol suggests a good binding activity between them, and <-7.0 kcal/mol indicates a strong binding activity [18]. In the present study, all of the active components bound to the target protein with good affinity at <-5.0 kJ·mol−1 (Fig. 6). Especially for AKT1–luteolin, AKT1–formononetin, AKT1–beta-carotene, AKT1–isorhamnetin, ESR1–formononetin, and ESR1–isorhamnetin, the binding affinity between them was excellent (Fig. 7A–F). In a nutshell, DGBXD worked by targeting multiple proteins with multiple drug-active components.

4. Discussion

As a traditional culture inherited for more than 2000 years, it is particularly necessary to promote the development of TCM with the help of modern technology. However, owing to the complicated biomolecular mechanisms of TCM, directly analyzing its mechanism

Table 1
Components of effective compounds in DGBXD.

Drug	Mol ID	Oral bioavailability (OB, %) (%)	Drug-like property (DL)
Hedysarum multijugum Maxim	MOL000211	55.38	0.78
	MOL000239	50.83	0.29
	MOL000296	36.91	0.75
	MOL000033	36.23	0.78
	MOL000354	49.60	0.31
	MOL000371	53.74	0.48
	MOL000374	41.72	0.69
	MOL000378	74.69	0.30
	MOL000379	36.74	0.92
	MOL000380	64.26	0.42
	MOL000387	31.10	0.67
	MOL000392	69.67	0.21
	MOL000398	109.99	0.30
	MOL000417	47.75	0.24
	MOL000422	41.88	0.24
	MOL000433	68.96	0.71
	MOL000438	67.67	0.26
	MOL000439	49.28	0.62
	MOL000442	39.05	0.48
	MOL000098	46.43	0.28
Angelicae sinensis Radix	MOL000358	36.91	0.75
	MOL000449	43.83	0.76
Cortex Eucommiae	MOL002058	57.20	0.62
	MOL000211	55.38	0.78
	MOL000358	36.91	0.75
	MOL000422	41.88	0.24
	MOL004367	62.23	0.41
	MOL000443	49.18	0.55
	MOL005922	43.35	0.77
	MOL006709	92.43	0.55
	MOL007059	32.16	0.41
	MOL000073	48.96	0.24
	MOL007563	57.53	0.81
	MOL009007	30.51	0.85
	MOL009009	87.19	0.62
	MOL009015	58.67	0.61
	MOL009027	55.42	0.82
	MOL009029	51.44	0.40
	MOL009030	30.10	0.24
	MOL009031	68.22	0.40
	MOL009038	45.58	0.83
	MOL009042	77.01	0.19
	MOL009047	33.29	0.62
	MOL009053	50.76	0.39
	MOL009055	49.81	0.37
	MOL009057	53.14	0.80
	MOL000098	46.43	0.28
	MOL002773	37.18	0.58
	MOL008240	56.32	0.36
	MOL011604	36.82	0.37
Drynariae Rhizoma	MOL001040	42.36	0.21
	MOL001978	53.42	0.24
	MOL002914	41.35	0.24
	MOL000449	43.83	0.76
	MOL000358	36.91	0.75
	MOL000422	41.88	0.24
	MOL004328	59.29	0.21
	MOL000492	54.83	0.24
	MOL005190	71.79	0.24
	MOL000569	61.85	0.26
	MOL000006	36.16	0.25
	MOL009061	39.25	0.76
	MOL009063	41.66	0.79
	MOL009075	40.57	0.79
	MOL009076	39.05	0.79
	MOL009078	62.65	0.51
	MOL009087	70.79	0.19

(*continued on next page*)

Table 1 (*continued*)

Drug	Mol ID	Oral bioavailability (OB, %) (%)	Drug-like property (DL)
	MOL009091	41.08	0.32
Radix Rehmanniae Praeparata	MOL000359	36.91	0.75
	MOL000449	43.83	0.76
Dipsaci Radix	MOL003152	64.06	0.21
	MOL000358	36.91	0.75
	MOL000359	36.91	0.75
	MOL009312	48.14	0.68
	MOL009317	43.32	0.81
	MOL008188	44.11	0.25
	MOL009322	48.02	0.53
	MOL009323	56.47	0.43

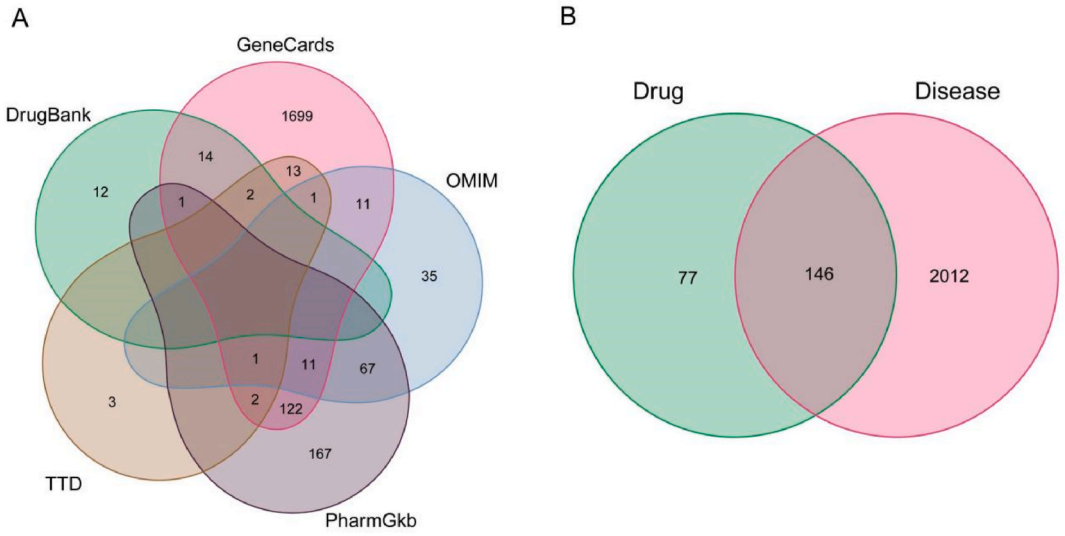

Fig. 2. GIOP target and common target prediction. A–B, Venn diagrams for the GIOP-related targets (A) and the common targets of DGBXD-GIOP (B). DGBXD, Modified Danggui Buxue Decoction; GIOP, glucocorticoid-induced osteoporosis.

through in vitro or in vivo experiments is challenging [19]. As a discipline that integrates system biology, pharmacology, bioinformatics, and computer science [20], network pharmacology has offered fresh perspectives and approaches for exploring the relationship between traditional herb compound and disease [21]. Presently, molecular docking technology and network pharmacology have already been employed to explore the mechanism of Chinese medicine prescriptions in many studies [22–24]. Through network pharmacology analysis, our study revealed that DGBXD acted on AKT1, JUN, MAPK3, MAPK1, TP53, and other targets through active compounds, such as kaempferol, quercetin, luteolin, naringenin and formononetin. Then, DGBXD was implicated in BPs (e.g., cell metabolism, proliferation, and differentiation) by regulating the TNF signaling pathway, IL−17 signaling pathway, AGE−RAGE signaling pathway in diabetic complications, and PI3K-Akt signaling pathway. These signaling pathways were selected for further investigation through a combination of our network pharmacology analysis and molecular docking studies, which identified that the key targets within these pathways were interacting with the bioactive compounds in DGBXD.

Quercetin, luteolin, kaempferol, naringenin, and formononetin stand out as the key active compounds in DGBXD, each underpinning its pharmacological efficacy in treating GIOP. Quercetin is found in *Hedysarum multijugum Maxim*, a component of DGBXD, but it also widely distributed in onions, apples, and tea. Quercetin and its related derivatives have anti-tumor, anti-oxidant, anti-inflammatory, and anti-microbial properties, and can be used as effective natural osteogenic agents [25]. Specifically, quercetin participates in osteoclast differentiation by blocking the expression of activator protein −1 (AP-1) and receptor activator of NF-κB ligand (RANKL). The skull of the New Zealand White rabbits were given a quercetin solution mixed with a collagen matrix by Wong et al. [26]. In their study, quercetin in a collagen matrix increased the formation of new bone locally, indicating the bone protective effect of quercetin. Luteolin can be sourced from *Dipsacus asper*, another ingredient in DGBXD, and it is also abundant in celery, parsley, and thyme. The mechanism of luteolin improving GIOP is related to the ERK/Lrp-5/GSK-3β signaling pathway. Zheng et al. [27] confirmed that luteolin could reverse the dexamethasone-induced decrease in OPG/RANKL ratio and suppress the osteoclast activity by affecting the ERK/Lrp-5/GSK-3β signaling pathway in an in vitro experiment. Kaempferol is present in Ginkgo biloba and is also a constituent of tea, broccoli, and kale, aligning with the phytochemical profile of DGBXD ingredients. Mesenchymal stem cells (MSCs) and osteoblasts have increased osteogenic differentiation after kaempferol treatment [28,29]. Runt-related transcription factor 2

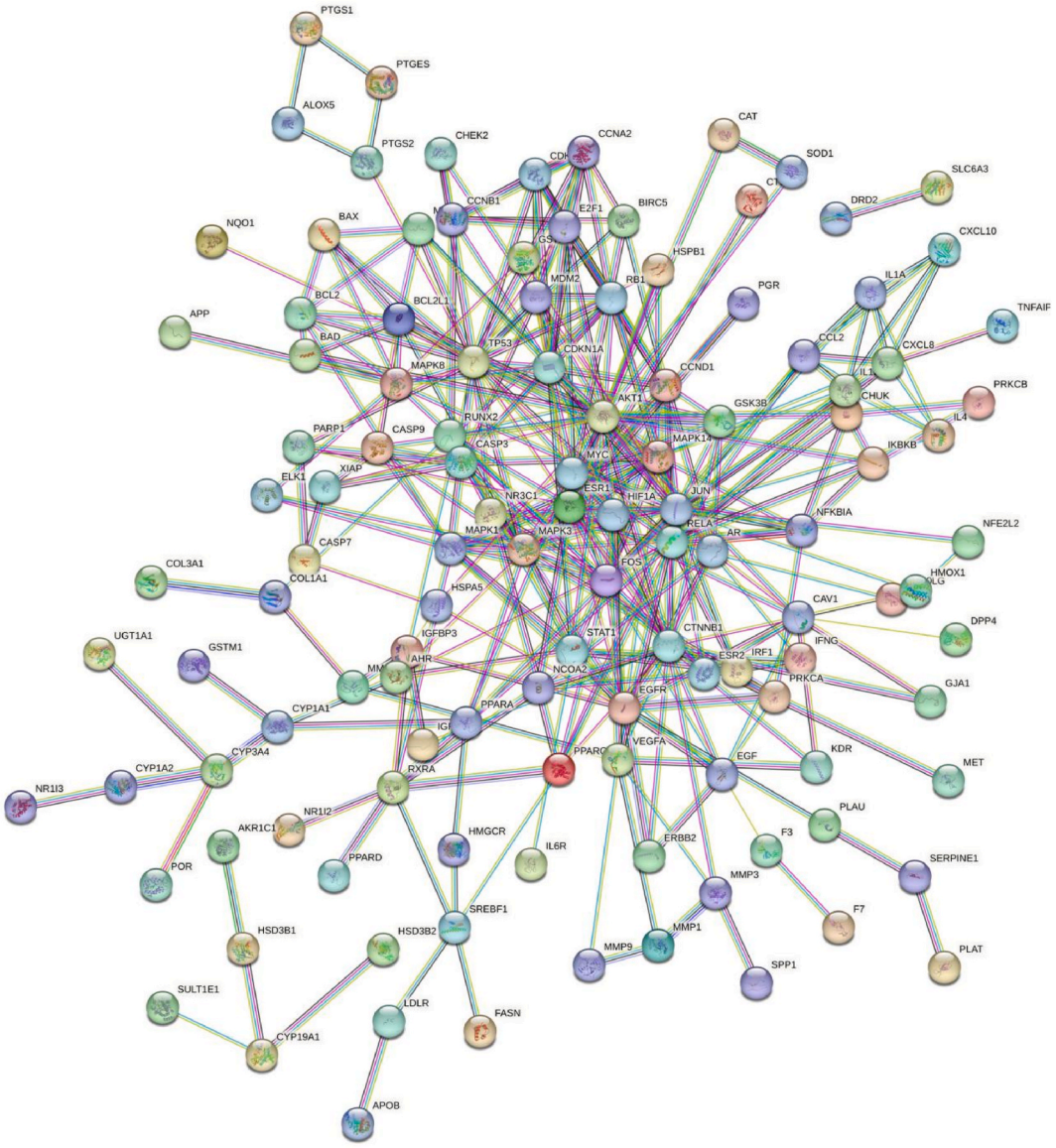

Fig. 3. Establishment of protein–protein interaction network. The edge represented the protein–protein interaction. The thickness of the edge indicated the intensity of the data support. All nodes were visualized with a degree value. Larger nodes with darker colors indicated higher degree values.

(Runx2) is critical in regulating osteoblast proliferation and differentiation [30]. Osterix, as a specific transcription factor of osteoblasts, regulates the expression of a series of genes during differentiation [31]. Yang et al.'s [32] studies have demonstrated that kaempferol could significantly enhance the Runx2 and osterix expressions in dexamethasone-treated MC3T3-E1 cells and activate the p38-MAPK and JNK signaling pathways. Naringenin, with anti-oxidative and anti-inflammatory characteristiics [33], is the main metabolite of naringin, which is present in citrus fruits. Compared with naringin, naringenin can better promote the osteogenic differentiation of osteoblasts in SD rats [34]. Besides, under the induction of dexamethasone, naringenin can lower the RANK and RANKL expressions, elevate the OPG expression, significantly inhibit osteoclast differentiation, and promote bone differentiation. Formononetin, an isoflavone, is derived from *Astragalus membranaceus*, used in DGBXD, and is also found in red clover. In vitro experiments have demonstrated that formononetin modulated the mRNA expression levels of RANKL and OPG, exhibiting an estrogen-like effect [35]. For instance, formononetin could compete with estrogen to bind to estrogen receptors. Moreover, formononetin inhibits osteoclast activity and slows down bone resorption and destruction by affecting the P38-MAPK/MMP-9 signaling pathway [36]. In summary, the active components of DGBXD have good bone protective effect and may be the pharmacological basis for its therapeutic effect.

In our exploration of DGBXD's therapeutic potential for GIOP, we identified several key proteins that play pivotal roles in bone

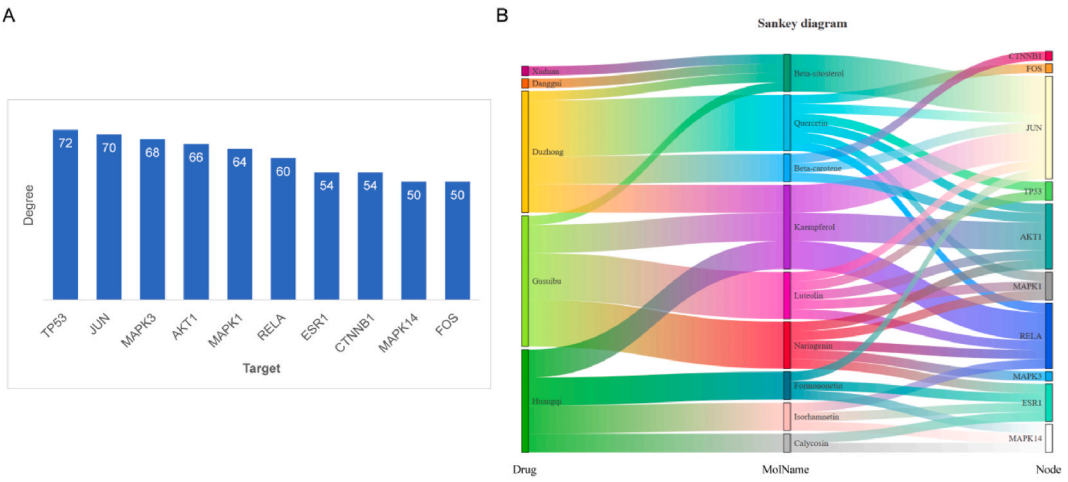

Fig. 4. Drug-active compound-disease-target network diagram. A, The top 10 key target genes; B, The Sankey diagram for the 10 main effective active compounds and 10 key target genes.

metabolism and repair. Our results demonstrate a correlation between these proteins and DGBXD in GIOP, aligning with previous literature. Among these, AKT1, a member of the serine/threonine protein kinases, enhances osteoblast proliferation and differentiation and reduces osteoclast apoptosis, highlighting its therapeutic relevance [37]. Further emphasizing the complexity of bone regulation, JUN, a component of the AP-1 transcription factor family, not only facilitates bone development and healing in osteoporotic fracture models but also promotes autonomous bone formation in various precursor subsets, significantly accelerating recovery in the drilling-defect models [38,39]. The roles of MAPK3 (also known as ERK1) and MAPK1 are also crucial. MAPK3, by responding to extracellular signals, regulates cellular proliferation and differentiation and plays a significant role in estrogen receptor and ERK/-MAPK signaling pathways, which are essential for apoptosis regulation in bone cells [40,41]. The suppression of MAPK3 expression can lead to increased apoptosis in pre-B and B cells, promoting osteoclast differentiation [42]. Meanwhile, MAPK1's involvement in reducing the differentiation capacity of bone marrow macrophages into osteoclasts [43] and its regulation by miRNA-483-5p in the development of osteoblasts from bone marrow MSCs [44] further underscore its importance. Additionally, the tumor suppressor protein p53, encoded by TP53, regulates cell growth and repair, with increased levels in osteoporosis patients suggesting its therapeutic potential [45]. P53's influence on bone marrow MSCs and its inhibition of osteogenesis through the microRNA signaling pathway further indicate its crucial role in bone health [46,47]. These insights collectively reveal that DGBXD targets multiple proteins integral to bone health, suggesting a robust multi-targeted approach for treating GIOP. The active components in DGBXD, including quercetin, luteolin, and kaempferol, may thus offer a comprehensive treatment solution by modulating these key pathways.

The results of the KEGG enrichment analysis suggested that the relevant targets of DGBXD in the treatment off GIOP were primarily enriched in AGE–RAGE signaling pathway, PI3K–Akt signaling pathway, lipid and atherosclerosis, IL-17 signaling pathway, and TNF signaling pathway. Specifically, the PI3K–AKT pathway, known for its roles in cell proliferation, metabolism, and survival, involves key proteins, including PI3K and AKT/protein kinase B [48,49]. Our study enhances the understanding of DGBXD's modulation of this pathway, which mirrors Ma et al.'s findings, where inhibiting PI3K-AKT reduced osteoclast differentiation and bone lacunae formation, emphasizing its therapeutic potential in increasing the OPG/RANKL ratio and promoting osteoblast differentiation [50]. Moreover, the AGE–RAGE signaling pathway, typically linked with diabetic complications, also impacts bone integrity through the deleterious effects of AGEs on the extracellular matrix and protein function, inducing osteoblastic activity and contributing to osteoporosis as demonstrated in the diabetic models [51,52]. Additionally, IL-17 plays a complex role through its impact on MSCs and osteoblast differentiation, mediated by ROS production, which aligns with our observations of DGBXD's effects in modulating this pathway to potentially protect against bone loss [53]. The TNF pathway, integral to bone metabolism through its regulation of osteoclast and osteoblast proliferation, is another area where DGBXD's effects were noted, particularly in how TNF-α promotes osteoclast differentiation and inhibits osteoblast formation from MSCs, which is critical in postmenopausal osteoporosis [54–56]. This integrated analysis not only confirms the involvement of these pathways in bone health as influenced by DGBXD but also provides a foundational insight into the potential multi-target therapeutic strategy offered by DGBXD for treating GIOP, thus reinforcing the connection of our results with established biochemical pathways and extending the implications for future therapeutic interventions.

Although our network pharmacology analysis has highlighted significant findings regarding the potential therapeutic applications of DGBXD for GIOP, the current study has several inherent limitations. First, the reliance solely on database-derived data limits our insights to previously documented compounds and interactions, potentially overlooking novel constituents and their synergistic effects. Second, our analysis was restricted to the primary active components of DGBXD, omitting a detailed examination of metabolites that may play critical roles in the decoction's pharmacological profile. The absence of metabolite analysis narrows our understanding of the complete spectrum of pharmacologically active agents within DGBXD, which might significantly influence both its efficacy and safety. Third, this study did not account for variations in herb dosage, nor the conditions and methods of their decoction, which are crucial factors for replicating and scaling these findings in clinical settings. Such parameters are vital for confirming DGBXD's

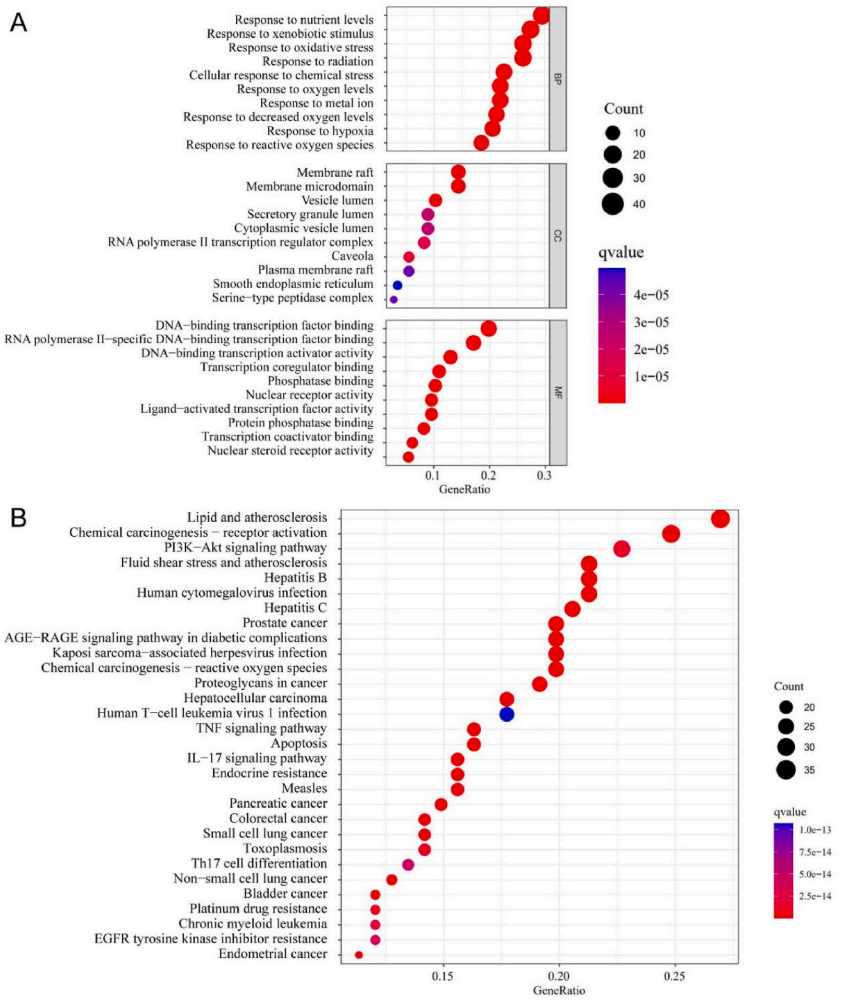

Fig. 5. GO and KEGG enrichment analyses. A, The results of the GO functional enrichment analysis. The ordinate included molecular functions (MFs), cell components (CCs), and biological processes (BPs), and the abscissa indicated the degree of enrichment. B, The results of the KEGG enrichment analysis. The ordinate included related diseases and involved pathways, and the abscissa meant the degree of enrichment. The size of each dot corresponds to the number of genes involved, with larger dots denoting a greater quantity of genes. The color gradient from blue to red reflects the increasing levels of enrichment significance, with red indicating the highest degree of enrichment. All displayed results represent findings with a p-value <0.05, emphasizing statistically significant enrichments.

therapeutic consistency and predictability. The unaddressed complexity of the herbal constituents and their preparation methods could lead to treatment outcome variations, underscoring the need for more controlled experimental designs. Building on this initial study, future research should include comprehensive metabolite profiling and rigorous validation of DGBXD's therapeutic targets. These steps are essential for effectively translating the theoretical benefits of DGBXD into viable clinical applications for treating GIOP.

5. Conclusion

To sum up, the multiple active components of DGBXD target and bind to multiple proteins to treat GIOP. The specific mechanism of DGBXD against GIOP may be associated with the PI3K–AKT, IL-17, and TNF signaling pathways. The methodological rigor and comprehensive analysis employed in our study exemplify the strength of combining traditional knowledge with contemporary scientific approaches, offering new insights into the development of innovative and holistic treatment strategies for osteoporosis and related conditions.

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Fig. 6. Molecular docking heat map of the 10 main active components and 10 target proteins of the Modified Danggui Buxue Decoction (DGBXD).

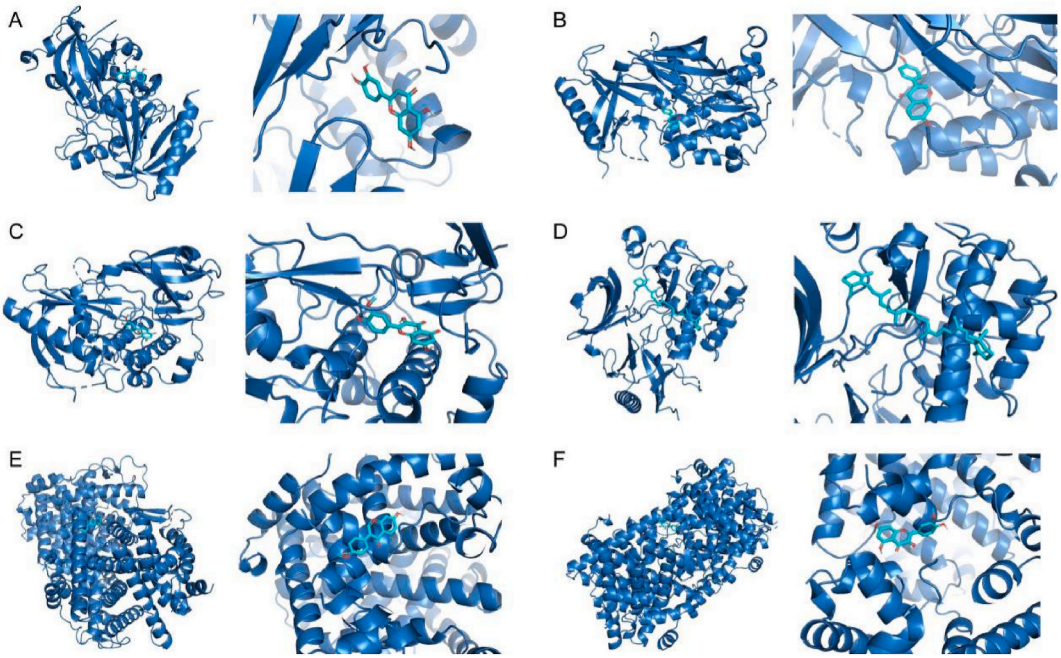

Fig. 7. Molecular docking results. (A) AKT1-luteolin; (B) AKT1-formononetin; (C) AKT1-beta-carotene; (D) AKT1-isorhamnetin; (E) ESR1-formononetin; (F) ESR1-isorhamnetin.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

CRediT authorship contribution statement

Yu-zhou Chen: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yi Zhou:** Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Jun-long Chen:** Resources, Methodology, Funding acquisition, Data curation. **Yi-ping Luo:** Visualization, Validation, Supervision, Project administration. **Cheng-zhi Feng:** Validation, Resources, Investigation, Formal analysis. **Xiao-hong Fan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation,

Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37249.

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