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Bone aging and osteoporosis: Recent evidence focusing on plant-based natural products

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Bone aging and osteoporosis: Recent evidence focusing on plant-based natural products

Topic editors

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Editorial: Bone aging and osteoporosis: recent evidence focusing on plant-based natural products

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Editorial on the Research Topic
Bone aging and osteoporosis: recent evidence focusing on plant-based natural products

Various active ingredients from plants, such as polyphenols, polysaccharides, and flavonoids, are considered potential sources of drugs for treating bone aging and osteoporosis (OP) and have been proven to have antioxidant, anti-inflammatory, and anti-bone resorption effects; thus, these compounds alleviate bone aging and OP (1, 2). However, unfortunately, studies on many active ingredients from plants with therapeutic potential for OP are still in the laboratory stage rather than being applied in clinical practice. Therefore, we proposed to summarize the research on this Research Topic to promote further discovery and research on plant natural products, and to promote the clinical application of plant-derived natural products. A total of 13 studies were included in this review, and these studies mainly presented the research progress and development of single plant medicines, monomeric compositions, and Chinese patent medicines for the treatment of bone aging-related diseases. The types of articles on this topic were mainly reviews, which can help us better understand the widespread progress of plant-derived natural products in the treatment of bone aging. In addition, we propose that more attention should be given to clinical research and mechanistic explorations of natural plant products.

Research on the mechanism of plant-based natural products

Basic research is key for promoting the application of natural plant products in the clinical treatment of bone aging and OP. A total of three basic research studies will be discussed. Jie et al. identified palmatine (PAL) as the main active ingredient of ErXian decoction by using liquid chromatography-tandem mass spectrometry and network

pharmacology screening and explored the therapeutic effect of PAL in the treatment of OP and osteoarthritis (OA) comorbidities in rats. Their results indicated that PAL can significantly improve the femoral microstructure and alleviate cartilage damage in OA-OP rats, and its mechanism of action may regulate the gut microbiome and serum metabolites to alleviate bone aging in OA-OP patients. Aconine has anti-inflammatory and analgesic pharmacological effects, but it must be used with caution due to its toxicity, and this limitation needs to be emphasized. Xue et al. reported that aconine can effectively reduce vertebral bone loss and restore the high level of bone turnover markers in ovariectomized mice. These authors further confirmed that aconitine can reduce the expression of the osteoclast-specific genes NFATc1, c-Fos, Cathepsin K, and Mmp9, thereby significantly inhibiting the occurrence of osteoclasts. The mechanism of action of aconitine may be related to the inhibition of NF-κB signaling pathway-mediated ferroptosis and the formation of osteoclasts. Liang et al. discovered through metabolomics techniques that Longbie capsules can regulate serum lipid metabolism, amino acid metabolism, and estrogen levels, thereby helping maintain the balance in bone metabolism in OA-OP rats and reducing bone loss in the articular cartilage. The research of Liang et al. provides a replicable strategy for elucidating the mechanism of traditional Chinese medicines in treating bone aging diseases.

Evidence-based therapeutic applications of plant-derived natural products

Evidence-based medicine is an indispensable part of clinical decision-making. The application of natural plant products from the laboratory into clinical practice requires a large amount of evidence-based data. Zhao et al. used quantitative data analysis methods to evaluate the efficacy of resveratrol in treating OP model rats. This meta-analysis included 15 animal experiments and evaluated multiple indicators, including bone density and serum marker levels. In this study, they found that resveratrol can increase bone density, improve bone microstructure, and regulate calcium and phosphorus metabolism in OP model rats. Resveratrol is a natural polyphenolic compound that has been proven to have anti-inflammatory, antioxidant, and anti-aging effects in multiple pharmacological studies (3, 4). Undoubtedly, Zhao et al.'s research provides reliable evidence for the use of resveratrol in the treatment of OP, which will promote its clinical application. Osteoporosis compression fracture, which is a common complication of OP, is a substantial economic and medical burden. The prevention and treatment of osteoporotic fractures cannot be ignored in public health. Fu et al. conducted a meta-analysis and confirmed that the Jintiange capsule has good safety and efficacy in the treatment of osteoporotic vertebral compression fractures (OVCFs). Specifically, Jintiange capsules can increase bone density, alleviate pain, and reduce the incidence of adverse events. The evidence provided by Fu et al. will further promote the clinical application of Jintiange capsules in the treatment of OVCFs.

Comprehensive review of plant natural products

A total of eight descriptive reviews will be summarized to help readers comprehensively understand the current state of the application of plant-based natural products to treat bone aging and OP. After analyzing and summarizing the pathological mechanisms of OP, Zhou C. et al. focused on the effects of traditional Chinese medicine formulas and their chemically active components on osteoblasts, osteoclasts, bone marrow mesenchymal stem cells, bone microstructure, angiogenesis, and the immune system; these findings provide a good perspective for comprehensively understanding of the mechanism of action of classical Chinese medicine formulas in the treatment of OP. Zhou G. et al. summarized the mechanism of action of traditional Chinese medicine formulas and monomers in the treatment of KOA combined with OP; these results will help readers understand the role of traditional Chinese medicine in the treatment of KOA combined with OP. During the aging process, cellular aging is an instinctive response of cells to various exogenous and endogenous stimuli. Therefore, targeting cellular aging may be a potential strategy for treating OP. Zhang et al. summarized the potential of traditional Chinese medicine in the treatment of senile OP (SOP) from the perspective of cell aging. Based on the current research, Zhang et al. suggested that the use of traditional Chinese medicine formulations and their active ingredients for targeting cellular aging in the treatment of SOP has broad application prospects. The potential of plant natural products to target cellular aging and exert anti-OP therapeutic effects is worthy of further exploration and research. Tang et al. comprehensively summarized the potential of *Cornus officinalis* for treating OP, and they also summarized the effective active ingredients of *Cornus officinalis*; this study provided a complete list of possible effective ingredients in *Cornus officinalis* for the treatment of OP. Wang et al. reviewed the impact of the pilose antler polypeptide on the mechanism of bone homeostasis; these results provided detailed evidence of the role of the pilose antler polypeptide in maintaining the dynamic balance of osteoblasts and osteoclasts. Gu et al. summarized research on the treatment of rheumatoid arthritis (RA) with OP using single traditional Chinese medicine formulations. Similar to the studies of Zhou G. et al., Gu et al.'s study provides additional information on the use of traditional Chinese medicine for the treatment of the comorbidity of RA combined with OP. Tea, as a leisure drink, is loved by many people worldwide. Xie et al. summarized the mechanism of tea in treating OP, OA, and RA, and the results provide comprehensive information on the use of tea in treating these three diseases related to bone aging. Zeng et al. offered significant research advancements that can be utilized as guidelines for the treatment of KOA with integrative medicine based on traditional Chinese medicine. The publication of guidelines will promote the standardization of clinical practice. We hope that these guidelines can provide scientific and effective guidance for the treatment of KOA and that the herbal therapies included in these guidelines will be thoroughly validated in a wider range of clinical applications.

In summary, multiple studies have investigated the use of plant-based natural products for the treatment of bone aging and

OP. These studies provide convenient and extensive information on the use of plant-based natural products, especially traditional Chinese medicines, in the treatment of diseases associated with aging bone. We thank all the authors, peer reviewers, and Frontiers staff for their rigorous and professional contributions. Without their help, successfully presenting this summary would not have been possible. We believe that all the information contained in this review will be beneficial for promoting scientific progress in the study of plant-based natural products for bone aging.

Author contributions

L-FZ: Conceptualization, Writing – original draft, Writing – review & editing. CL: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The mechanism of palmatine-mediated intestinal flora and host metabolism intervention in OA-OP comorbidity rats

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Background: ErXian decoction is a Chinese herbal compound that can prevent and control the course of osteoarthritis (OA) and osteoporosis (OP). OP and OA are two age-related diseases that often coexist in elderly individuals, and both are associated with dysregulation of the gut microbiome. In the initial study, Palmatine (PAL) was obtained by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and network pharmacological screening techniques, followed by 16S rRNA sequencing and serum metabolomics of intestinal contents, to explore the mechanism of PAL in the treatment of OA and OP.

Methods: The rats selected for this study were randomly divided into three groups: a sham group, an OA-OP group and a PAL group. The sham group was intragastrically administered normal saline solution, and the PLA group was treated with PAL for 56 days. Through microcomputed tomography (micro-CT), ELISA, 16S rRNA gene sequencing and non-targeted metabonomics research, we explored the potential mechanism of intestinal microbiota and serum metabolites in PAL treatment of OA-OP rats.

Results: Palmatine significantly repair bone microarchitecture of rat femur in OA-OP rats and improved cartilage damage. The analysis of intestinal microflora showed that PAL could also improve the intestinal microflora disorder of OA-OP rats. For example, the abundance of Firmicutes, Bacteroidota, Actinobacteria, Lactobacillus, unclassified_f_Lachnospiraceae, norank_f_Muribaculaceae, Lactobacillaceae, Lachnospiraceae and Muribaculaceae increased after PAL intervention. In addition, the results of metabolomics data analysis showed that PAL also change the metabolic status of OA-OP rats. After PAL intervention, metabolites such as 5-methoxytryptophol, 2-methoxy acetaminophen sulfate, beta-tyrosine, indole-3-carboxylic acid-O-sulfate and cyclodopa glucoside increased. Association analysis of metabolomics and gut microbiota (GM) showed that the communication of multiple flora and different metabolites played an important role in OP and OA.

Conclusion: Palmatine can improve cartilage degeneration and bone loss in OA-OP rats. The evidence we provided supports the idea that PAL improves OA-OP by altering GM and serum metabolites. In addition, the application of GM and serum metabolomics correlation analysis provides a new strategy for uncovering the mechanism of herbal treatment for bone diseases.

KEYWORDS

osteoarthritis, osteoporosis, palmatine, gut microbiota, metabonomics

1. Introduction

Osteoarthritis (OA) and osteoporosis (OP) have gradually become an urgent global public health problem and a large number of middle-aged and elderly people, an important component of the burden of social medical costs (1, 2). According to previous studies, OA would become the fourth global pandemic by 2020 (3). Furthermore, OP is a very common disease that affects 200 million people worldwide (4). Importantly, OA and OP are two kinds of bone diseases that are closely related and have the noteworthy feature of abnormal reconstruction of subchondral bone (5). Although clinically OP and OA have different pathological characteristics, their risk factors are similar and closely related and include aging, metabolic changes and inflammation (6). Therefore, both diseases are described as age-related. Due to the high prevalence of OA and OP and their heavy burden on patients and the function of social medical services, OA and OP have gradually become an ongoing focus in current scientific research (7). Studies have shown that both OA and OP are closely related to the equilibrium state of the gut microenvironment, suggesting a potentially important factor imbalance in the gut microbiota (8). An increasing number of human and animal studies, especially in recent years, have shown the existence of the intestinal bone axis and have revealed that there are some correlations between the transgenes and their metabolites and the pathogenesis of OA and OP, which may be a potential target for intervention (9).

There is increasing evidence that there is an inextricably linked relationship between the gut microbiome (GM) and bone homeostasis involving host microbiome crosstalk (8). The GM is a very diverse ecosystem consisting of 10 to 100 trillion microorganisms and plays an important role in the body's metabolic system. The effects of the intestinal flora on OP and OA share many common mechanisms, including influencing nutrient absorption, changing hormone levels, altering the intestinal mucosal barrier and mediating immunity (10–13). Studies on OP have shown that the consumption of specific metabolites, such as Glucagon-like peptide-1 and peptide YY, by gut microbes affects the endocrine functioning of the host, and these metabolites play a regulatory role in OP processes, regulates the differentiation of bone marrow Mesenchymal stem cell between bone cells and adipocytes (14, 15). In OA, inflammatory status, obesity, metabolic syndrome and intestinal flora disorder are strongly correlated and closely related to OA risk. For example, disruption of the GM may slow OA by reducing inflammatory states and reducing the expression of Wnt signaling regulatory proteins (16). People pay more and more attention to the disease of bone metabolism from the perspective of intestinal flora. GM affects joints by regulating inflammation and metabolism, but GM alone does not fully explain the phenomenon. Thus, the combined application of omics techniques, such as metabolomics and 16S RNA sequencing, to explore this complex problem becomes particularly important.

Traditional Chinese medicine decoction ErXian decoction (EXD) was included in the famous Ming Dynasty medical book Wenbing Tiaobian (17). In clinic, erxian decoction has a good curative effect (18). Our previous research results showed that joint disease from both OA and OP produced more severe cartilage damage than that from OA alone. Notably, EXD can significantly improve cartilage damage and reverse the protein expression of SOX9, COL2A1, and COMP. In addition, This benign intervention involves cysteine, deoxycholate, and D-turanose, as well as their associated glycolysis/gluconeogenesis, pantothenic acid, and CoA biosynthesis (19). In this study, in combination with network pharmacology, we screened the possible monomer components of EXD in the treatment of OP and OA with palmatine (PAL). PAL has been found to have unique pharmacological effects, including anticancer, antioxidant, anti-inflammatory, antibacterial, antiviral and lipid-modulating effects (20). It has been shown that PAL can decrease the differentiation of osteoclasts by inhibiting the expression of RANKL and OPG in osteoblasts (21). In addition, In the treatment of OA, PAL can improve the pathological progression of OA by inhibiting Wnt/β-catenin and Hedgehog signaling pathway (22). Therefore, PAL may be an effective drug for the treatment of OA and OP. In this study, we used a variety of technical approaches to explore the potential mechanisms of Pal in the treatment of OA and OP. Moreover, 16S rRNA gene sequencing and metabolomics techniques were used to study the mechanism of PAL treatment

Abbreviations: OA, osteoarthritis; OP, osteoporosis; PAL, palmatine; micro-CT, microcomputed tomography; H & E, hematoxylin and eosin; GM, gut microbiota; TCMSP, the traditional Chinese medicine systems pharmacology database and analysis platform; EXD, ErXian decoction; OB, oral bioavailability; BATMAN-TCM, a bioinformatics analysis tool for molecular mechanism of traditional Chinese medicine; ACLT, anterior cruciate ligament resection; DL, drug similarity; PPI, protein–protein interaction; OVX, ovariectomy; LDA, linear discriminant analysis effect size (LEfSe); OARSI, osteoarthritis research society international; OPLS-DA, orthogonal least partial squares discriminant analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; VIP, variable importance in the projection.

of OA and OP from the perspective of intestinal flora and host metabolism.

2. Materials and methods

2.1. Screening of potential components of EXD in the treatment of OA and OP

2.1.1. Construction of the drug activity component library

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP)[1] and the Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine (BATMAN-TCM)[2] databases were used to retrieve the active ingredients of EXD. The chemical composition information of EXD were retrieved from TCMSP. A screening condition of 30% oral bioavailability (OB) and 0.18 drug similarity (DL) was established to achieve the active ingredients. The BATMAN-TCM procedure set the similarity score between drug and target as score cutoff + 20 and adjusted *P*-value 0.05.

2.1.2. Disease target data set

Search and filter relevant gene targets using the keyword "Osteoarthritis" and "Osteoporosis" on GeneCard,[3] DisGeNET,[4] OMIM,[5] DrugBank,[6] and PharmGKB[7] databases. Using Venn diagrams, drugs and disease targets were shown logically. Drug targets were mapped to disease targets, and the common targets of EXD for OA and OP were screened.

2.1.3. PPI network and core target analysis

STRING database was used to analyze the protein interaction networks based on the common targets obtained, and a protein-protein interaction diagram (PPI) was created. Multiple proteins, Homo sapiens, with a confidence level of 0.7 were the conditions. The "CytoNCA" plug-in in Cytoscape software was used for topology attribute analysis to obtain the topology parameters of betweenness centrality (BC), closeness centrality (CC), degree centrality (DC), eigenvector centrality (EC), network centrality (NC), and local average connectivity (LAC). To form the core PPI network, nodes with BC, CC, DC, EC, NC, and LAC greater than the median were screened out.

2.1.4. Networking of drugs and diseases

A target network for EXD treatment for OA and OP was constructed using Cytoscape (3.7.2). For the visual analysis of the target network, each node represents the relevant target of EXD and OA-OP, and the edge represents the interaction between these biological analyses.

1 https://old.tcmsp-e.com/tcmsp.php
2 http://bionet.ncpsb.org.cn/batman-tcm/
3 https://www.genecards.org/
4 https://www.disgenet.org/
5 https://omim.org/
6 https://go.drugbank.com/
7 https://www.pharmgkb.org/

2.2. Experimental verification

2.2.1. Drugs and reagents

Palmatine (PAL, purity >98%, HY-N0110A) was provided by MCE (NJ, USA); Osteopontin (OPN) Antibody (#AF0227)\ MMP13 Antibody (#AF5355)\MMP3 Antibody (#AF0217) was provided by Affinity Biosciences (Jiangsu, China). RUNX2 Antibody (sc-390351) was provided by Santa Cruz Biotechnology (CA, USA). The enzyme-linked biotechnology company (Shanghai, China) provided Rat Estradiol (E2) ELISA Kit (YJ002871),Rat Bone gla protein; Osteocalcin (BGP; OCN) ELISA Kit (YJ420711),Rat alkaline phosphatase (ALP) ELISA Kit (YJ003360), Rat 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$) ELISA Kit (YJ403912),Rat phosphorus (P) ELISA Kit (YJ103904) and Rat calcium (Ca) ELISA Kit (YJ103924).

2.2.2. Instruments

Multifunctional microplate reader (Enspire, Perkin Elmer, USA); Skyscan 1276 Micro-CT Imaging System (Skyscan, Kontich, Belgium); triple Tof5600 triple quadrupole mass spectrometry, AB SCIEX; ExionLC AD liquid chromatography System, AB SCIEX; HSS T 3 column (100 mm × 2.1 mm I.D., 1.8 MM), waters, USA; Abi Geneamp 9700 PCR machine (ABI, USA); the Milli-Q ultra-pure water system (Millipore, Billerica, MA, USA); centrifuge 5702 low-speed centrifuge (Eppendorf, Germany); Leica DMI8 fluorescence inversion microscope (Leica, Germany).

2.2.3. Animal experiment and sample collection

Under the approval of the Animal Ethics Committee of Nanjing University of Chinese Medicine (202209A034), female Sprague Dawley (SD) rats (6 to 8 weeks of age) were purchased from GemPharmatech Co., Ltd. The animals were raised in a controlled temperature, humidity and 12 h/dark environment. As previously reported, the OA-OP rat model was established through the combination of anterior cruciate ligament resection (ACLT) and ovariectomy (OVX) (19). Two week after recovery from surgery, Three groups of rats were assigned randomly: blank (n = 6), OA-OP + PAL (n = 6) and OA-OP (n = 6) (23). In the PAL group, PAL (100 mg kg^{-1}) was administered by gavage once a day for 56 days (24, 25). All rats were sacrificed on the 56th day to obtain femurs, tibias and blood. The rats were first weighed and anesthetized with a 3% sodium pentobarbital solution at a dose of 100 mg/kg according to body weight, and then the rats were euthanized by treatment of cervical dislocation. The collected blood samples were coagulated and centrifuged (2000 rpm, 10 min, 4°C) to obtain serum.

2.2.4. Microcomputed tomography

The lower limbs of rats were fixed with 4% paraformaldehyde for 24 h and then soaked in PBS solution until used. We performed Micro-CT scans using the Skyscan 1276 Micro-CT system (Skyscan, Kontich, Belgium). Bone trabecular volume fraction (BV/TV,%), trabecular number (TB. N,/MM), trabecular thickness (TB. Th, MM) and trabecular space (TB. SP, MM) were calculated.

2.2.5. ELISA Assay

According to the manufacturer's instructions, Ca, P, $1,25(OH)_2D_3$, E_2, BGP, OCN, and ALP were determined in rat serum with ELISA kits.

2.2.6. Testing and evaluation of histopathology

After the intervention, the rats were euthanized and the samples of knee joints were collected. Fixed with 4% paraformaldehyde for 24 h. Decalcification was carried out with 10% EDTA solution. The sections were made after paraffin embedding. H & E, Lycopene O/rapid green and TRAP staining were performed. After staining was completed, image acquisition was performed using an upright light microscope (Eclipse E100, Nikon, Japan) and an imaging system (DS-U3, Nikon, Japan).

2.2.7. Measurement of serum metabolites using LC–MS/MS

2.2.7.1. Metabolite extraction

A liquid sample of methanol: acetonitrile (1:1, V/V) solution was used. The mixture was then sonicated at 5°C at 40 khz. The sample was placed at −20°C to precipitate the protein. The supernatant was evaporated and dried under the flow of nitrogen after centrifugation at 4°C for 10 min at 12000 × *g*. For UHPLC-MS/MS analysis, samples were reconstructed in a loading solution (1:1, V/V) of acetonitrile: water by transient sonication in a 5°C water bath. The extracted metabolites were centrifuged at 4°C, 12000 × *g* for 1 min, and the cleared supernatant was transferred to sample vials for LC-MS/MS analysis.

2.2.7.2. UPLC–MS/MS analysis

2.2.7.2.1. Chromatographic conditions

The samples were separated on HSS T 3 column before entering into mass spectrometry. Using 0.1% formic acid in water: acetonitrile (95:5, V/V) as mobile phase A, 0.1% formic acid in acetonitrile: Isopropanol: water (47.5:47.5, V/V) as mobile phase B, the volume of each sample was 10 μl and the analysis time was set at 5 min, the flow rate of 0.4 mL/min and the column temperature of 40°C were maintained at the same time.

2.2.7.2.2. MS conditions

The UPLC system was designed under the following conditions: the source temperature was 550°C, the curtain gas (CUR) was 30 psi, the ion source GAS1 and Gas2 were 50 psi, the ion spray voltage floating (ISVF) was −4000 V, the positive mode was 5000 V, the potential of de-clustering was 80 V, and the voltage of ion spray was −4000 V The collision energy (CE) of 20–60e V rolling MS/MS data acquisition was adopted in the information dependent acquisition (Ida) mode. Samples were tested in the range of 50–1000 m/z.

2.2.7.3. Data processing and annotation

liquid chromatography-tandem mass spectrometry data were preprocessed by Progenesis QI (Waters Corporation, Milford, CT, USA) following mass spectrometry detection, and a three-dimensional data matrix in comma separated value (CSV) format was exported. At the same time, the metabolites were searched and identified in the Human Metabolome Database (HMDB), Metlin MassBank and MzCloud databases.

Perform variance analysis was conducted on the matrix file after data preprocessing. The R package ropls (Version 1.6.2) was used to perform orthogonal least partial squares discriminant analysis (OPLS-DA) and 7-cycle interactive validation was used to evaluate the stability of the model. The selection of significantly different metabolites was determined based on the variable importance in the projection (VIP) obtained by the OPLS-DA model and the *p*-value of Student's *t*-test, and the metabolites with VIP > 1 and $p < 0.05$ were significantly different metabolites. Differential metabolites among the two groups were summarized and mapped into their biochemical pathways through metabolic enrichment and pathway analysis based on a Kyoto Encyclopedia of Genes and Genomes (KEGG) database search.

2.2.8. 16S RNA sequencing

2.2.8.1. DNA extraction

A soil DNA kit (Omega Bio Tek, Norcross, GA, USA) was used to extract the total genomic DNA from the microbial community.

2.2.8.2. Library preparation and sequencing

Samples meeting quality control criteria were subjected to amplification of the V3-V4 region of the 16S rRNA gene by Abi GeneAmp 9700 PCR thermal cycler (ABI, CA, USA) using primers from 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), Purified amplicons were pooled in equimolar amounts and were paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, CA, USA). Raw sequencing reads were deposited in NCBI's Sequence Read Archive (Accession Number: SUB12688536).

TABLE 1 A total of 17 common active components.

Name	Formula	Class	MZMED
Palmitic acid	C16H32O2	Fatty acyls	255.2325528
Vanillin	C8H8O3	Phenols	153.0544604
Dictamnine	C12H9NO2	Alkaloids	200.0706866
Adenine	C5H5N5	Alkaloids	136.0618078
Ligustilide	C12H14O2	Dihydrofurans	191.1068483
Palmatine	C21H22NO4 +	Alkaloids	352.1546634
Jatrorrhizine	C20H20NO4 +	Alkaloids	338.1387217
Berberine	C20H18NO4 +	Alkaloids	336.1233456
Baohuoside I	C27H30O10	Flavonoids	515.1902982
Kaempferol	C15H10O6	Flavonoids	287.0548828
Epimedin A	C39H50O20	Flavonoids	839.289447
Icariin	C33H40O15	Flavonoids	677.2423439
Hyperoside	C21H20O12	Flavonoids	465.1021701
Isomangiferin	C19H18O11	Xanthones	423.0916829
Mangiferin	C19H18O11	Xanthones	423.0919346
Sarsasapogenin	C27H44O3	Terpenoids	417.3354335
Anhydroicaritin	C21H20O6	Flavonoids	369.1328378

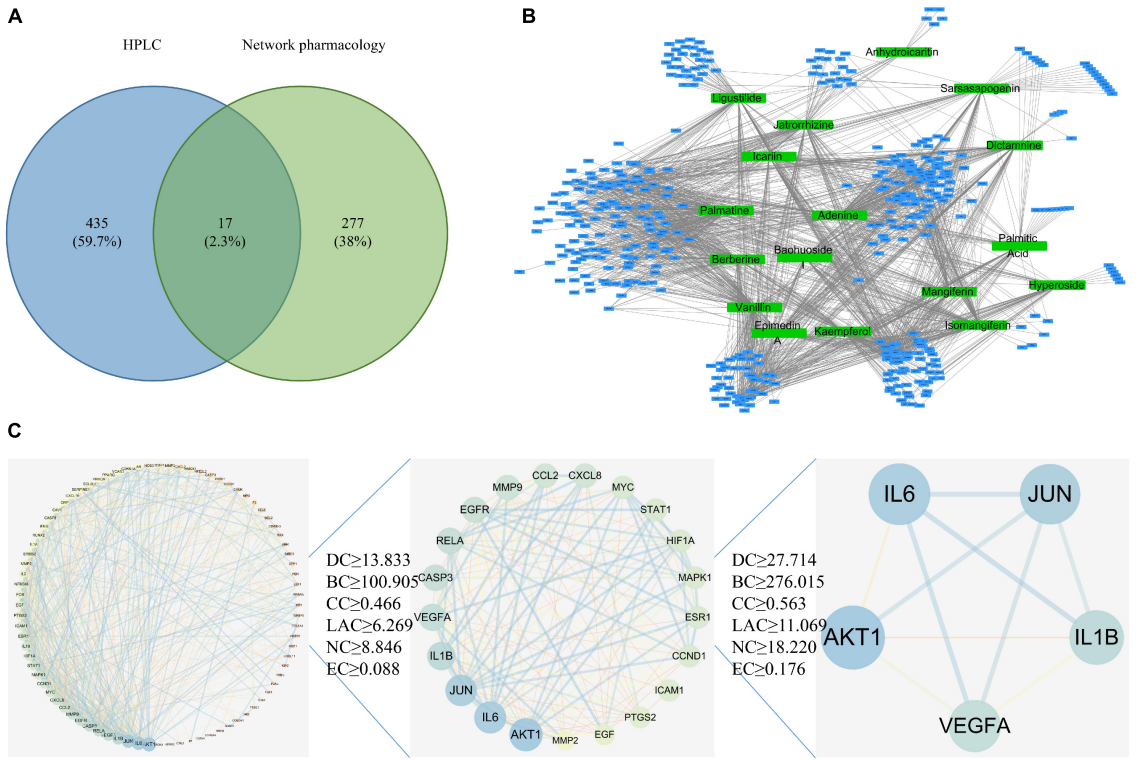

FIGURE 1
Network pharmacology analysis. **(A)** Common target acquisition. **(B)** Drug-disease target network construction. The blue circles represent common genes, and the green circles represent active ingredients of EXD. **(C)** PPI network diagram.

2.2.8.3. Analysis of sequencing data

Similarity of microbial communities between samples was determined by principal coordinate analysis (PCOA) based on Bray-curtis dissimilarity, using the vegan v2.5-3 package. Permutation multivariate analysis of variance (Permanova) test was used to assess the percentage of variance explained by treatment of vegan v2.5-3 packages and its statistical significance. According to the LEfSe linear discriminant analysis analysis, there were significantly abundant taxa (phylum genus) in different groups of bacteria (LDA score >2, $p < 0.05$). Species were selected for correlation network plot analysis according to Spearman correlation $|r| > 0.6$ $p < 0.05$.

2.2.9. Western blotting

Rat cartilage and femur were ground in liquid nitrogen and homogenized with Ripa buffer. The protein concentration was determined by BCA method. Proteins were first separated using 10% PAGE and then transferred to PVDF membranes. PVDF membranes were blocked with TBST buffer containing 5% skim milk powder for 1 h at room temperature and then rinsed with TBST. After incubation with the first antibody at 4°C overnight, the membrane was incubated with the corresponding second antibody for 1 h. Protein bands were displayed using chemiluminescent reagents, and ImageJ was used to quantify protein intensity.

2.2.10. Statistical analysis

SPSS software 22.0 and GraphPad Prism 8.0 were used for data analysis. The results are expressed as the mean ± standard deviation ($x \pm s$). Group comparisons were assessed with one-way ANOVA, and $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Screening of active ingredients of drugs

According to the screening conditions, 317 kinds of effective ingredients from EXD were obtained. After deleting invalid and repeated targets, 294 active ingredients were included in this analysis. According to our previous research, 452 monomer components were obtained by HPLC analysis. The network pharmacology screening results and the identification results of HPLC were mapped, and 17 common components were obtained, as shown in Table 1 and Figure 1A.

Based on 17 common components and the GeneCard, DisGeNET, OMIM, DrugBank, and PharmGKB databases, 447 drug targets were screened. A network diagram of the common targets of the drug active ingredients was constructed by using Cytoscape software and included 464 points and 1677 edges (Figures 1B , C). With the degree value as the main reference for topological analysis, the top five active ingredients were palmatine, adenine, vanillin, kaempferol, and isomangiferin. These top drug ingredients may be the key compounds of EXD in the treatment of OA and OP. According to the topological analysis results and previous studies (Supplementary Table 1), we chose PAL

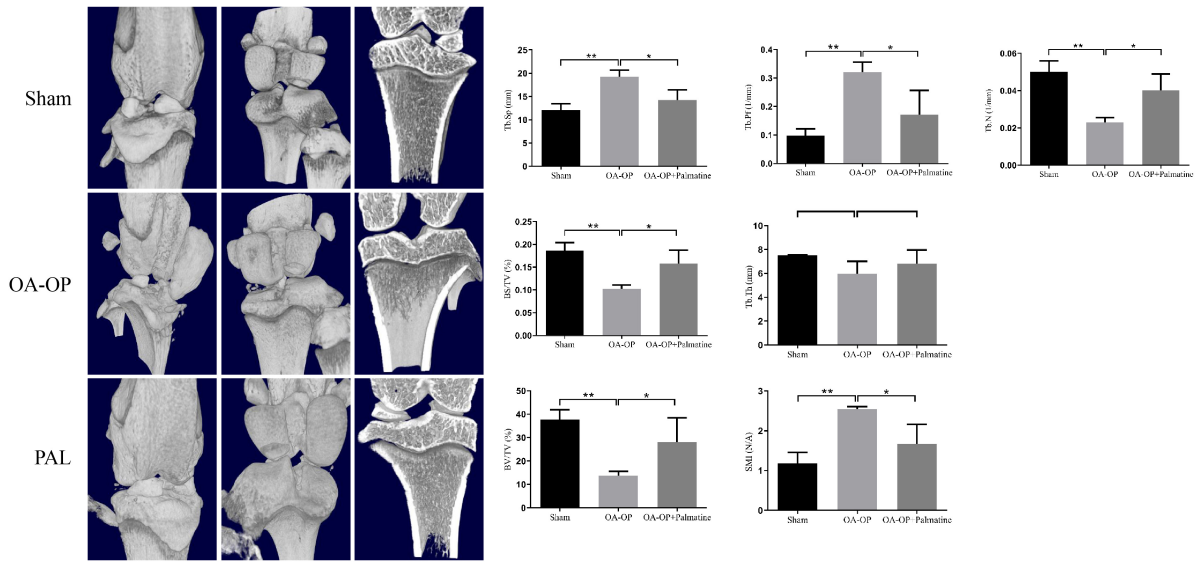

FIGURE 2
Effect of PAL on bone trabecular morphometric parameters in OA-OP rats. Micro-CT images of the distal femur and proximal tibia 56 days after PAL administration; Tb. N, BV/TV, BS/TV, Tb. Th, Tb. Pf, Tb. Sp, and SMI, 56 days after PAL administration Micro-CT analysis. *Statistically significant difference ($P < 0.05$). **Statistically significant difference ($P < 0.01$).

to carry out follow-up experiments (19). A detailed procedure how palmatine was identified was illustrated in the flow chart (**Supplementary File 1**).

3.2. Palmatine improves the bone structure of rats

Micro-CT has become a key tool for evaluating bone microstructure in animal models of OP (26). The isolated femur samples from each group were scanned with micro-CT, and the area of interest was reconstructed in three dimensions (**Figure 2**). The results showed that compared with the sham group, the OA-OP group trabecular bone was reduced and broken, and the distance between the trabecular bone was widened, indicating that the bone microstructure in the OA-OP group was destroyed, and that bone loss and osteoporosis occurred. However, after PAL intervention, it was observed that the number of bone trabecule increased, the distance between bone trabecule decreased, the thickness of bone trabecule increased, and the bone strength partially recovered, preventing the process of bone loss. In addition, compared with the sham group, femur Tb. N, BV/TV, BS/TV, and Tb. Th in the model group were significantly decreased ($p < 0.05$), while Tb. Pf, Tb. Sp, and SMI were significantly increased ($p < 0.05$). Tb. Pf and SMI can measure the degree of rod-like and plate-like bone trabecula, and their increase indicates the change in bone trabecula from plate-like to rod-like. Combined with the decrease in bone volume and bone trabecular thickness and number, the rat OVX model was successfully established. Compared with the OA-OP group, Tb. N, BV/TV, BS/TV, and Tb. Th of the femur in the PAL group was significantly increased ($p < 0.05$), Tb. Pf and SMI were significantly decreased ($p < 0.05$).

3.3. Effect of palmatine on bone metabolism in rats

The results show (**Figure 3**), compared with the sham group, the OA-OP group serum E2, ALP, BGP, and $1,25(OH)_2D_3$ contents decreased, while the Ca content increased ($p < 0.05$). The levels of E2, ALP, BGP, and $1,25(OH)_2D_3$ in serum of OA-OP rats were increased in PAL group ($p < 0.05$).

3.4. Palmatine improved OA and OP processes in rats

Compared with the OA-OP group, the sham group cartilage surface was smooth, while the PAL group showed less cartilage destruction. The OARSI score was consistent with that of solid green staining, and OA-OP group's score was significantly higher than SHAM group's, the OARSI score of the PAL group was lower than that of the OA-OP group. Compared with the sham group, in the OA-OP group, H & E staining of femur showed that PAL could ameliorate the destruction of extra-articular matrix induced by OA-OP. In TRAP staining, the number of positive cells in SHAM group was less than that in OA-OP group, while PAL reduced the number of positive cells in OA-OP group. The results of WB (**Figure 4**) showed that the protein expression levels of MMP3 and MMP13 in the cartilage of the OA-OP group were increased ($p < 0.05$), while PAL could decrease the protein expression levels of MMP3 and MMP13 in the cartilage of the KOA group ($p < 0.05$). In the OA-OP group, the protein expression levels of Runx-2 and OPN were decreased ($p < 0.05$), whereas PAL could increase the protein expression levels of RUNX-2 and OPN in KOA cartilage ($p < 0.05$).

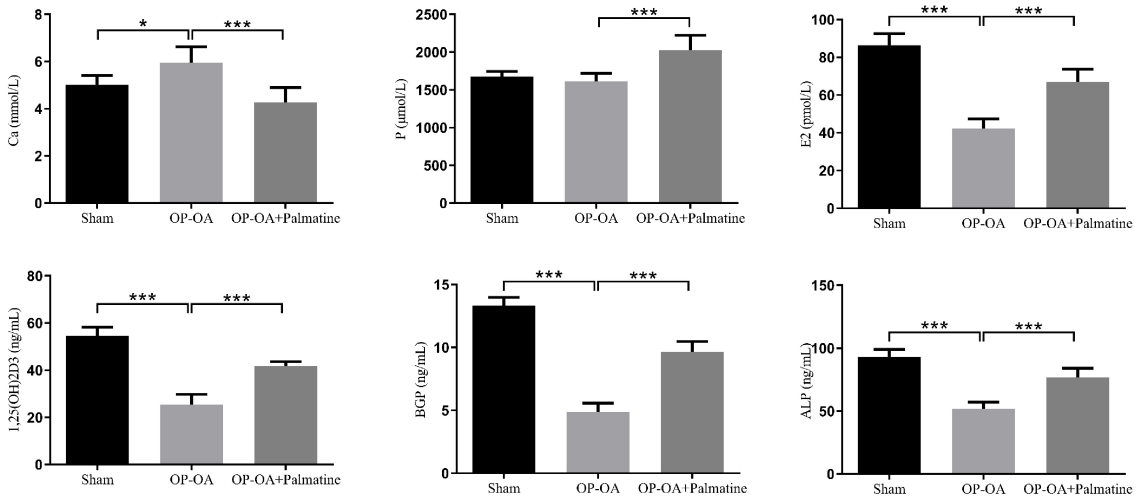

FIGURE 3
Effect of PAL on bone metabolism in OA-OP rats. Quantitative analysis of serum levels of E2, ALP, BGP, 1,25(OH)2D3, Ca, and P. *Statistically significant difference ($P < 0.05$). **Statistically significant difference ($P < 0.01$). ***Statistically significant difference ($P < 0.001$).

3.5. PAL effects on OA-OP rats' intestinal flora composition

To further study the effect of PAL on intestinal microflora, fecal microflora of 18 samples from 3 groups were analyzed by 16S RNA sequencing. For the α-diversity, there were significant differences in the ACE, Chao1 and Shannon indexes among the three groups (Figure 5A), suggesting that PAL treatment may have a significant effect on the intestinal microflora of OA-OP rats. Using the PCoA method, we analyzed β-diversity. As shown in Figure 5B, PCoA showed a significant clustering of microbiota composition in each group and showed that the microbiota community of rats in the OA-OP group was significantly different from that in the sham and PAL groups, suggesting that PAL treatment may improve OA and OP in rats by regulating GM imbalance. Next, to assess specific changes in GM, the relative abundance of dominant groups was further analyzed (Figure 5C). At the phylum level, Firmicutes, Bacteroidota and Actinobacteriota were predominant. At the genus level, Lactobacillus, unclassified_f_Lachnospiraceae and norank_f_Muribaculaceae were predominant. At the family level, Lactobacillaceae, Lachnospiraceae and Muribaculaceae were predominant. To further explore the differences in intestinal microbiota among the sham, OA-OP and PAL groups, we used LEfSe to identify specific changed bacterial phenotypes at each phylogenetic level. As shown in Figure 5D, $p < 0.05$ and LDA > 3.0 were biomarkers of significant differences in the screening rank sum test. A total of 27 specific bacteria were divided into 3 groups, including 5 specific bacteria in the sham group, 19 in the OA-OP group and 3 in the PAL group.

3.6. Effect of PAL on host metabolism in OA-OP rats

Blood samples were analyzed by LC-ESI-MS/MS system to study the metabolic status of each group of hosts. PLS-DA was used to assessed differences in blood metabolic profiles between the two groups. The PAL group was significantly separated from the OA-OP group, indicating that PAL could regulate the metabolism of model rats (Figures 6A, B). The PAL group was significantly separated from the OA-OP group, indicating that PAL could regulate the metabolism of model rats. In the next step, potential biomarkers were screened between the sham and OA-OP groups based on OPLS-DA. Moreover, the PLS-DA model was verified by a displacement test, which showed that the fit was good. The online HMDB, Metlin, MassBank, MzCloud, and databases were used to screen 93 potential biomarkers, including 46 upregulated and 47 downregulated metabolites (Figure 6C and Supplementary Table 2). We performed a data clustering analysis of the top 30 metabolites by VIP value to show differences in metabolite expression, (Figure 6D). The horizontal coordinate indicates the sample name, and the vertical coordinate indicates the top 30 differential metabolites according to VIP value. In the figure, red represents upregulation, and green represents downregulation of metabolites. The results showed that there were significant differences in the levels of metabolites between the groups. Furthermore, the metabolic pathways of 93 different metabolites were analyzed to explore the metabolic pathways regulated by PAL. When the $p < 0.05$, the metabolic pathway was considered to be significantly correlated with PAL intervention. These pathways are mainly involved in tyrosine metabolism, phenylalanine metabolism, ubiquinone and other terpenoid-quinone biosynthesis and so on (Figure 6E and Supplementary Table 3).

3.7. Correlation analysis between metabonomics and GM

In Spearman correlation analysis, different metabolites with the top 20 abundance and family, genus and phylum flora were analyzed to determine the effect of PAL on intestinal flora and metabolic relationships in OA-OP

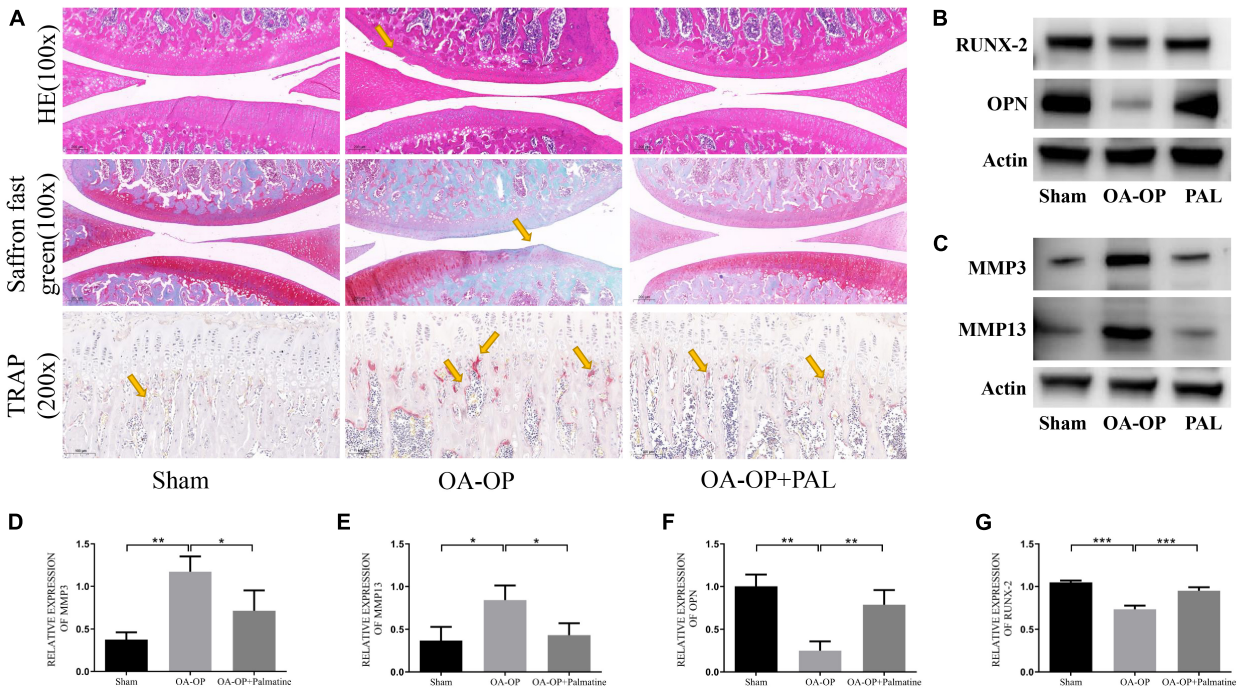

FIGURE 4
Effect of PAL on bone pathological section of Op Oa Rats. **(A)** Representative pictures of rat knee tissue sections stained with H & E, Saffron O/Fast Green and TRAP. **(B)** Representative images of protein bands in rat tissues. **(C)** Representative images of protein bands in rat cartilage. **(D)** The relative expression levels of MMP3 in cartilage. **(E)** The relative expression levels of MMP13 in cartilage. **(F)** The relative expression levels of OPN in femur. **(G)** The relative expression levels of RUNX-2 in femur. *Statistically significant difference ($P < 0.05$). **Statistically significant difference ($P < 0.01$). ***Statistically significant difference ($P < 0.001$).

rats. The data showed that at the family level, PAL treatment changed the abundance of Desulfovibrionaceae, Eggerthellaceae, Peptostreptococcaceae, Ruminococcaceae and norank_o_Clostridia_UCG-014 and affected the metabolic levels of 4-ethylphenylsulfate, LysoPC (20:4(5Z, 8Z, 11Z, 14Z)/0:0), 2-hydroxycinnamic acid, Henicosanoylcarnitine, 1-O-hexadecyl-sn-glycero-3-phosphocholine and deoxycytidine (Figure 7A). At the genus level, the abundance changes in Romboutsia, norank_f_norank_o_Clostridia_UCG-014, Desulfovibrio, and Marvinbryantia affected the levels of Henicosanoylcarnitine, 2-hydroxycinnamic acid, 1-O-hexadecyl-sn-glycero-3-phosphate, deoxycytidine and 4-ethylphenylsulfate (Figure 7B). At the phylum level, the abundance changes of Campilobacterota, Cyanobacteria, Desulfobacterota, Firmicutes, and Patescibacteria affected the levels of 1-O-isopentyl-3-O-octadec-2-enoyl glycerol, 4-ethylphenylsulfate, deoxycytidine, P-coumaric acid, 2-hydroxycinnamic acid, (12Z)-10-hydroxy octadecenoyl carnitine, 1-O-hexadecyl-sn-glycero-3-phosphocholine and isenicosanoylcarnitine (Figure 7C).

4. Discussion

In this study, we first conducted drug monomer screening based on previous research (19), and then used network pharmacology to further refine the selection. Active compounds in EXD were retrieved and screened from TCMSP and BATMAN-TCM databases. These active compounds were matched with UPLC-QTOF-MS data from previous studies (19), resulting in 17 common active ingredients. We then searched and screened gene targets for OA and OP based on GeneCard, DisGeNET, OMIM, DrugBank, and PharmGKB databases. By conducting topological analysis on the 17 common active ingredients and gene targets of OA-OP, and through analysis of the results and previous literature research, we ultimately chose palmatine for further experiments. For a more detailed description of the selection process for palmatine, please refer to Supplementary File 1.

Network pharmacology, metabolism, and intestinal flora are all interconnected in the human body. The relationship between network pharmacology and metabolism is that drugs can interact with metabolic enzymes, which can affect their pharmacokinetics and pharmacodynamics. Drug may be metabolized by a specific enzyme, and genetic variations in that enzyme may affect how the drug is metabolized, leading to differences in drug efficacy and toxicity. The relationship between metabolism and intestinal flora is that the gut microbiome can also affect drug metabolism. Some gut bacteria can produce enzymes that metabolize drugs, which can affect drug efficacy and toxicity. Additionally, the gut microbiome can affect the absorption of drugs in the intestine, which can also impact drug efficacy and toxicity.

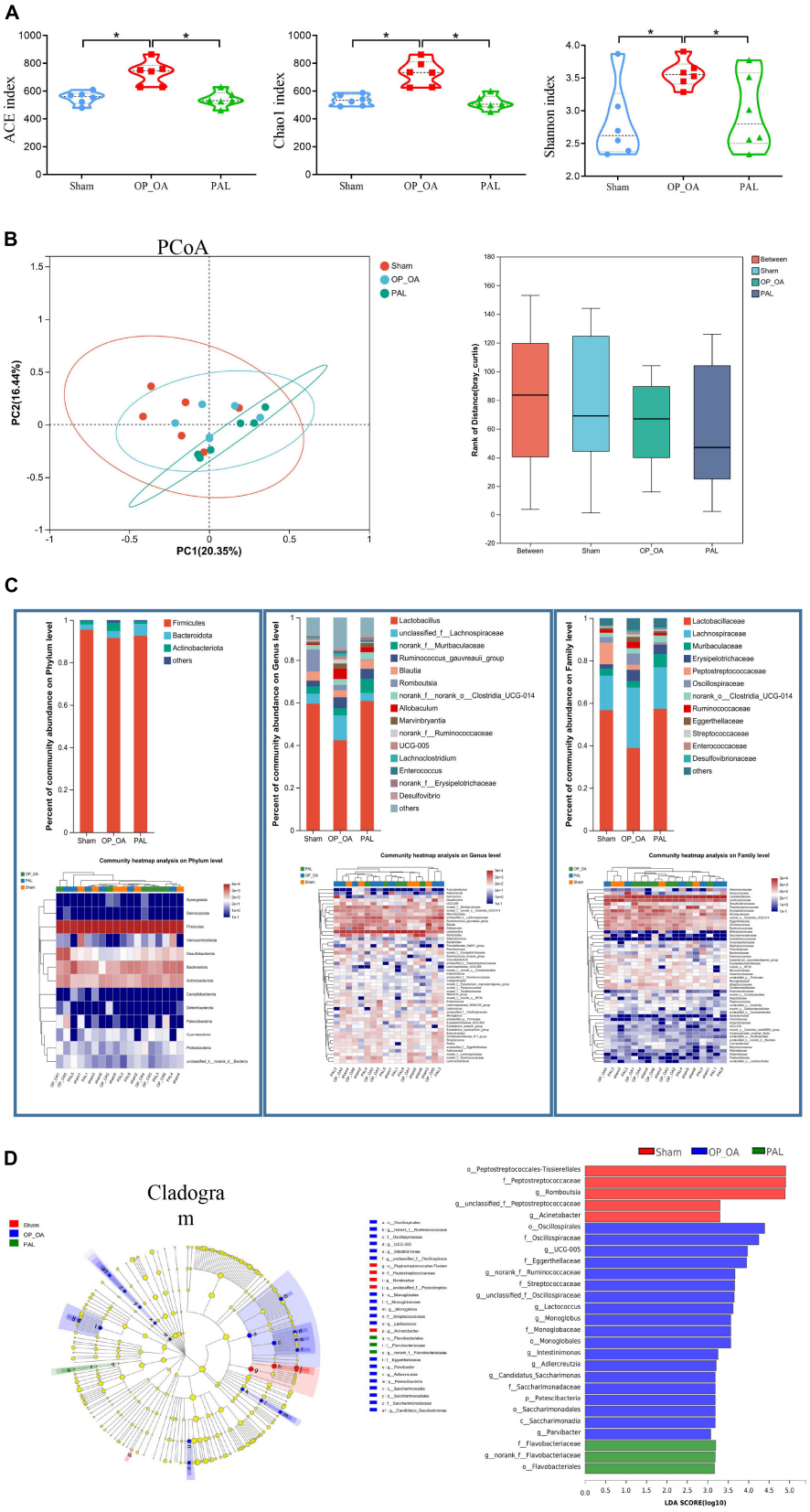

FIGURE 5
Intestinal flora analysis. **(A)** The alpha diversity of intestinal microorganisms was evaluated through Chao1, ACE, and Shannon; **(B)** pCoA scoring chart and box diagram of distance between groups; **(C)** species composition abundance map and abundance clustering heatmap of phylum, genus and family level; **(D)** intestinal microbial LEfSe from domain to species and LDA showed scores of these specific bacteria. *Statistically significant difference ($P < 0.05$).

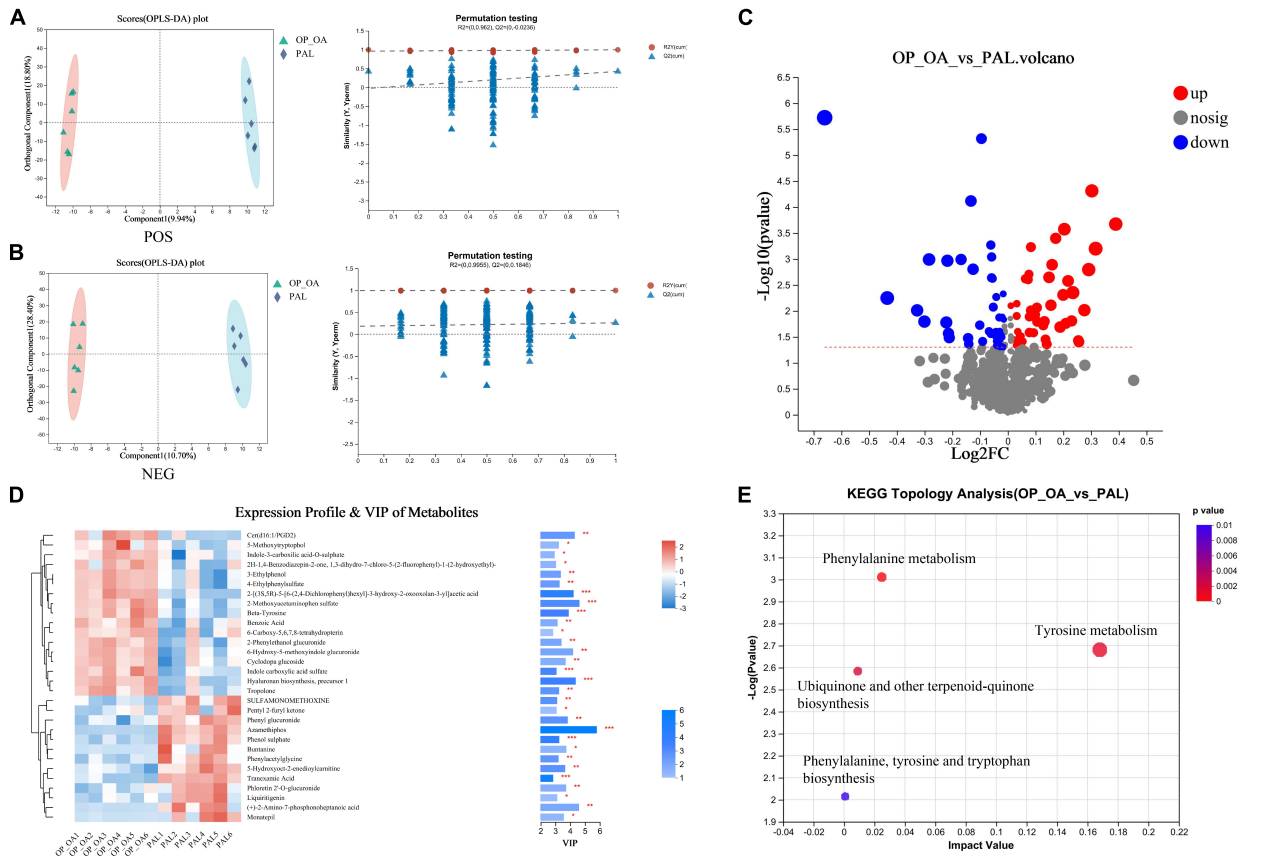

FIGURE 6
Metabonomic analysis. **(A)** OPLS-DA score plot and OPLS-DA permutation test chart (POS); **(B)** OPLS-DA score plot and OPLS-DA permutation test chart (NEG); **(C)** comparison between OA-OP and PAL single-dimensional metabolite volcano map. Threshold setting $p < 0.05$ and $|\log2fc| \geq 1$ (FC, fold change); **(D)** VIP analysis chart. The left side is the metabolite cluster tree, and the color represents the relative expression amount of this metabolite in this group of samples. The right side is a VIP bar graph of metabolites. The bar length indicates the contribution value of the metabolite to the difference between the two groups. The larger the value, the greater the difference between the two groups. The bar color indicates the significant difference in metabolites between the two groups of samples. **(E)** Metabolic pathway analysis of plasma differential metabolites.

In this study, the effects of PAL on the intestinal flora and serum metabolite profiles of OA-OP rats were determined by non-targeted LC–MS/MS metabonomics and 16S rDNA sequencing techniques. The results showed that PAL treatment could affect the serum metabolite profile and intestinal flora of rats. Metabolites including 5-methoxytryptophol, 2-methoxy acetaminophen sulfate, beta-tyrosine, indole-3-carboxylic acid-O-sulfate and cyclodopa glucoside were upregulated in the KOA group compared to the blank group; however, these metabolites were dialed back after PAL treatment. These metabolites are involved in tyrosine metabolism, phenylalanine metabolism, ubiquinone and other terpenoid-quinone biosynthesis. In terms of the intestinal flora, the abundances of Firmicutes, Bacteroidetes, Actinobacteria, Lactobacillus, unclassified_f_Lachnospiraceae, norank_f_Muribaculaceae, Lactobacillaceae, Lachnospiraceae, and Muribaculaceae were similar to those of the sham group after PAL administration. The results showed that PAL affected the levels of metabolites and the abundance of some intestinal flora in OA-OP rats through multiple targets, thereby improving the physical signs of OA-OP mice.

5-methoxytryptophan (5-MTX) has been found to have potential for radical scavenging and antioxidant activity (27). 5-methotrexate also coordinate the circadian rhythms of a variety of mammals (28). In addition, 5-MTX has important biological functions as an antioxidant, immunomodulator and anticancer agent (29). Interestingly, a study indicated that 5-MTX can inhibit osteoclast formation and promote osteoblast differentiation (29). In addition, 5-MTX can inhibit proinflammatory cytokines, reduce the levels of MMP-2 and MMP-9, and improve synovial inflammation in rats (30). 2-Methoxy acetaminophen sulfate is a member of the acetamide group.

Acetamide has long been considered to be related to the levels of glutathione and N-acetylcysteine. N-acetylcysteine can regulate a variety of pathophysiological processes, including oxidative stress, apoptosis, mitochondrial dysfunction, and imbalance of glutamate and dopamine neurotransmitter systems (31). Other studies have shown that 2-methoxy acetaminophen sulfate has an intervention effect on neurodegenerative diseases (such as amyotrophic lateral sclerosis and frontotemporal dementia) (32). Phenylalanine is one of the essential aromatic amino acids of the human body and can only be obtained from the outside world by

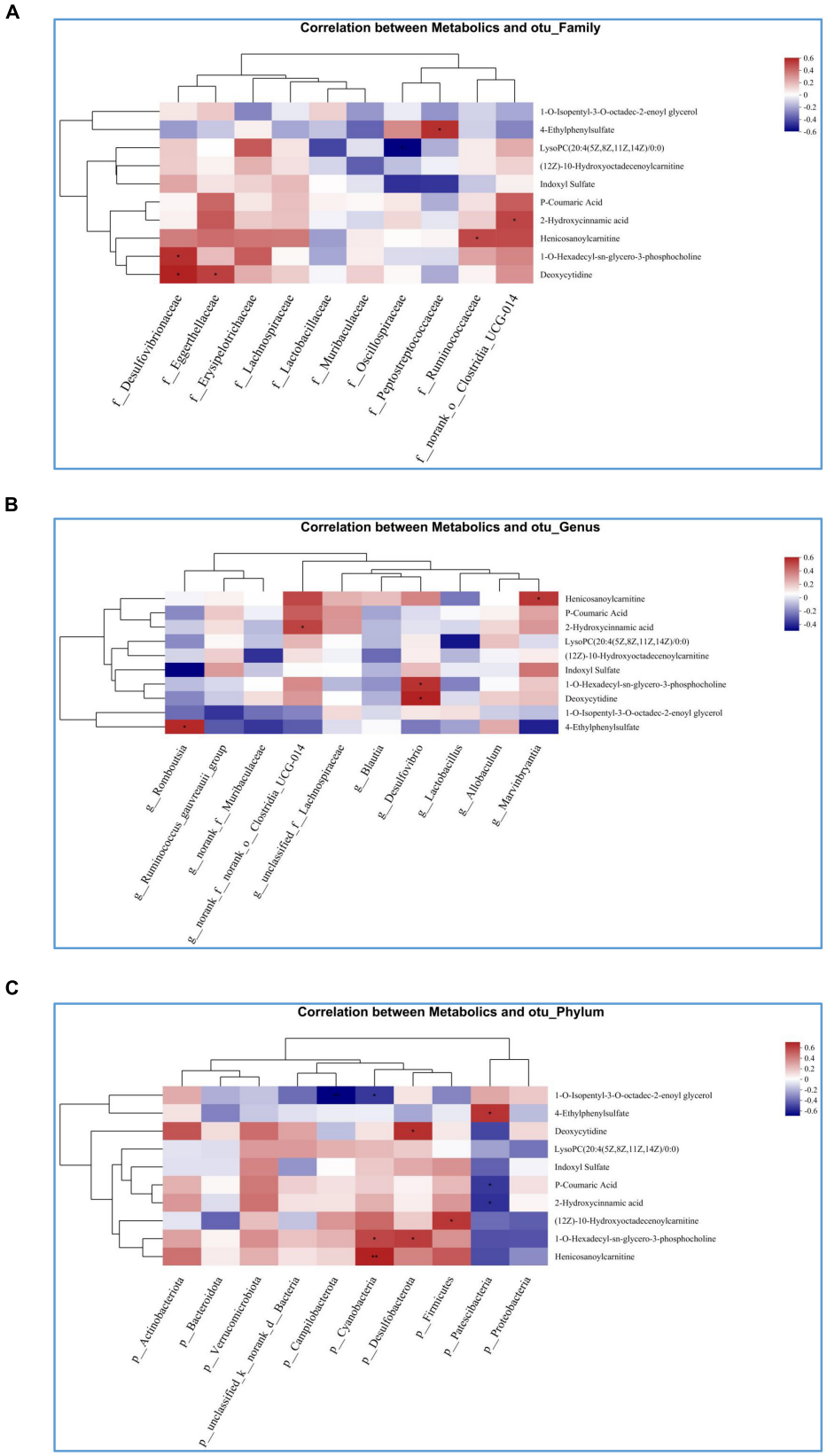

FIGURE 7
Heatmap of the correlation analysis between gut microbiota and targeted metabolic profiling. **(A)** Correlation between intestinal flora and metabolites at the family level; **(B)** correlation between intestinal flora and metabolites at genus level; **(C)** correlation between intestinal flora and metabolites at phyla level. *Statistically significant difference ($P < 0.05$). **Statistically significant difference ($P < 0.01$).

food, because there is no relevant synthesis pathway in the human body. Phenylalanine is catabolic mainly in the liver and is catalyzed to form tyrosine by phenylalanine hydroxylase (33). Tyrosine is a non-essential amino acid found in humans and other mammals. Tyrosine catabolism is catalyzed by a variety of metabolic enzymes, including tyrosine aminotransferase, 4-hydroxyphenylpyruvic acid, 4-hydroxyphenylpyruvate dioxygenase, homogentisic acid, homogentisate 1,2-dioxygenase, fumarylacetoacetase, and fumaric acid (34). Depletion of tyrosine metabolizing enzymes leads to the accumulation of metabolites, which further causes DNA damage, tissue damage, and depletion of intracellular glutathione, eventually leading to apoptosis (35). In addition, excessive accumulation of tyrosine in the body can also cause changes in the functions of several key enzymes in the TCA cycle, such as citrate synthase, malate dehydrogenase and succinate dehydrogenase, resulting in the disorder of energy metabolism in the body and the oxidative stress of mitochondria (36). Our results suggest that PAL can improve disease progression in OA-OP rats by regulating tyrosine and phenylalanine metabolism.

A growing number of studies have shown that an imbalance in intestinal homeostasis may induce several extrenteral immune and metabolic diseases (such as osteoporosis, OA, psoriasis, and systemic lupus erythematosus) (37–39). The intestinal flora is a key factor in activating and maintaining intestinal physiological functions and plays an indelible role in maintaining the health and homeostasis of the host (7). In recent years, an increasing number of human and animal studies have indicated the presence of the gut axis and recognized that the gut joint axis and gastrointestinal microbiome-induced immune and inflammatory responses play an important role in joint health (40). It has been shown that the normal human gut microbiome consists of two main phyla, Bacteroidetes and Firmicutes (41). Bacteroides play a role in bone protection by promoting osteoblast differentiation and inhibiting osteoclast differentiation (42, 43). In our results, Bacteroidetes and Firmicutes were downregulated in the OA-OP group compared with the sham group; however, this situation was restored after PAL administration. Lactobacillus is thought to be a beneficial bacterium that potentially affects immune-related bone health by regulating proinflammatory cytokines and markers related to bone metabolism (44). Muribaculaceae belongs to the phylum Bacteroides and was renamed by Ilias et al. Studies have shown that intermittent parathyroid hormone (PTH) can increase the abundance of Muribaculaceae and increase bone mass in OVX rats (45). An epidemiological analysis showed that Lachnospiraceae abundance was reduced in populations with low bone mineral density (BMD). Our study showed that PAL use increased the abundance of Actinobacteriota, Lactobacillus, Lachnospiraceae and Muribaculaceae in OA-OP rats.

Our results demonstrate the potential of PAL to improve OA and OP dual models in rats, where a variety of gut microbes and metabolites may play a role in the recovery mechanism. However, the study has some limitations, including a limited sample size and identified bacteria and metabolites that could not be described as biomarkers in OA-OP rats. Therefore, the results of this study can only provide some references for exploring the mechanism of bone mass loss and cartilage degeneration in rats with osteoporosis and osteoarthritis inflammation, which needs to be verified in a large sample size study. Further, in animal studies, we will further add different dosing concentrations and positive control drugs in the future to improve the reliability of our conclusions. In addition, we will explore the impact of GM and metabolites on OA-OP through additional experiments in the future.

5. Conclusion

In conclusion, PAL can improve cartilage degeneration and bone mass loss in OA-OP rats. The potential mechanism of action may be related to the improvement of intestinal microbiome ecological imbalance and to balance of phenylalanine/tyrosine metabolism disorder. Moreover, there is a potential role of the GM as a shared mechanism for two common age-related diseases. In addition, the key genera of the GM detected in this study may help identify potential therapeutic targets for joint degradation and bone mass loss in the GM.

Data availability statement

The data presented in this study are deposited in the NCBI repository, accession number PRJNA929116.

Ethics statement

This animal study was reviewed and approved by the Animal Ethics Committee of Nanjing University of Chinese Medicine.

Author contributions

LJ and ZM conceived the study, designed the experiments, participated in the literature searched, and extracted the data. PW and JM participated in study design, drafted the manuscript, devised the study, and oversaw the research program. XS, YG, and LY participated in data analysis, performed the statistical analysis, and drafted the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1153360/full#supplementary-material

SUPPLEMENTARY TABLE 1
Topological analysis based on network pharmacology.

SUPPLEMENTARY TABLE 2
Potential metabolites.

SUPPLEMENTARY TABLE 3
Potential metabolic pathways.

SUPPLEMENTARY FILE 1
The flow chart of a detailed procedure how palmatine was identified.

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Effects of resveratrol in an animal model of osteoporosis: a meta-analysis of preclinical evidence

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Background: Resveratrol is a natural polyphenol compound that is widely present in herbal medicines such as *Reynoutria japonica Houtt.*, *Veratrum nigrum L.*, and Catsiatora Linn and is used in traditional Chinese medicine to treat metabolic bone deseases. Animal experiments have shown that resveratrol may have a strong treatment effect against osteoporosis (OP). The purpose of this study was to explore the efficacy of resveratrol in treating OP animal models based on preclinical research data.

Methods: This study was completed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We searched the PubMed, Embase, Cochrane Library, and China National Knowledge Infrastructure (CNKI) databases from inception to May 8, 2023, to identify animal experiments on the treatment of OP with resveratrol. The effect sizes of bone mineral density (BMD), parameters of micro-CT, serum calcium, phosphorus, alkaline phosphatase (ALP) and osteocalcin were expressed as the mean differences (MDs) and 95% confidence intervals (CIs). RevMan 5.4 software was used for data analysis.

Results: This meta-analysis included a total of 15 animal experiments, including 438 OP rats. The meta-analysis results showed that compared with the control group, resveratrol (<10, 10–25, 40–50, ≥ 60 mg/kg/day) significantly increased femoral and lumbar bone mineral density (BMD) in OP rats ($p < 0.05$). Resveratrol (<10 mg/kg/day) significantly increased the BMD of the total body (MD = 0.01, 95% CI: 0.01 to 0.01, $p < 0.001$). In terms of improving the parameters related to micro-CT, resveratrol (40–50 mg/kg/day) can increase trabecular thickness and trabecular number and reduce trabecular spacing ($p < 0.05$). Compared with the control group, resveratrol can reduce the concentration of calcium and phosphorus in serum but has no significant effect on serum ALP and osteocalcin ($p > 0.05$). The results of subgroup analysis showed that resveratrol increased the whole-body BMD of SD rats ($p = 0.002$) but did not improve the whole-body BMD of 3-month-old rats ($p = 0.17$).

Conclusion: Resveratrol can increase BMD in OP rat models, and its mechanism of action may be related to improving bone microstructure and regulating calcium and phosphorus metabolism. The clinical efficacy of resveratrol in the treatment of OP deserves further research.

KEYWORDS

resveratrol, plant-based natural products, osteoporosis, bone mineral density, meta-analysis, evidence-based medicine

1. Introduction

Osteoporosis (OP) is a systemic bone disease characterized by low bone mass, damage to the microstructure of bone tissue, and increased bone fragility (1). The increased risk of bone fragility and fracture caused by OP poses a heavy economic burden to society and patients (2, 3). The aetiology of OP is complex and diverse, including the interactions between endocrine, nutritional, genetic, physiological, and immune factors (4). Among them, postmenopausal osteoporosis (PMOP) is considered strongly correlated with oestrogen deficiency (5). The increase in bone resorption and the decrease in bone formation lead to an imbalance in bone homeostasis (1, 6), which is closely related to the occurrence of OP. An epidemiological study has shown that the prevalence of OP in people over 50 years old in Europe and America is 4–6%, while in Asian populations, it is over 15% (7). According to the diagnostic criteria of the World Health Organization (WHO), the latest epidemiological research results show that the global prevalence of OP is as high as 19.7% (8, 9). The OP prevalence rates in different countries (4.1% in Netherlands to 52.0% in Türkiye) and continents (8.0% in Oceania to 26.9% in Africa) vary greatly (8, 9). As the population continues to age, OP is recognized as a major public health issue (7). At present, the treatment of OP mainly includes bisphosphonates, parathyroid drugs, or oestrogen replacement therapy (10, 11), all of which have inevitable adverse reactions. Therefore, researching and developing more alternative drugs with fewer side effects and better therapeutic effects is an important topic for OP treatment.

Botanical or traditional medicine have always been breakthrough points in new drug development, mainly due to their higher potential for drug conversion and lower incidence of adverse reactions. Traditional Chinese medicine is also commonly used for the treatment of OP, and its pharmacological mechanism usually has the characteristics of "multiple components, multiple targets, and multiple pathways." Resveratrol is a natural polyphenol compound with a structure similar to oestrogen diethylstilbestrol, which is widely present in herbs such as *Reynoutria japonica Houtt.*, *Veratrum nigrum L.*, and *Catsiatora Linn* (12, 13). Research has shown that resveratrol competitively binds to oestrogen receptors *in vitro*, similar to phytoestrogens, and exerts anti-OP effects (14). Another study showed that resveratrol can affect the metabolism of bone cells and has the ability to regulate bone turnover (15). It is a natural antioxidant that can effectively prevent bone loss caused by oxidative stress in the body. Previous clinical studies have suggested that resveratrol can reduce bone loss and fracture risk in postmenopausal women or diabetes patients (16, 17). However, there is currently a lack of advanced evidence for the use of resveratrol in the treatment of OP; therefore, there is a lack of clarity regarding the application value of resveratrol.

The pre-clinical studies conclusions of animal experiments can provide key information for clinical practice and enhance the understanding of disease mechanisms among clinical and scientific researchers. At present, the clinical evidence for the treatment of OP with resveratrol is very limited, and thus, there is little information regarding the potential medicinal value of resveratrol in OP treatment. However, in the experimental field, studies have examined resveratrol treatment of OP animal models. This systematic review and meta-analysis aimed to evaluate the efficacy of resveratrol in treating OP animal models in order to provide evidence for future research on the anti-OP clinical efficacy of resveratrol.

2. Materials and methods

The implementation of this study strictly followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (18). The data source for this meta-analysis is publicly published papers, which means that ethical review was not needed.

2.1. Eligibility criteria

The inclusion criteria for this meta-analysis were as follows: (1) the study design was a controlled experiment, which means that the study protocol included both an experimental group and a control group, (2) the research object was a female rat model; the species of rats were Albino rats, SD rats, or Wistar rats; the age of rats did not exceed 6 months, and the modelling method was ovariectomy (OVX), (3) the intervention for the experimental group was resveratrol, but the dosage of resveratrol was not limited, (4) comparison: the intervention measures for the control group can be blank control (tap water or normal saline) or other drug treatments, and (5) outcome index: bone mineral density (BMD) (g/cm^2) is the primary outcome measure, and secondary outcomes included trabecular thickness (Tb. Th), trabecular number (Tb. N), trabecular spacing (Tb. SP), serum calcium (mmol/l), serum phosphorus (mmol/l), serum alkaline phosphatase (ALP) (U/l), and serum osteocalcin (nmol/l); furthermore, all outcome indicators must clearly report the results of the measurement data, and the data reporting format must be mean ± standard deviation. There were no restrictions regarding publication language.

Abbreviations: OP, osteoporosis; CNKI, China National Knowledge Infrastructure; MD, mean difference; CI, confidence interval; BMD, bone mineral density; PMOP, postmenopausal osteoporosis; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta analyses; NR, not reported; Tb. Th, trabecular thickness; Tb. N, trabecular number; Tb. SP, trabecular spacing; ALP, alkaline phosphatase; SYRCLE, Systematic Review Center for Laboratory Animal Experience; OVX, ovariectomy; SD, Sprague–Dawley.

2.2. Exclusion criteria

The exclusion criteria were as follows: (1) review, meeting abstract, and case report, (2) incomplete experimental data, and (3) *in vitro* studies or clinical studies.

2.3. Search strategies

We searched the following four databases to obtain animal experimental studies on the treatment of OP with resveratrol: PubMed, Embase, The Cochrane Library, and China National Knowledge Infrastructure (CNKI). The search was performed from database inception to May 8, 2023. The search strategy included a combination of MeSH terms and free words, and the strategy was adjusted based on the characteristics of each database. The keywords related to resveratrol included "Resveratrol" OR "trans-Resveratrol" OR "3 5 4 trihydroxystilbene" OR "cis-Resveratrol" OR "3 4 5 stilbenetriol" OR "trans-Resveratrol-3-O-sulfate" OR "trans-Resveratrol-3-O-sulfate" OR "SRT-501" OR "trans-Resveratrol" OR "SRT501" OR "SRT-501" OR "cis-Resveratrol" OR "Resveratrol-3-sulfate" OR "3 4 5 trihydroxystilbene" OR "Resveratrol-3-sulfate." The keywords for OP include: "Osteoporosis" OR "OP" OR "Osteoporoses" OR "bone loss" OR "bone density" OR "bone mineral density" OR "bone mass density."

2.4. Data extraction

Two researchers independently conducted literature screening and data extraction and cross-checked the results. Disagreements were resolved by discussion or by consulting a third researcher. The following data were extracted: (1) basic information of the included study: author, title, year of publication, animal species, weight, age, and sample size, (2) specific details of intervention measures, including medication dosage and duration, (3) the various information elements of bias risk assessment, and (4) outcome indicators and outcome measurement data.

2.5. Quality evaluation of the included studies

We used the risk of bias tool for animal studies provided by the Systematic Review Center for Laboratory Animal Experience (SYRCLE) to conduct a literature quality evaluation of the included studies (19, 20). This evaluation tool has a total of 9 items, including random group allocation, groups similar at baseline, blinded group allocation, random housing, blinded interventions, random outcome assessment, blinded outcome assessment, reporting of drop-outs, and other biases. Each item can be judged as having low bias risk, high bias risk, and unclear bias risk (19, 20).

2.6. Statistical analysis

RevMan 5.4 software was used for data analysis. The outcome measures included in this study were all continuous variables, so all combined effects are expressed as the mean difference (MD) and 95% confidence interval (CI). This meta-analysis used the random-effects model for pooled data analysis. To clarify the anti-OP effect of resveratrol at different doses, we divided the drug doses of resveratrol into four groups: >10, 10–25, 40–50, and ≥ 60 mg/kg/day. In each included study, if there were 2 or more sets of satisfactory measurement data within the same dose range (the same study), the group with the lowest dose was selected for meta-analysis. Considering that differences in race and age of rats may affect the reliability of the conclusion, we conducted subgroup analyses based on those two factors. In particular, the resveratrol group used in the subgroup analysis was the lowest-dose group in each included study. We also constructed funnel plots for each outcome indicator to evaluate potential publication bias.

3. Results

3.1. Literature screening results

After removing duplicate literature, we initially obtained 339 articles. In the initial screening, we excluded literature that clearly did not meet the inclusion criteria based on the information provided by the title and abstract. After applying the inclusion and exclusion criteria and screening full texts, a total of 15 studies on the treatment of OP animal models with resveratrol that met the requirements of this meta-analysis were ultimately included (21–35). The search process and details are shown in Figure 1.

3.2. Characteristics of the 15 included studies

This meta-analysis included 15 experimental studies on the treatment of OP rats with resveratrol. A total of 438 rats were included in this study, including 295 in the resveratrol group and 143 in the control group. There are three types of rat strains, namely, Albino rats, SD rats, and Wistar rats. The modelling method for OP is OVX. The dosage of resveratrol varies greatly, with a minimum dosage of 625 μg/kg/day and a maximum dosage of 500 mg/kg/day. The course of medication is between 4 and 24 weeks. The specific details and characteristics of each included study are shown in Table 1.

3.3. Literature quality evaluation

Most of the 15 studies included in this meta-analysis were evaluated for unclear risk bias. Only 2 studies used the random number table method (30, 33); 2 studies did not use random assignment (21, 27); and the remaining studies did not provide sufficient information to determine whether the experimental animals were randomly assigned. One study used a blinding method for the evaluators of results (22). One study did not provide a detailed explanation of missing data (29), which may lead to potential data reporting bias. The quality evaluation results of the literature included in the study are shown in Figure 2.

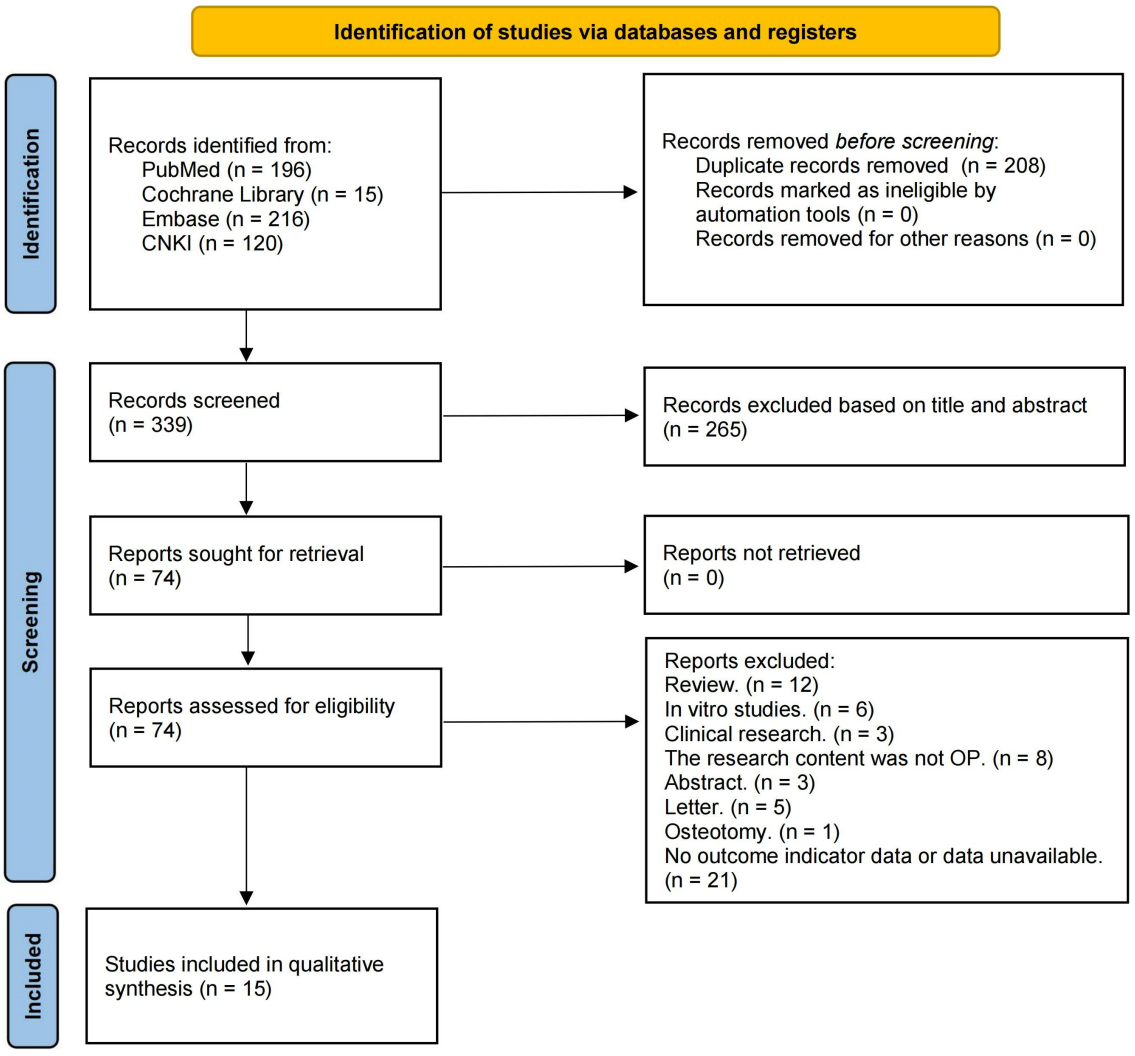

FIGURE 1
Summary of the process for identifying candidate studies.

3.4. Results of meta-analysis

3.4.1. Primary outcomes

3.4.1.1. BMD of the total body

A total of 5 studies (22, 25, 27, 30, 33) reported total-body BMD (Figure 3). The meta-analysis results showed that compared with the control condition, <10 mg/kg/day resveratrol significantly increased the total-body BMD of the OP rat model (MD = 0.01, 95% CI: 0.01 to 0.01; $p < 0.001$), and there was no heterogeneity among the studies in this subgroup ($I^2 = 0\%$). However, resveratrol doses of 10–25 mg/kg/day and 40–50 g/kg/day showed no significant difference in total-body BMD compared with the control condition ($p > 0.05$).

3.4.1.2. BMD of the femur

A total of 5 studies (26–29, 35) reported FBMD (Figure 4). The meta-analysis results showed that compared with the control group, four doses of resveratrol (<10, 10–25, 40–50, ≥ 60 mg/kg/day) all increased FBMD in OP rats, with MDs (95% CIs) of 0.01 (0.01, 0.01), 0.01 (0.00, 0.02), 0.02 (0.02, 0.03), and 0.02 (0.01, 0.03), respectively.

3.4.1.3. BMD of the lumbar vertebrae

Three studies (27, 29, 35) reported LBMD (Figure 5). The meta-analysis results showed that resveratrol <10 (MD = 0.02, 95% CI: 0.01 to 0.03), 10–25 (MD = 0.02, 95% CI: 0.01 to 0.03), 40–50 (MD = 0.03, 95% CI: 0.02 to 0.04), and ≥ 60 (MD = 0.02, 95% CI: 0.01 to 0.03) mg/kg/day significantly increased LBMD compared to the control group ($p < 0.05$).

3.4.2. Secondary outcomes

3.4.2.1. Parameters of micro-CT

This meta-analysis analysed three parameters related to micro-CT, namely, Tb. Th (Supplementary material 1), Tb. N (Figure 6), and Tb. Sp (Supplementary material 2). The meta-analysis results showed that resveratrol (40–50 mg/kg/day) significantly increased Tb. Th

TABLE 1 Characteristics of the 15 included studies.

Study	Model (method)	Species	Age	Weight (g)	Intervention		Duration	Sample size	
					Resveratrol	Control		Resveratrol	Control
Elseweidy et al. (21)	OVX	Albino rats	3 months	200–220	80 mg/kg/day	Tap water	8 weeks	10	10
Feng et al. (22)	OVX	SD rats	3 months	280–350	5/25/45 mg/kg/day	Tap water	8 weeks	8/8/8	8
Feng et al. (23)	OVX	SD rats	3 months	220 ± 19.27	40 mg/kg/day	Sesame oil	10 weeks	10	10
Guo et al. (24)	OVX	Wistar rats	8 weeks	180–200	500 mg/kg/day	Tap water	60 days	10	10
Khera et al. (25)	OVX	SD rats	3 months	NR	625 μg/kg/day	Tap water	4 weeks	6	6
Li et al. (26)	OVX	SD rats	6 months	250 ± 20	10/20/40 mg/kg/day	Tap water	12 weeks	6/6/6	6
Lin et al. (27)	OVX	SD rats	3 months	254.91 ± 18.01	5/15/45 mg/kg/day	Tap water	90 days	8/8/8	8
Liu et al. (28)	OVX	Wistar rats	NR	220–250	0.7 mg/kg/day	Tap water	12 weeks	11	11
Wang et al. (29)	OVX	SD rats	NR	NR	10/20/40 mg/kg/day	Tap water	8 weeks	8/8/8	8
You et al. (30)	OVX	Wistar rats	6 weeks	NR	8.4 mg/kg/day	Tap water	8 weeks	10	10
Zhang et al. (31)	OVX	SD rats	6 months	220 ± 10	5/15/45 mg/kg/day	Tap water	12 weeks	12/12/12	12
Zhang et al. (32)	OVX	SD rats	6 months	NR	50/100/200 mg/kg/day	Carboxymethyl cellulose	12 weeks	8/8/8	8
Zhang et al. (33)	OVX	SD rats	6 weeks	241.06 ± 32.81	40 mg/kg/day	Tap water	8 weeks	10	10
Zhao et al. (34)	OVX	Wistar rats	3–4 months	200–220	20/40/80 mg/kg/day	Tap water	12 weeks	10/10/10	10
Zhou et al. (35)	OVX	SD rats	6 months	360 ± 10	60/80/100 mg/kg/day	Tap water	24 weeks	16/16/16	16

OVX, ovariectomy; SD, Sprague–Dawley; NR, not reported.

(MD = 0.01, 95% CI: 0.01 to 0.01) in the OP rat model. Compared with the control group, resveratrol (<10, 40–50, ≥ 60 mg/kg/day) increased Tb. N ($p < 0.05$). Resveratrol (10–25, 40–50, ≥60 mg/kg/day) was more effective in reducing Tb. Sp compared to the control group, and the differences were statistically significant ($p < 0.05$).

3.4.2.2. Serum calcium

Four studies (24, 26, 29, 31) reported changes in serum calcium concentration (Figure 7). The meta-analysis results showed that resveratrol at concentrations of <10 (MD = -0.24, 95% CI: −0.32 to-0.16), 10–25 (MD = -0.35, 95% CI: −0.43 to-0.27), and 40–50 (MD = -0.37, 95% CI: −0.45 to-0.29) mg/kg/day significantly reduced serum calcium concentration compared to the control group ($p < 0.001$).

3.4.2.3. Serum phosphorus

Similarly, four studies (24, 26, 29, 31) reported changes in serum phosphorus concentration (Supplementary material 3). The meta-analysis results showed that resveratrol was more effective in reducing serum phosphorus concentration compared to the control group, and the differences were statistically significant ($p < 0.05$).

3.4.2.4. Serum ALP

A total of 6 studies (21, 22, 24, 26, 29, 31) reported serum ALP levels (Figure 8). The meta-analysis results showed that there was no statistically significant difference in the effect of resveratrol and the control group on serum ALP ($p > 0.05$).

3.4.2.5. Serum osteocalcin

A total of 4 studies (22, 24, 30, 33) reported changes in serum osteocalcin levels (Supplementary material 4). The meta-analysis results showed that there was no significant difference in the effect of resveratrol on serum osteocalcin compared to the control group ($p > 0.05$).

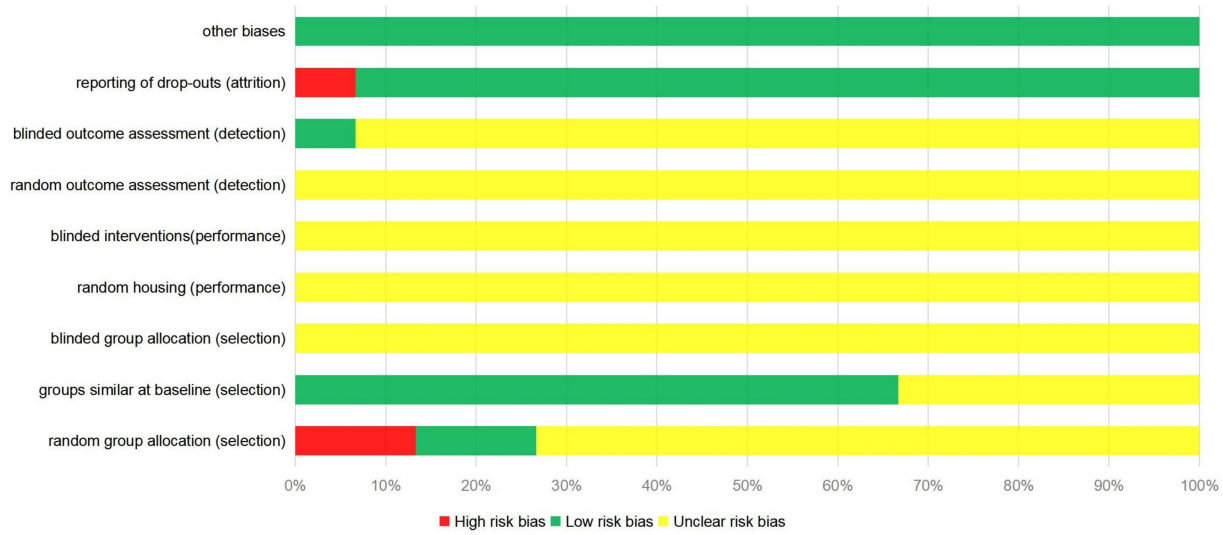

FIGURE 2
Risk of bias of the 15 included studies.

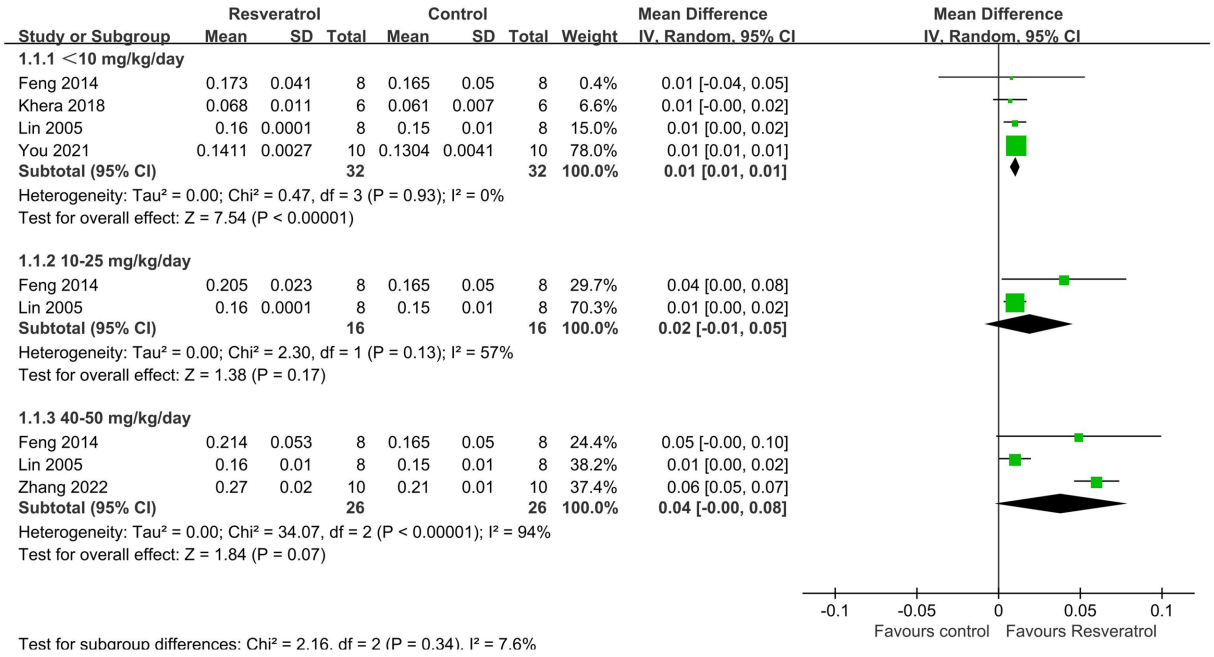

FIGURE 3
Forest plot of the total body BMD.

3.4.3. Subgroup analysis of BMD of the total body

3.4.3.1. Resveratrol in SD rats

A total of 3 studies were included in the subgroup analysis (22, 25, 27). Meta-analysis results showed that compared with the control condition, resveratrol treatment resulted in a statistically significant increase in total-body BMD (MD = 0.01, 95% CI: 0.00 to 0.01; $p = 0.002$) (Figure 9).

3.4.3.2. Resveratrol in 3-month-old rats

A total of 2 studies were included in the subgroup analysis (22, 25). Meta-analysis results showed that compared with the control treatment, resveratrol treatment had no statistically significant effect on improving total-body BMD ($p = 0.17$) (Figure 10).

3.5. Publication bias

We plotted corresponding funnel plots for all outcome indicators to evaluate publication bias. The funnel plot results show that the funnel plots of BMD of the total body, Tb. Th, serum phosphorus, and serum osteocalcin are asymmetric, indicating that there may be publication bias in these outcome indicators. The funnel plot of all outcome indicators is shown in Supplementary material 5.

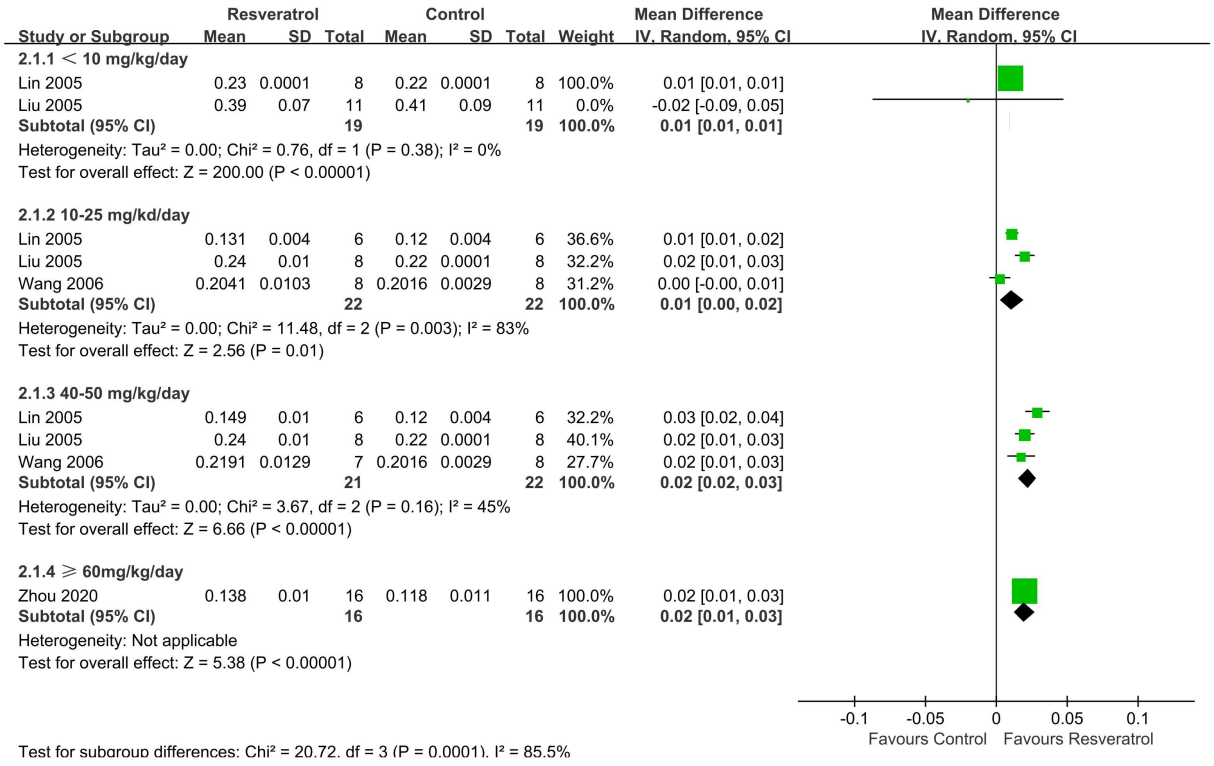

FIGURE 4
Forest plot of femur BMD.

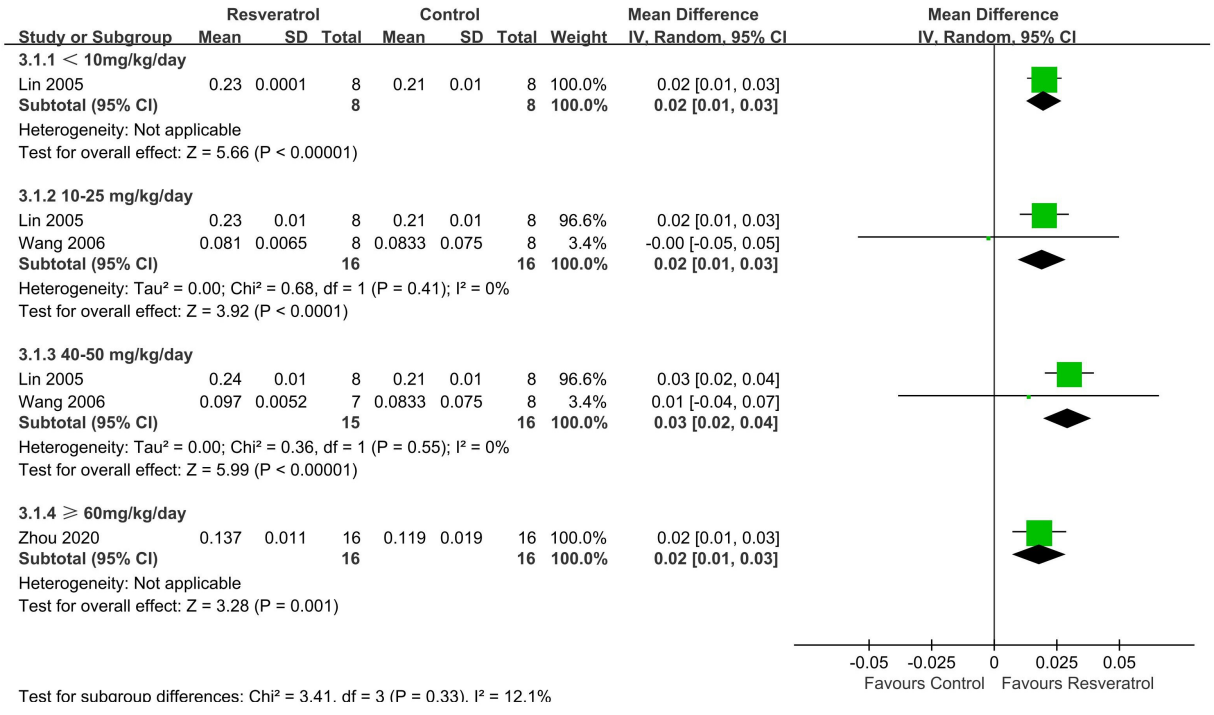

FIGURE 5
Forest plot of lumbar vertebrae BMD.

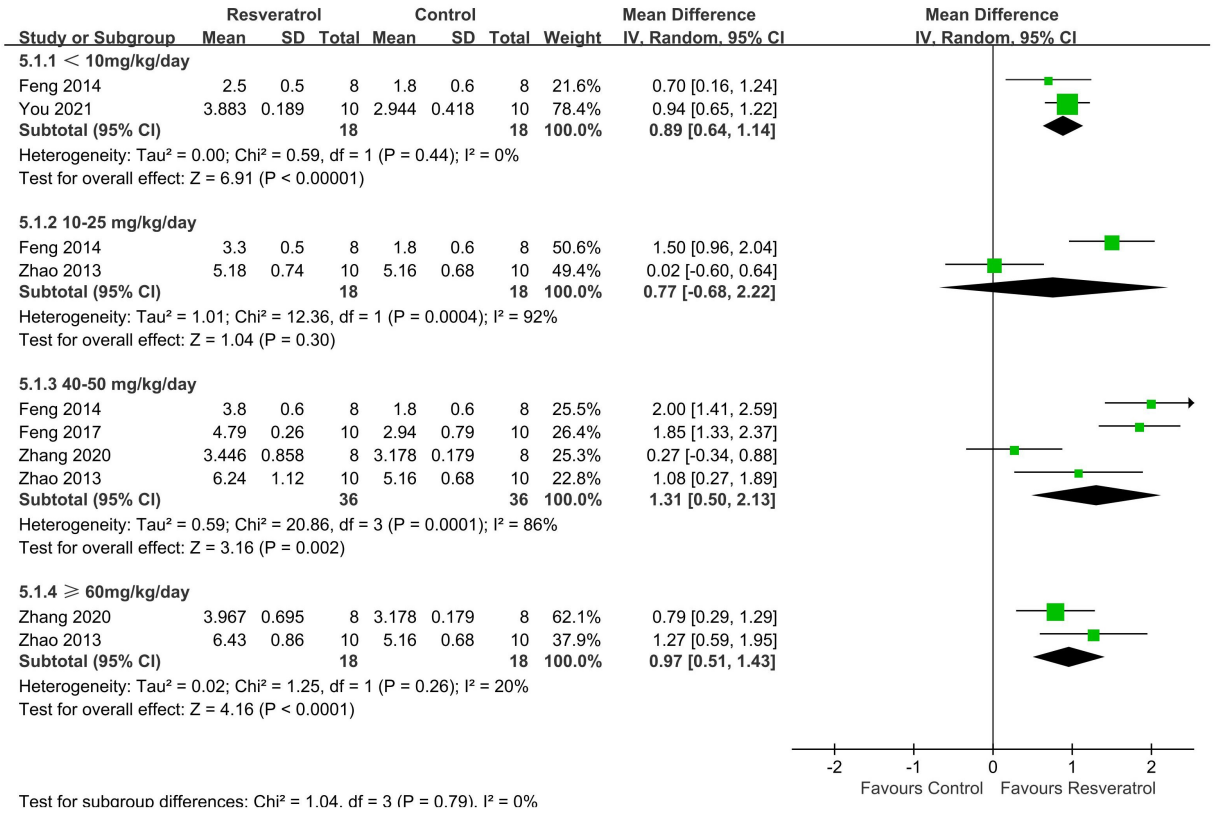

FIGURE 6
Forest plot of trabecular number.

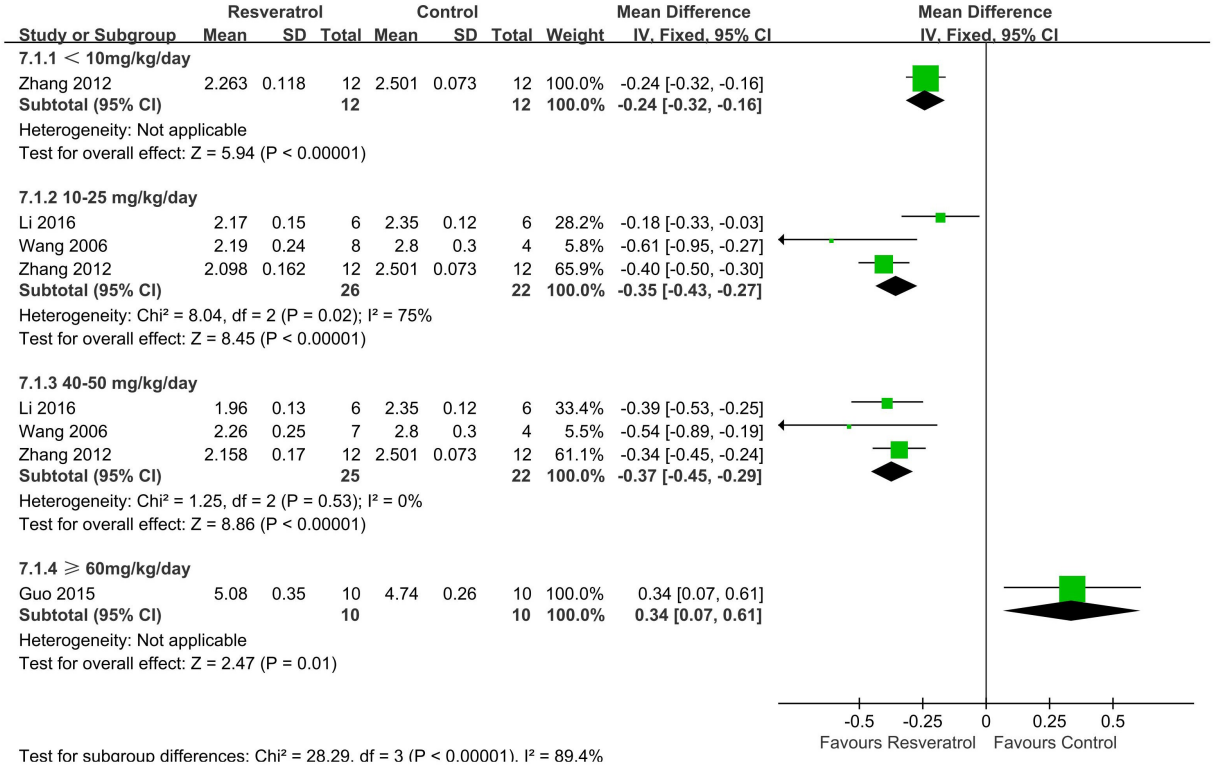

FIGURE 7
Forest plot of serum calcium.

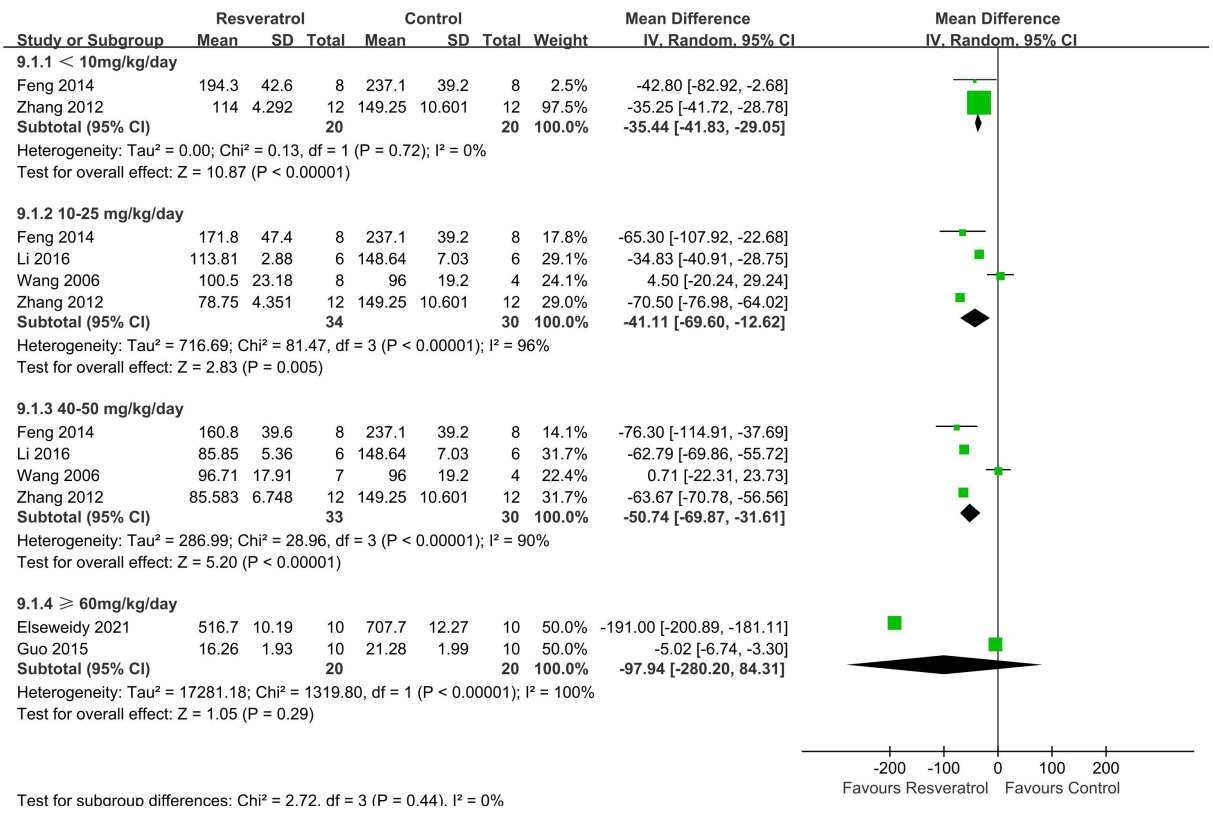

FIGURE 8
Forest plot of serum alkaline phosphatase.

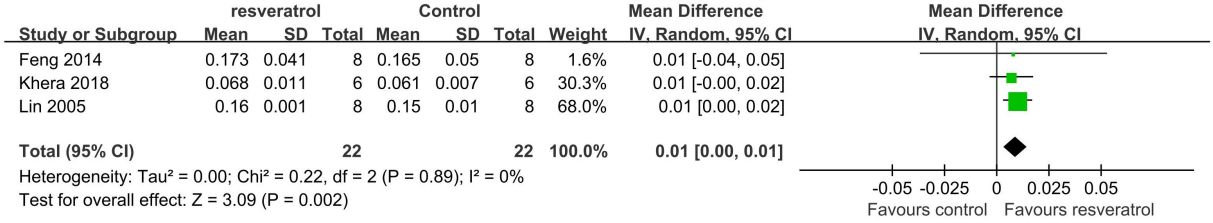

FIGURE 9
Subgroup analysis of total-body BMD in SD rats.

4. Discussion

OP is known as the silent killer, and osteoporotic fractures are a serious complication of OP, which means that the prevention and treatment of OP are important aspects to which public health needs to pay attention. Ethnic medicine or botanical medicine has always been the focus of drug conversion. In recent years, the therapeutic effect of resveratrol on OP has received considerable attention, but research on its anti-OP efficacy or mechanism is mostly limited to animal or cell experiments, which seriously limits the progress of resveratrol in clinical application. To further clarify the anti-OP efficacy of resveratrol, this study summarizes preclinical evidence to provide support to proceed with clinical trials. This meta-analysis found that resveratrol can significantly increase FBMD and LBMD in OP rats, and this conclusion remained consistent at concentrations <10, 10–25, 40–50, and ≥ 60 mg/kg/day. In the improvement of BMD of the total body, resveratrol (<10 mg/kg/day) showed better efficacy than the control group. In terms of improving the parameters related to micro-CT, resveratrol can increase Tb. Th and Tb. N and reduce Tb.Sp. The concentration of resveratrol at 40–50 mg/kg/day can all improve these three bone microstructure indicators. In addition, resveratrol can reduce the concentration of calcium and phosphorus in serum but has no significant effect on serum ALP and osteocalcin, which was also verified in this meta-analysis. Based on preclinical animal research data, we found that resveratrol may have enormous clinical application potential in the treatment of OP, which means that

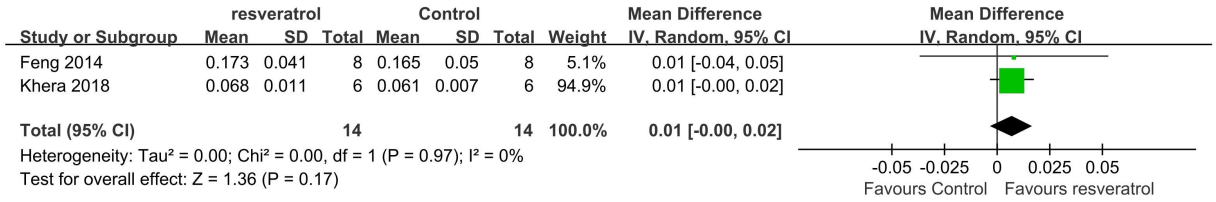

FIGURE 10
Subgroup analysis of total-body BMD in 3 months aged rats.

resveratrol may become a candidate drug for OP treatment, but this still needs to be verified through large-scale clinical studies in the future.

Resveratrol has the characteristics of multiple targets, low cost, and low toxicity (36), and its therapeutic effect in OP is receiving increasing attention. The dynamic balance between osteoblasts and osteoclasts has always been considered the core content of OP research. An experimental study found that resveratrol can activate the osteogenic transcription factor CBFA-1 (37) and enhance the transcription of bone-specific type I collagen in a CBFA-1-dependent manner, stimulate the proliferation and differentiation of osteoblasts, and activate Sirt-1 to transform osteoblasts into osteoblasts. Research shows that resveratrol can upregulate the expression level of Sirt-1 and then upregulate the expression of FoxO1 protein to inhibit the differentiation of osteoclasts (38). The occurrence of oxidative stress can cause damage to bone cells and osteoblasts (39, 40) and lead to bone resorption activity exceeding bone formation. Resveratrol is a natural antioxidant and can effectively prevent bone loss caused by oxidative stress in the body (15), which may be the potential mechanism of its anti-OP effect. In addition, resveratrol can bind to oestrogen receptors and exert oestrogenic effects (32), thus compensating for bone loss caused by oestrogen deficiency. In addition, this meta-analysis showed that resveratrol achieved better efficacy in improving biochemical markers. Serum biochemical indicators reflect the essence of bone metabolism and the direct reflection of bone formation and bone resorption. This meta-analysis found that resveratrol has a better effect than the control treatment in reducing serum calcium concentration, which may be because resveratrol inhibits oxidative stress and reduces bone loss, thereby reducing the content of calcium entering the serum. Oxidative stress may lead to oxidative damage to bone cells and osteoblasts in the bone microenvironment, leading to imbalanced bone remodelling. The antioxidant effect of resveratrol can maintain bone homeostasis, thus stabilizing bone microstructure. Based on the undeniable regulatory role of resveratrol in bone metabolism, its clinical application in OP deserves in-depth attention.

5. Limitations

This study has limitations that should be considered when interpreting the results. First, the animal models included in the study may exhibit significant differences in factors such as species of rats, drug dosage, and sample size, which may lead to heterogeneity in the experiment and compromise the reliability of the conclusions of this study. Second, the included animal experiment reports focus on the construction of animal models and outcome evaluation, but the report on experimental design, implementation, and measurement methods is relatively brief, which may lead to poor methodological quality in literature reports, difficulty in estimating potential bias risks, and reduced data credibility. Third, there may be differences in the BMD measurement tools and serum markers used in all 15 included studies, which may lead to measurement errors between studies. Given the limitations of animal experimental design, future clinical studies targeting the treatment of OP with resveratrol should avoid these situations, which would be beneficial for improving the reliability of evidence-based data on this research topic.

6. Conclusion

This study found that resveratrol can increase BMD in OP rat models, and its mechanism of action may be closely related to improving bone microstructure and regulating calcium and phosphorus metabolism. Given that this study focuses on an OP rat model, the efficacy of resveratrol in treating OP still needs to be further validated through clinical studies in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

JZ: conceptualization, validation, data curation, writing – original draft, writing – review and editing, visualization, supervision, and project administration. GZ and JY: writing – original draft, and writing – review and editing. JP: investigation, data curation, and formal analysis. BS and ML: investigation and data curation. WY: conceptualization, methodology, software, validation, formal analysis, and data curation. JL: conceptualization, supervision, validation, and funding acquisition. LZ: conceptualization, methodology, software, validation, formal analysis, data curation, writing – original draft, writing – review and editing, visualization, supervision, and funding

acquisition. JZ and GZ: contributed equally to this work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1234756/full#supplementary-material

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Mechanisms of action and synergetic formulas of plant-based natural compounds from traditional Chinese medicine for managing osteoporosis: a literature review

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Osteoporosis (OP) is a systemic skeletal disease prevalent in older adults, characterized by substantial bone loss and deterioration of microstructure, resulting in heightened bone fragility and risk of fracture. Traditional Chinese Medicine (TCM) herbs have been widely employed in OP treatment owing to their advantages, such as good tolerance, low toxicity, high efficiency, and minimal adverse reactions. Increasing evidence also reveals that many plant-based compounds (or secondary metabolites) from these TCM formulas, such as resveratrol, naringin, and ginsenoside, have demonstrated beneficial effects in reducing the risk of OP. Nonetheless, the comprehensive roles of these natural products in OP have not been thoroughly clarified, impeding the development of synergistic formulas for optimal OP treatment. In this review, we sum up the pathological mechanisms of OP based on evidence from basic and clinical research; emphasis is placed on the *in vitro* and preclinical *in vivo* evidence-based anti-OP mechanisms of TCM formulas and their chemically active plant constituents, especially their effects on imbalanced bone homeostasis regulated by osteoblasts (responsible for bone formation), osteoclasts (responsible for bone resorption), bone marrow mesenchymal stem cells as well as bone microstructure, angiogenesis, and immune system. Furthermore, we prospectively discuss the combinatory ingredients from natural products from these TCM formulas. Our goal is to improve comprehension of the pharmacological mechanisms of TCM formulas and their chemically active constituents, which could inform the development of new strategies for managing OP.

KEYWORDS

osteoporosis, traditional Chinese medicine herbs, plant-based natural products, bone homeostasis, active ingredients, anti-osteoporosis drug

Introduction

Osteoporosis (OP) as a chronic systemic skeletal disease, is considered to be a growing silent epidemic in the 21st century, and it is estimated to affect approximately 200 million individuals worldwide (1, 2). It is characterized by a decline in bone mineral density (BMD) and degradation of bone tissue microstructure, which results in heightened bone fragility and a higher risk of fractures (3). Several factors, including hormonal imbalances, medication use (like glucocorticoids), smoking, lack of exercise, and insufficient calcium and vitamin D intake, contribute to bone loss in OP progression (4, 5). Epidemiology research has shown that 1/3 of women and 1/5 of men over the age of 50 are prone to osteoporotic fractures, with the risk increasing with age, particularly in women over 60 (6). Current OP treatments vary from non-pharmacological options (such as muscle tone and balance-improving exercises) to pharmacological therapies (such as bisphosphonates, denosumab, teriparatide, abaloparatide, and romosozumab) (7–9). Certain patients with low BMD or those who respond poorly to treatment may still have extremely low BMD despite receiving bone-formation therapy. The safety risks connected with these therapeutic agents have resulted in investigations into safer and more effective alternatives for treating OP.

Traditional Chinese medicine (TCM) has been used for over 2000 years to manage a broad spectrum of medical diseases or conditions and relies on plant-based natural products, including TCM formulas and their compounds and extracts (10–12). These products have been pivotal in maintaining the health of Chinese individuals, providing effective treatments for diseases and reducing adverse effects, compared to Western medicine or placebo, for instance, 5-Ling Granule produced significantly greater improvement in the severity of tic symptoms than placebo in an 8 weeks, double-blind, randomized, controlled trial and Pingchuan Yiqi granule significantly improves lung function and symptoms of acute asthma in a randomized, double-blind, placebo-controlled trial (13–17). As to OP, in clinical practice and animal experiments, TCM formulas, such as Bushen Huoxue decoction (BSHXD), Er Xian Decoction (EXD), and Liuwei Dihuang Pill (LWDHP), have been proven to be effective in treating OP (18–20).

Meanwhile, various natural compounds, which are present in TCM formulas, have exhibited diverse biological effects in treating osteoarticular degenerative diseases, including OP. Many of these compounds have been proven to have effects akin to those of the original formulas for treating OP (21–26). Of note, chemically active ingredients from plant-based compounds (or secondary metabolites), like resveratrol, naringin, and ginsenoside, have been extensively employed for preventing and treating OP, as corroborated by their beneficial effects in decreasing bone resorption, increasing bone formation, repairing bone microstructure, enhancing angiogenesis, and improving the immune system (27–29). Nevertheless, the full extent of the roles played by these TCM herb formulas and their natural products in OP remains to be systematically elucidated, which hampers the development of synergistic formulas for more effective OP treatment.

Based on evidence from clinical and basic research, this review outlines the underlying pathological mechanisms of OP, and offers a concise summary of the most recent discoveries on the anti-OP mechanism of TCM formulas and their chemically active ingredients, highlighting their detailed effects on osteoblasts and osteoclasts, bone marrow mesenchymal stem cells and bone microstructure, angiogenesis, and the immune system. Furthermore, through the prospective discussion of the unique ingredient combinations in these TCM formulas, we seek to offer valuable information for devising fresh OP treatment strategies.

Mechanism of OP

OP is diagnosed when an individual's BMD T score falls at or below −2.5, according to WHO diagnostic criteria (30). Mechanistically, various factors contribute to OP progression, including bone and hormone metabolism disorders, modulation of signaling pathways, dysregulation of the vasculature, immune dysfunction, and other factors that can vary (31–33). Here, we mainly summarize the potential pathological mechanisms of OP from various aspects mentioned above (Figure 1).

Bone homeostasis

The balance of bone-forming and bone-resorbing activities is essential to preserve bone homeostasis. Multiple bone cell types, including osteoblasts (OBs), osteoclasts (OCs), and bone marrow mesenchymal stem cells (BMSCs) are involved in the multifaceted process of bone homeostasis (34, 35). OBs and BMSCs are responsible for bone formation, whereas OCs are the primary functional cells involved in bone resorption (36). This interdependent mechanism is referred to as coupling, where OCs break down the organic matrix and dissolve bone minerals, followed by the recruitment of OBs to deposit fresh bone matrix that later mineralizes. An imbalance in the bone remodeling process, leading to excessive bone resorption relative to bone formation, is the principal factor contributing to the greater occurrence of OP (37, 38). The normal bone remodeling equilibrium can be disrupted by various factors, such as hormones, endocrine regulation, cytokines, and others, which interfere with OBs and OCs differentiation and activity (39, 40). Furthermore, the imbalance in bone remodeling also deteriorates the bone microstructure, making it a target for therapeutic approaches to improve bone strength and decrease the risk of fractures (41).

Besides, the regulation of OP initiation and development involves various signaling pathways, incorporating genetic elements, and modulatory molecules, like TGF-β, RANKL, as well as BMP, which have been studied in great detail over the past few decades (42). These signal transduction pathways are implicated in regulating the differentiation and growth of OBs, OCs, or BMSCs. For example, the Wnt/β-catenin signaling pathway plays a crucial role in the differentiation of BMSCs into OBs by determining their directionality, whereas knocking down *β-catenin* expression significantly elevates OCs numbers and accelerates bone resorption rates, resulting in the development of OP (42, 43). In addition, TGF-β signaling not only inhibits OBs maturation, mineralization, and transition into osteocytes, but also inhibits OCs differentiation by decreasing RANKL/OPG secretion ratio (44, 45). Other signaling pathways, including Notch, and BMP2/SMADs, also participate in bone homeostasis and are highly correlated to the differentiation, growth, and maturation of OBs, OCs, and BMSCs (46–48). In general, dysregulation of signaling pathways mentioned above is associated with the risk of OP.

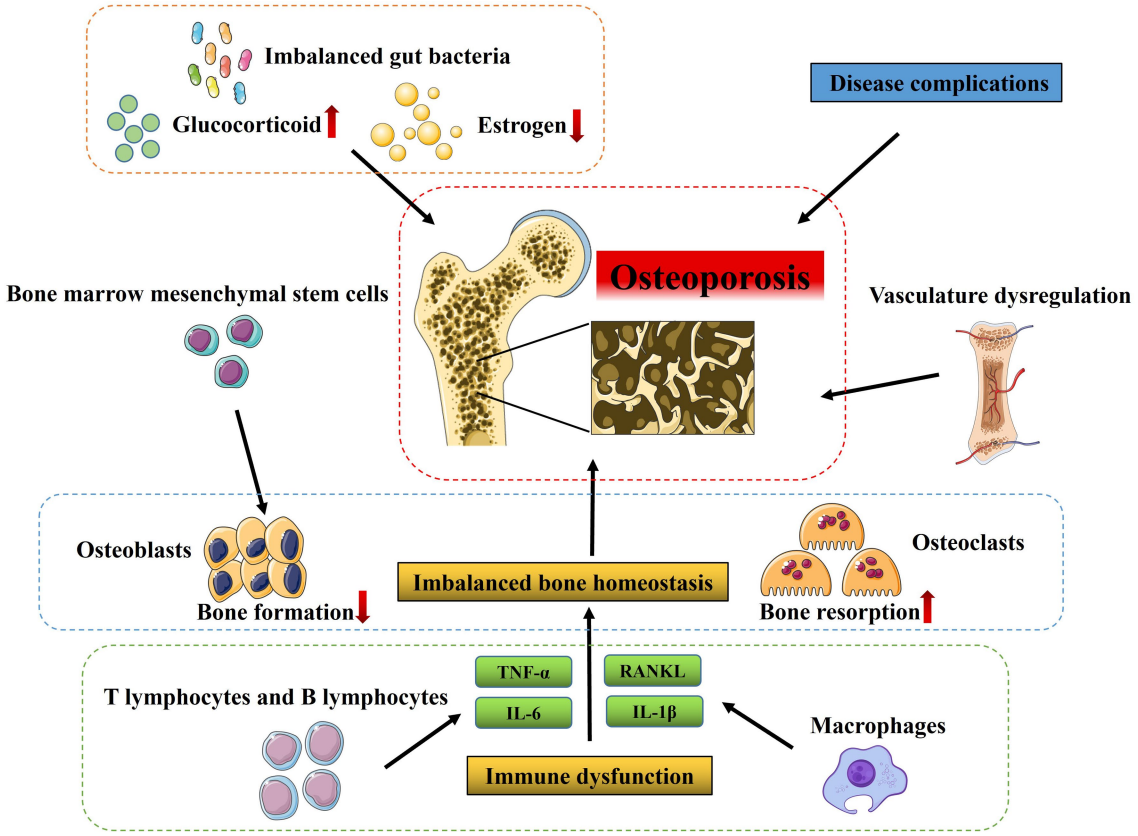

FIGURE 1
Pathological mechanisms of osteoporosis. Various factors contribute to OP progression, including imbalanced bone homeostasis regulated by osteoblasts (responsible for bone formation), osteoclasts (responsible for bone resorption), bone marrow mesenchymal stem cells as well as vasculature dysregulation, and immune dysfunction and many other factors such as estrogen deficiency, long-term and high-dose glucocorticoid treatment and imbalanced gut bacteria.

Vasculature

The existence of blood vessels in the bone microenvironment is necessary for appropriate bone development and growth, post-fracture healing, and maintaining healthy bones. Angiogenesis in bone tissues involves the proliferation, migration, and tube formation of endothelial cells, which establishes blood flow conduits that deliver essential nourishment, vital oxygen, crucial growth-promoting substances, and hormones to bone cells (31). On the contrary, disruption in angiogenesis in bone tissues can disturb the equilibrium within the bone niche, which is intimately linked to the pathological progression of OP (49, 50). Emerging clinical evidence reveals that individuals with OP have comparatively decreased blood supply to the bone than those with normal bone density, suggesting a significant correlation between bone mineral density and bone vascularization (51). Thus, a significant hallmark of OP is a reduction in the number of sinusoidal and arterial capillaries located in the bone marrow, resulting in decreased delivery of blood to the bone tissue (52). Moreover, the reduction in blood perfusion within the bone marrow is also linked to a decline in BMD and an increased incidence of fractures in elderly women (53). Similarly, age and ovariectomized (OVX) mice show decreased levels of PDGF-B in serum and bone marrow, along with a marked decrease of type H vessels in long bones (54, 55). All these findings indicate a cause-and-effect connection between the number of type H vessels and OP, given the type H vessels' capability to promote angiogenesis and bone formation, and maintain the delicate equilibrium between bone resorption and formation (54, 56). Therefore, activating angiogenesis might be a vital therapy strategy in the prevention of OP.

Immune system

Bone surrounds the bone marrow, which serves as an essential center for the immune system's maturation and hematopoiesis. It is well-documented that there is a significant connection between the immune system and the skeletal system. T cells and B cells are the two types of lymphocytes responsible for adaptive immunity. They and their cytokines can impact the functions and activities of both OBs and OCs, with additional indirect implications on OCs due to an increase in RANKL expression via OBs proliferation (57–59). Moreover, these two systems share several regulatory molecules, including cytokines and signaling molecules. The innate immune cells are major producers of pro-inflammatory mediators, with several noteworthy cytokines such as IL-6, TNF-α, IL-1β, as well as ROS that have a crucial impact on OP (60). For instance, TNF-α can promote OCs formation by directly interacting with both OCs along with their precursors, synergistically with RANKL (61). Noteworthy, the regulation of bone homeostasis

involves the contribution of multiple immune factors, such as T cells, B cells, NK cells, macrophages, and many other cytokines (37, 62). Different subsets of T cells, namely $CD4^+$ T cells and $CD8^+$ T cells, all exert crucial impacts in regulating bone health. Of these, Th17 cells stand out as a significant cause of bone loss since they produce significantly more RANKL and TNF-α (63, 64). Besides, the presence of B cells and their production of RANKL could be involved in the pathogenesis of ovariectomy-induced bone loss (65). Likewise, M1 macrophages exert a significant impact on promoting osteoclastogenesis by producing abundant ROS and pro-osteoclastogenic cytokines such as TNF-α and IL-1β (66). In contrast, unlike M1 macrophages which promote osteoclastogenesis, M2 macrophages contribute to bone mineralization by promoting the differentiation of MSCs and precursor OBs into mature OBs, thus supporting bone health (67).

Other factors

Accumulating evidence reveals that the development of OP is also affected by various other factors, such as disease complications, estrogen levels, and gut microbiota (68). Among them, endocrine systemic diseases, hematological diseases, or other metabolic diseases, have been shown to be closely related to the occurrence and development of OP (68). It has been reported that diabetic patients may suffer reduced bone turnover and bone loss due to deleterious impacts on bone metabolism and degradation of the extracellular matrix. And the development of brittle bone in diabetes is controlled by many contributing elements, such as obesity, insulin resistance, dysglycemia, myopathy, and the use of certain medications. In parallel, certain comorbidities, including thyroid dysfunction, gonadal disorders, and maldigestion, may disrupt bone metabolism and contribute to an imbalance in affected individuals (69, 70). Given the close relationships between bone and bone marrow, hematological diseases exert a significant influence on the secondary forms of OP; there is an increased rate of bone resorption among patients with hemophilia (71). Additionally, estrogen is considered to act directly on bone cells in preventing a decline of bone mass (72, 73), and it directly or indirectly influences immune cells to impact OBs and OCs via additional intermediaries like OPG/RANKL and chemokines such as TNF-α and IL-1β (74). Moreover, growing evidence indicates there is a connection between a balanced microbiome and stable bone health, and that an imbalance in gut bacteria might worsen the function of OCs, thus resulting in OP (68).

Furthermore, clinical evidence reveals that long-term and high-dose glucocorticoid treatment is another significant contributor to OP (75). Accumulating studies have demonstrated that glucocorticoids can inhibit OBs proliferation and increase rates of apoptosis of OBs, inhibit calcium uptake in the gastrointestinal tract, act as an antagonist to vitamin D, upregulate RANK ligand, and suppress OPG (76, 77). Importantly, glucocorticoid therapy also has been shown to adversely affect muscle mass and muscle strength, increasing the risk for OP fractures (78, 79).

Anti-OP effects of TCM and plant-based natural compounds

Compared with chemically synthesized drugs, TCM possesses beneficial characteristics such as fewer adverse reactions, long-term use, and stable treatment effect, therefore, TCM is increased in clinical trials to treat OP, among them, Bushen Zhuanggu tablet was demonstrated to be effective in the postmenopausal OP treatment by increasing the BMD, as well as regulating calcium and phosphorus metabolism in case-control studies (80–84). TCM formulas, including LWDHP, BSHXD, and other classic formulas, have demonstrated significant clinical effectiveness in both preventing and treating OP (20, 85–88). Given the multifaceted and diverse pathological mechanisms of OP, comprehensively understanding the pharmacological mechanisms of diverse TCM approaches for OP treatment is imperative. Here, we summarize *in vitro* and preclinical *in vivo* evidence-based anti-OP mechanisms of TCM formulas and their chemically active ingredients from various perspectives such as OBs and OCs, BMSCs, as well as bone microstructure, angiogenesis, and immune system regulation.

The anti-OP effects of TCM formulas

In general, many TCM formulas have the effect of improving OP phenotypes to some extent by promoting bone homeostasis, and angiogenesis, and regulating the immune system (Table 1). In terms of TCM formulas for the treatment of OP, LWDHP increases the BMD of femurs and improves the biomechanical capability of the vertebral body in OVX rats, mechanically, it could upregulate Runx2 and Osx expression and augment the number of calcified nodules in OBs by activating canonical Wnt/β-catenin cascade through the increase of Lrp-5 and β-catenin expressions (89). Bioinformatics analysis also provides potential therapeutic targets of LWDHP against OP such as AKT1, ATF2, and FBXW7 from a systemic perspective (90, 96). Qing'e Pill (QEP) inhibits OB ferroptosis and increases the transcription of osteogenesis-related genes, such as *BMP2*, *Runx2* to increase the number of trabecula and trabecular connections by activating PI3K/AKT pathway and inhibiting ATM Serine/Threonine Kinase expression, thereby exerting a therapeutic role in OVX rats (97). In parallel, *in vitro* studies of OBs cultured with Danggui Buxue Decoction (DGBXD) led to a significant improvement in mitochondrial function, antioxidation, and anti-inflammatory, leading to increased OBs activity (91, 92).

As another part of bone homeostasis, OCs are also extensively studied in treated with TCM formulas. TCM formulas, BSHXD, and Jianpi Qingchang Bushen decoction (JQBD) can alleviate bone loss, decrease bone volume, and limit osteoclastogenesis by reducing the RANKL-stimulated NF-κB, ERK, JNK signal transduction pathways in OP model mice (93, 98). Bushen Tongluo decoction (BSTLD) and You Gui Yin (YGY) can promote bone generation and attenuate OC activity and bone resorption in the OVX-induced OP mouse model by reducing RANKL/OPG ratio (53, 99).

Meanwhile, numerous studies have demonstrated that TCM formulas can promote the BMSCs' growth and migration to alleviate OP symptoms by enhancing the ability of BMSCs to differentiate into OBs. Duhuo Jisheng Decoction (DHJSD) was found to promote the activity of BMSCs, leading to upregulation of the expression of *BMP2* and *Runx2* genes, and OBs differentiation through activating SMAD1/5/8 and ERK signaling pathway (94). Taohong Siwu Decoction (THSWD) enhances the proliferation, and migration, together with bone-forming potentiality by upregulating VEGF expression and the phosphorylation of FAK and Src (100). Zuogui Pill

TABLE 1 The efficacy of TCM formulas for treating OP *in vivo*.

TCM formula	Model	Dosage and duration	Results	References
LWDHP	OVX	2 mL/100 g/day; 12 weeks	Increases expression of Lrp-5, β-catenin, Runx2, Osx and the BMD of femurs	(89)
QEP	OVX	4.5 g/kg/day; 2 weeks	Promotes the osteogenic protein expression and calcium-phosphate metabolism and inhibits osteoclast function	(90)
BSHXD	GIOP mice	16 mg/kg, every 2 days; 6 weeks	Inhibits osteoclast formation and function by suppressing ERK, JNK, and NF-κB signaling	(91)
JQBD	IBD-induced bone loss model	16.5 g/kg/day; 2 weeks	Inhibits activation of the RANK/RANKL/OPG signaling pathway	(92)
YGY	OVX	20 mL/kg/day; 12 weeks	Inhibits bone loss and osteoclastogenesis, decreased the expression of RNAKL and NF-κB signaling genes	(93)
ZGP	GIOP rats	1.62 g/kg/day; 3 months	Inhibits bone resorption and accelerated bone formation through the activation of let-7f and regulation of autophagy-associated genes	(94)
EXD	OVX	300 mg/kg/day; 12 weeks	Ameliorates OVX-induced bone loss and bone microstructure deterioration, upregulates the level of serum estrogen	(95)
BSTLD	OVX	6 g and 12 g/kg/day; 12 weeks	Reduces osteoclasts activation and bone resorption, reduced mRNA and protein levels of calcitonin receptor and CTSK	(53)

(ZGP), another classical TCM prescription, could up-regulate *let-7f* and *Runx2* mRNA expression and down-regulate *Beclin-1*, *ATG12*, *ATG5*, *LC3*, and *CTSK* mRNA expression in BMSCs to promote osteogenic differentiation of BMSCs, thus improving the OP progression (95), while another study indicated it can also slow down the senescence of BMSCs through modulating Wnt/β-catenin signaling pathway (101).

Moreover, EXD, which has been extensively used for the treatment of OP (102), can improve the bone microstructure and deterioration of bone loss in the OVX-induced OP model through lipid metabolism and the IGF1/PI3K/AKT pathway (103). BSTLD treatment results in a significant promotion in angiogenesis in OVX-induced OP rats, which could be associated with the promotion of HIF-1α/VEGF-regulated angiogenesis signal transduction and inhibition of the RANKL/OPG ratio (53). In a clinical trial, LWDHP has been found to promote the expression of immune-related cardiotrophin-like cytokine factor 1 (CLCF1) in the peripheral blood of postmenopausal osteoporosis patients with kidney Yin deficiency by activating the JAK/STAT signaling pathway, thereby improving immunocompetence, which provides a promising therapeutic approach for postmenopausal OP (104). Other classic Chinese herbal medicine like Xianling Gubao Capsule, Zhuanggu Zhitong Capsule, and Guilu Erxian Glue were also demonstrated to provide potential therapeutic benefits for the treatment of OP through *in vivo* and *in vitro* research (105–108).

The anti-OP effects of herbs and chemical ingredients in TCM formulas

Extensive *in vivo* studies have demonstrated the anti-OP effects of various Chinese herbs and their extracts, including *Rhizoma Drynariae* homogenous polysaccharide, mixed extracts of *Fructus Corni* and *Radix Achyranthis Bidentatae* (the main components of LWDHP and ZGP), *Rhizoma Drynariae*. These herbs have been shown to ameliorate the OP phenotype by increasing BMD and bone mineral content, which is closely related to the activity of OBs (109–111). Resveratrol, derived from *Reynoutria japonica*, has been found to exhibit significant antioxidant capacity in OBs by triggering the NRF2/SIRT1/FoxO1 signaling pathway and improving excessive iron-induced oxidative stress (112–114). Puerarin, another bioactive ingredient extracted from the root of *Pueraria lobata (Willd.) Ohwi*, has been found to significantly upregulate the expression of *ALP* and *OPG* mRNA levels while inhibiting OBs apoptosis (115, 116). Similarly, Icariin, a compound rich in TCM formulas like EXD, can also promote OBs proliferation, differentiation, and mineralization, as demonstrated by increased expression of ALP and Col I, and bone nodule formation but inhibit their apoptosis by activating ERK and JNK signaling but not p38 signaling (117). Intriguingly, in another study, MC3T3-E1 subclone 14 cell line administered with Icaritin, a hydrolytic product of Icariin, increased mRNA levels and protein expression of ALP, COL1, OC, OPN, and RUNX2 to enhance preosteoblastic cell differentiation, and upregulated the bone nodule formation and collagen synthesis to improve mineralization through the activation of ERK and p38 signalings but not the JNK signaling (118). These discrepancies in reported signaling activation can be attributed to several factors, including differences in the compounds used, varying concentrations, and inconsistent timing of signal detection between the studies (119).

OCs and a major osteoclastogenic molecule, RANKL, have also been studied in relation to the effective monomer and active ingredients from TCM formulas. For instance, it has been suggested that dendrobium, a traditional medicinal plant, exhibits potent suppression of OCs by inhibiting the increase in ROS, the expression of c-fos, and NFATC1, which are mediated through RANKL, and significantly blocking *Mmp9* RNA transcription to reduce LPS-stimulated inflammatory bone loss (120). Moreover, Schisandrin A, which is rich in BSTLD, can inhibit the advantage of ROS induced by RANKL on OCs to improve bone resorption in OVX-induced OP mice via stimulating Nrf2 activity (121). Ellagic acid inhibits the expression of genes and proteins exclusive to OCs and hinders OCs differentiation, which limits bone loss, by blocking RANKL-RANK ligation, along with RANKL-conducted osteoclastogenesis (122, 123).

In parallel, Aconitine, Berberine, and dihydro-artemisinin can inhibit RANKL-mediated osteoclastogenesis and bone resorption activity, leading to the promotion of osteogenic recovery (124–126).

Moreover, some TCM has been suggested to have dual effects. For example, Icariin can alleviate mitochondrial membrane potential dysfunction and oxygen-free radical production, promote Runx2, ALP, and OPN expression in OBs caused by excessive iron accumulation, and additionally inhibit the cellular differentiation and activity of OCs (127).

As for BMSCs, Artemisinin exerts antioxidant effects on BMSCs by inhibiting hydrogen peroxide-induced Caspase 3 activation and apoptosis of BMSCs (128). Likewise, Leonurine attenuates oxidative stress-induced COX2 and NOX4 mRNA expression in BMSCs and up-regulates mitochondrial membrane potential levels through the PI3K/Akt/mTOR signaling cascade to enhance the growth and differentiation capability of rat BMSCs (129, 130). Salvianolic acid B, extracted from *Salvia miltiorrhiza*, has been investigated in human BMSCs that can increase the expression of ALP, OPN, and Runx2 while promoting the formation of bone minerals (131). In addition, Panax notoginseng saponins dose-dependently increased ALP activity and ALP, *Cbfa1*, *OC*, and *BSP* mRNA levels of BMSCs, and decreased the mRNA and protein expression of PPARγ2, so as to accelerate proliferation and osteogenic differentiation of BMSCs (132). Meanwhile, it can also downregulate the number of OC precursor cells to inhibit bone resorption, together with enhancing BMSCs differentiation activity in the direction of osteogenesis (133). Oral administration of Ginkgolide has been identified as increasing the OPG-to-RANKL ratio and thus improving bone mass in three animal OP models, including the aging-, OVX-, and glucocorticoid-induced OP models (134, 135).

The destruction of bone microstructure is one of the typical characteristics of OP, and thus, the repair of bone microstructure is a standard to measure medicinal effectiveness. α-asarone, which is extracted from a TCM herb *Acorus tatarinowii*, is capable of inhibiting osteoclastogenesis and strengthening bone microstructure in OP models induced by estrogen deficiency (136). Cuscuta extract, which is from TCM formulas such as EXD and BSHXD, indicates an anti-OP effect by increasing bone density and bone microstructure via the c-fos/c-Src kinase/NFATC1 signaling pathway in OVX-induced OP mice (137). As mentioned above, the Wnt/β-catenin signaling pathway exerts a key role in the development of OP. Studies have shown that Ginsenoside Rc and Rhizoma drynariae total flavonoids noticeably hinder the decline of bone mass and bone volume, and promote osteogenesis and bone formation via the Wnt/β-catenin signaling pathway (138, 139). Moreover, Bionic Tiger-Bone Powder promotes osteogenesis, inhibits OCs, and increases collagen content in OVX-induced OP mice, improving bone microstructure and biomechanical strength. In line with the *in vivo* study, it also increases BMD in postmenopausal patients with OP in clinical (140). In different animal OP models, current evidence also suggests that TCM has preventive effects on OP by improving bone microstructure. Ganoderma lucidum contained in EXD can prevent extra bone loss and improve bone microstructure in OVX-induced OP mice by regulating bone and adipose tissue homeostasis, which can prevent bone loss caused by estrogen deficiency (141). Radix Achyranthis Bidentatae with Eucommiae Cortex herbal pair ameliorates glucocorticoid-induced OP by modulating the expressions of Runx2, OP-1, and β-catenin (142). In terms of the disuse-induced OP model, Scutellaria extract can significantly improve bone density and mechanical strength to prevent OP in the hindlimb unloading tail-suspended rat model (143). And oral administration of total flavonoid extracts from epimedium increase maximum BMD in normal-growing rats (144).

There is increasing evidence that angiogenesis is intimately associated with bone generation and remodeling to ensure adequate bone homeostasis. Thus, the promotion of angiogenesis-dependent osteogenesis and the reduction of bone loss are essential for preventing and managing OP (54). Studies have shown that Naringin derived from the traditional Chinese medicinal plant *Drynaria* regulated the function of endothelial cells and promoted angiogenesis, thereby exhibiting an anti-osteoporotic effect in postmenopausal osteoporotic rat models (145). As an active component found in many TCM herbs, vitexin modulates both osteogenesis and angiogenesis in an OVX-induced OP rat model through vitamin D receptor and endothelial NO synthase pathway (146). It is well-recognized that diabetes mellitus raises the risk of OP, and TCM also contributes to treating diabetic OP via angiogenesis. Curcumin prevents bone loss and promotes angiogenesis in diabetic OP mice by suppressing the hyper glucose-activated NF-κB pathway to restore osteogenic and angiogenic coupling of BMSC in hyperglycemia (147). In addition, Ginsenoside Rg1 facilitates the secretion of VEGF, triggers the Noggin/Notch signaling pathway, and enhances the interaction between H-type blood vessels and bone formation, leading to an improvement in bone structure in a mouse model of diabetic OP (148). Besides, Salvianolic acid B and Salidroside were also reported to prevent bone loss by enhancing angiogenesis in Glucocorticoid-induced and OVX-induced OP model, respectively, (149, 150).

The term "osteoimmunology" was coined in a commentary in *Nature* to highlight the connection point between osteology and immunology (151). In recent decades, mounting evidence suggests that TCM herbs and their extracts can regulate the immune system, which is essential for effective OP treatment. Ganoderma lucidum mycelium extract Ling-Zhi-8, an immunomodulatory protein, can effectively alleviate glucocorticoids-induced OP phenotypes in mice, as indicated by the increase in the OPG/RANKL ratio, suppression of osteoclastogenesis, and improvement in serum mineral metabolism, bone formation, and absorption markers expression, as well as expressions of hormone molecules such as estradiol and parathyroid hormone (152). Osthole, a type of coumarin compound present in various medicinal plants, including *Cnidium monnieri*, can enhance the immunosuppressive capacity of osteoporotic BMSCs to improve the therapeutic effect of BMSCs transplantation on colitis as well as OP (153). Likewise, *Cissus quadrangularis* markedly promotes the production of immune cells that counteract osteoclastogenesis, including Th1, Th2, and Tregs, in the bone marrow, while simultaneously decreasing the number of OC-promoting Th17 cells. This effectively inhibits RANKL's ability to generate OCs and hinders the capacity of OCs in bone resorption (154).

Given the importance of proinflammatory cytokines, including TNF-α, IL-1β, as well as IL-17 in osteoimmunology, there is accruing evidence from recent studies suggesting that TCM herbs and their active ingredients such as Sinomenine (155), and Galanin (156) have therapeutical effects on OP by regulating the inflammatory reaction and inflammation-related signaling pathways. We have summarized the chemically active constituents in TCM herbs for treating OP in Table 2.

TABLE 2 The chemically active constituents in TCM herbs for the therapy of OP.

Active constituents	Medicinal materials	Antiosteoporotic action	References
Resveratrol	*Reynoutria japonica*	Exhibits antioxidant capacity in OBs and improves excessive iron-induced oxidative	(105–107)
Puerarin	*Pueraria lobata (Willd.) Ohwi*	Increases the expression of *ALP* and *OPG* mRNA levels and inhibits OBs apoptosis	(108, 109)
Icariin	*Herba epimedii*	Inhibits the apoptosis of OBs by activating ERK and JNK signaling	(110)
Schisandrin A	*Schisandrae chinensis* Fructus	Improves bone loss in OVX mice by stimulating the activity of Nrf2	(114)
Aconitine	*Aconitum*	Inhibits RANKL-induced OCs formation and bone resorption activity	(118)
Artemisinin	*Artemisia annua* (Asteraceae)	Exerts antioxidant effects on BMSCs and inhibits hydrogen peroxide-induced apoptosis of BMSCs	(121)
Leonurine	*Herb leonuri*	Up-regulates mitochondrial membrane potential levels and improves the proliferation and differentiation ability of rat BMSCs	(122, 123)
Salvianolic acid B	*Salvia miltiorrhiza*	Increases the expression of ALP, OPN, Runx2, and Osx, and promotes osteogenic mineralization	(124)
Panax notoginseng saponins	*Panax notoginseng*	Increases ALP activity, downregulates the number of OC precursor cells to inhibit bone resorption, and promotes the differentiation of BMSCs	(125, 126)
Ginkgolide B	*Ginkgo biloba*	Increases the ratio of OPG-to-RANKL and improves the bone mass	(127, 128)
α-asarone	*Acorus tatarinowii*	Inhibits OCs generation and improves the bone microstructure	(129)
Ginsenoside Rc	*Panax ginseng*	Increases bone density and bone volume fraction, and promotes bone formation and osteogenic differentiation through the Wnt/β-catenin signaling pathway	(131)
Naringin	*Drynaria*	Regulates the function of endothelial cells and promotes angiogenesis	(138)
Vitexin	*Crataegus pinnatifida* (hawthorn)	Regulates angiogenesis and osteogenesis in ovariectomy-induced OP rats through VDR/eNOS signaling pathway	(139)
Curcumin	*Curcuma longa* L	Prevents bone loss and promotes angiogenesis by inhibiting the NF-κB signaling pathway	(140)
Ginsenoside Rg1	*Panax ginseng*	Promotes VEGF secretion, activates Noggin/Notch pathway and improves bone structure	(141)
Ling-Zhi-8	*Ganoderma lucidum*	Alleviates the symptoms of glucocorticoids-induced OP, and increase the OPG/RANKL ratio to limit the production of OCs	(145)
Osthole	*Cnidium monnieri*	Enhances the immunosuppressive capacity of osteoporotic BMSCs to improve the therapeutic effect of BMSCs transplantation	(146)

Conclusion and perspectives

The main feature of OP is the pathological decrease of BMD caused by the imbalance in bone remodeling, which involves a sophisticated interplay of endogenous, exogenous, mechanical, biological, and immunological mediators. TCM formulas are rich in natural compounds and display diverse biological effects in treating various diseases. Importantly, clinical trials as well as *in vivo* and *in vitro* findings demonstrate that TCM formulas such as LWDHP, QEP, EXD, YGY, and their chemically active constituents such as Resveratrol, Puerarin, Icariin, Schisandrin A possess the potential to deliver beneficial outcomes in the management of OP. Mechanically, they primarily reverse the OP progression by regulating bone remodeling, bone homeostasis, and angiogenesis, as well as OP-related signaling pathways and immune factors (Figure 2).

Compared to chemical drugs, TCM formulas and their active ingredients possess the advantages of producing fewer adverse effects and being appropriate for sustained usage. TCM formulas containing multiple herbs, in which each single herb medicine often contains anti-OP constituents, have the potential to advance our understanding of traditional medicine's role in modern healthcare and accelerate the screening of new anti-OP drugs. Also, the combination of chemical drugs and TCM formulas might contribute to discovering effective therapies for treating OP.

It should be noted that treating OP requires a comprehensive understanding of its etiology. Thus, various animal models of OP, including OVX-induced OP models, chemically-induced OP models, and disuse-induced OP models, are frequently utilized to study the underlying mechanisms of this bone disorder. TCM formulas and their chemically active ingredients should be tested in multiple models to comprehensively elucidate their anti-OP effects. Although estrogen substitution therapy can prevent OP, the anti-OP mechanisms of many estrogen-like TCM formulas (such as Shugan Liangxue decoction, Qibao Meiran formula) or monomers (such as *Radix astragali*, *Curcuma comosa Roxb*) are still unknown (157–160). Further studies elucidating the anti-OP mechanism of these formulas or monomers targeting estrogen-like effects will greatly promote the treatment of OP with TCM. Moreover, investigating the effects of TCM on the factors that regulate estrogens and their receptors will aid in screening new anti-OP drugs. In the past few years, non-coding RNAs, like *LncRNA HOTAIR*, *LncRNA ROR*, and *miRNA-14* have been linked to the onset and progression of OP (161, 162), and the expression levels of these

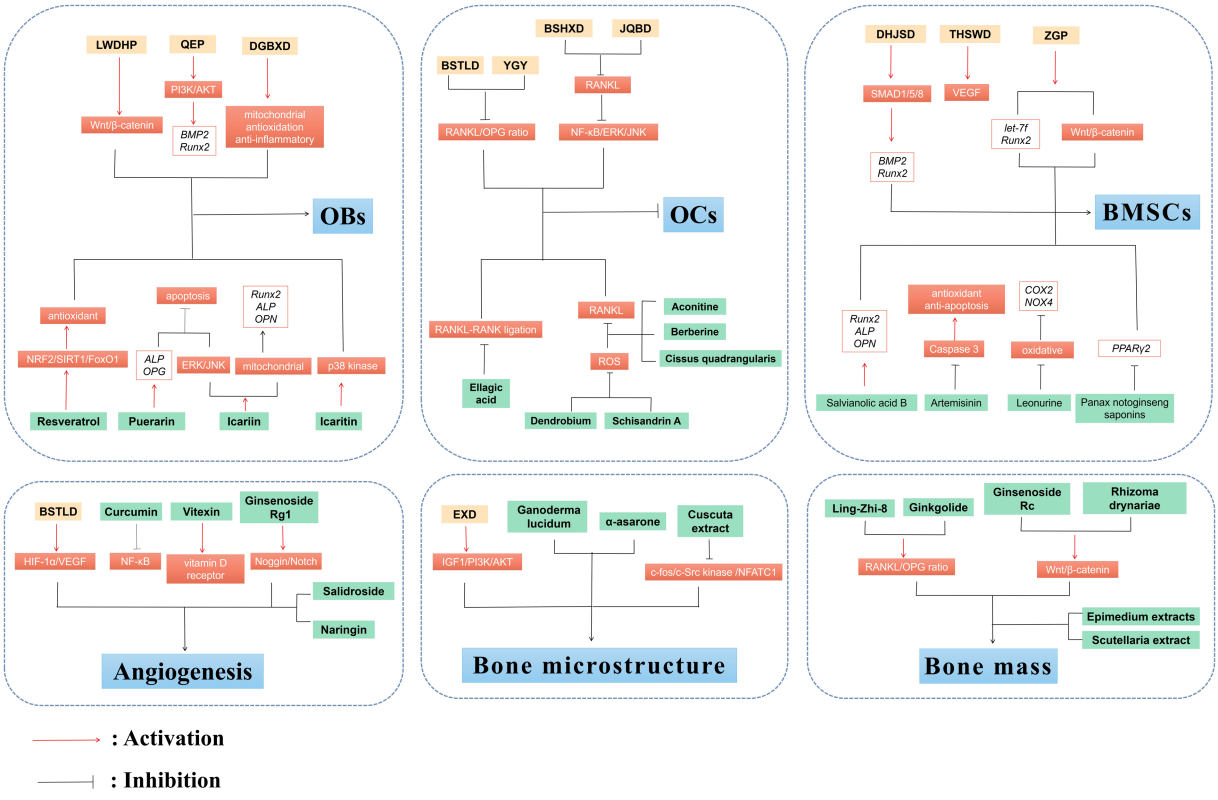

FIGURE 2
Anti-OP effects of TCM and plant-based natural compounds. TCM and plant-based natural compounds play a therapeutic role on OP from various perspectives such as OBs and OCs, BMSCs, bone microstructure, angiogenesis and bone mass.

ncRNAs have been discovered to be modulated by various active components in TCM, such as ginsenoside, curcumin and baicalin (163). Therefore, ncRNAs may serve as promising molecular targets for clinical therapeutic options of OP with TCM.

Some clinical trials focused on TCM in the treatment of OP have been carried out (82, 164–167), however, most of the clinical studies only contain a small sample size and short treatment duration, as well as the clinical parameters and biomarkers for analysis differ from each other, which results in a limited amount of robust evidence from clinical trials regarding effective approaches for preventing and treating OP. Thus, a sequential combination of TCM and Western medicine is the only way to solve these problems. In fact, some clinical trials on the treatment of OP by the combination of TCM and Western medicine have been carried out, such as Dihuang Decoction plus alendronate (83), Bushen Zhuanggu tablet plus calcium supplement and vitamin D (84), EXD plus caltrate tablets and calcitriol (102). A diverse array of sources from TCM formulas and their chemically active ingredients, along with ample anti-OP mechanisms, further enhance our assurance, eagerness, and motivation to discover new anti-OP drugs, but screening is imminent.

Author contributions

YZ, CW, LZ, and HR: conceptualization. CZ, SS, and MZ: writing original draft preparation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

OP	Osteoporosis
TCM	Traditional Chinese Medicine
BMD	Bone mineral density
BSHXD	Bushen Huoxue decoction
EXD	Er Xian Decoction
LWDHP	Liuwei Dihuang Pill
QEP	Qing'e Pill
DGBXD	Danggui Buxue Decoction
JQBD	Jianpi Qingchang Bushen decoction
BSTLD	Bushen Tongluo decoction
YGY	You Gui Yin
DHJSD	Duhuo Jisheng Decoction
THSWD	Taohong Siwu Decoction
ZGP	Zuogui Pill
OBs	Osteoblasts
OCs	Osteoclasts
BMSCs	Bone marrow mesenchymal stem cells
OVX	Ovariectomize
CLCF1	Cardiotrophin-like cytokine factor 1
GIOP	Glucocorticoid-induced osteoporosis

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Research progress on the treatment of knee osteoarthritis combined with osteoporosis by single-herb Chinese medicine and compound

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Knee osteoarthritis (KOA) is a degenerative disease with synovial inflammation, articular surface cartilage degeneration, meniscus degeneration, ligament and muscle changes, subchondral bone changes, and osteophyte formation around the joint as the main pathological changes. Osteoporosis (OP) is a disease characterized by low bone mass and deterioration of the microstructure of bone tissue. KOA and OP are both geriatric diseases, and the incidence of KOA combined with OP is high, but there is a lack of specific drugs, and the major treatments are limited to drug therapy. Most traditional Chinese medicine (TCM) treatments use plant-based natural products, and they help patients obtain good clinical benefits and at the same time provide researchers with ideas to study the mechanism of disease occurrence and the relationship between the two diseases. This article summarizes the research progress of TCM monomers and TCM compounds that are frequently used to treat KOA combined with OP to provide ideas for future clinical treatments and related basic research.

KEYWORDS

geriatric medicine, knee osteoarthritis, osteoporosis, bone aging, metabolism, plant-based natural products, signaling pathway

Introduction

Knee osteoarthritis (KOA) and osteoporosis (OP) are common bone and joint degenerative diseases in middle-aged and elderly people. The pathological changes of KOA include synovial inflammation, articular surface cartilage degeneration, meniscal degeneration, ligament and muscle pathologic changes, subchondral bone changes, and periarticular osteophyte formation. OP is a disease characterized by low bone mineral density (BMD) and deterioration of the microstructure of bone tissue. OP results in increasing bone fragility, which increases the risk of fracture. In 1994, the World Health Organization (WHO) established the criteria for measuring BMD, which allows the diagnosis of OP before a fracture occurs. This practical definition is based on its primary and known risk factor, reduced bone strength or density, and includes individuals who are at high risk but do not have fractures (1).

Regarding the relationship between KOA and OP, with the efforts of researchers, the following three viewpoints have widely emerged in recent years: the first viewpoint is that there is a positive correlation between the two diseases, that is, OP can lead to the occurrence of KOA; the second view is that there is a negative correlation between the two diseases, that is, the presence of KOA leads to bone growth, which reduces the occurrence of OP; and the third view is that there is no correlation between the two diseases. However, most studies support a strong correlation between KOA and OP. The two diseases are closely related in epidemiological, pathological, and therapeutic aspects. The majority of patients with KOA are also diagnosed with OP or osteopenia. KOA and OP can interact and eventually form a vicious cycle, which is one of the main causes of knee pain and incapacity for the work of patients (2). A study has shown that the prevalence of KOA combined with OP in China is as high as 30% (2, 3). Therefore, there is a demand for the prevention and treatment of KOA with OP, which we cannot ignore (2).

Materials and methods

Two researchers conducted a comprehensive search of four databases: China National Knowledge Infrastructure (CNKI), Wanfang Data, China Science and Technology Journal Database (VIP) and PubMed. The search period was up to June 2023 for each database. The four databases were searched by using subject words and free words. The literature retrieval strategy was as follows: "osteoporosis" and "knee osteoarthritis" or "osteoarthritis" and "Chinese medicine."

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) articles mentioned KOA or OP; (2) articles used traditional Chinese medicine (TCM) monomers or TCM compounds for treatment; and (3) there was no restriction on the language of the literature reports.

Exclusion criteria: (1) No reference to TCM treatment; (2) The article only refers to a unique group (e.g., postmenopausal KOA and OP).

Advantages of TCM in treating KOA and OP

KOA in the understanding of TCM theories belongs to the "Xi Bi" or "Gu Bi" (4, 5), *Huangdi Neijing* recorded: Once external evil invades the human body from the pores or skin, people will have performances of chilling and fear of cold. When the evil invades deep into the bones and remains in the bones, people will have Gu Bi. TCM doctors believe that the occurrence of KOA mostly involves kidney deficiency, external evil, and blood stasis, and kidney deficiency is the primary factor. In TCM theories, OP belongs to the category of "Gu Wei" (5), and TCM practitioners believe that the occurrence of OP also has a certain relationship with the kidney. *Taiping Shenghui Fang* mentioned: The kidney is the residence of shen and jing, and the fullness of yuan qi is closely related to kidney function. If the kidney qi is full, the bones of the human body will be strong. If the qi and blood of the human body is insufficient, viscera dysfunction, the human body's ability to resist evil, and evil invasion will lead to weakness of kidney qi and then lead to bone marrow that cannot fill the bone, which will lead to Gu Wei and a thin body. Because of the theory that the kidney dominates bone and generates marrow, the treatment of the combination of two diseases in the theories of TCM can start from kidney tonifying, which is a common clinical treatment idea of KOA combined with OP in TCM (6).

TCM has the advantages of flexible means, diverse methods, stable curative effects, etc., in treating KOA and OP. For example, acupuncture, massage, acupotomy, external application of Chinese medicine, and fumigation of Chinese medicine (7, 8). At the same time, TCM drug matching can also take into account other secondary symptoms of patients. If there is a bad appetite in KOA or OP patients caused by spleen and stomach qi stasis, appetizing Chinese medicine such as Radix Aucklandiae, *Citrus reticulata* Blanco or Amomum villosum Lour can be chosen to help patients regulate the spleen and stomach qi and then help restore appetite.

There are many pathogenic factors for the comorbidity of KOA and OP, and the common factors are inflammatory factors, hormonal factors, genetic factors, biomechanical factors, etc. KOA and OP are not immune diseases, but inflammatory factors are involved in bone resorption and cartilage degradation during their course. Related studies have mainly focused on inflammatory factors and hormonal factors. Some researchers have found that nuclear transcription factor-κB (NF-κB) is associated with the pathogenesis and progression of OP and KOA by stimulating the production of various proinflammatory cytokines (such as tumor necrosis factor-α [TNF-α] and interleukin-6 [IL-6]) (9–11). In addition, parathyroid hormone (PTH), estrogen, calcitonin (CT), and other hormones can improve the inflammatory environment of the knee joint, prevent bone mass loss, and delay the progression of the disease (3, 12). At present, nonsurgical treatment is the first choice for the clinical treatment of KOA combined with OP. TCM has obvious advantages in treatment, as it has significant curative effects and fewer side effects than Western medicine treatment, so it has a greater potential promotion value (2). This paper mainly introduces the research progress of TCM monomers and TCM compounds, used frequently by orthopedics and traumatology clinicians, in the treatment of KOA combined with OP and provides directions for future research.

Treatment of KOA with OP by TCM monomers

The main use method of TCM monomers lies in the effect of tonifying kidney and bone, especially the effect of tonifying kidney Yin or kidney essence. In TCM theories, yang represents the function, and yin represents the substance, so the strength of bone and bone marrow mainly comes from the abundance of yin. This is the reason why TCM doctors should pay attention to the use of bone-tonifying drugs or kidney-tonifying drugs while treating KOA combined with OP. There are many therapeutic active ingredients in TCM monomers, and different effective ingredients play different roles in the treatment of different diseases. The main effective ingredient of TCM monomers mentioned in this paper refers to the known or possible effective active ingredient in the treatment of KOA or OP.

Eucommia ulmoides Oliv

TCM doctors think that *Eucommia ulmoides* Oliv can nourish the liver and kidney, strengthen muscles and bones, and prevent miscarriage. The effective ingredients of *Eucommia ulmoides* Oliv in OP treatment are myrcetin, geniposide, nipinylic acid, etc. Modern pharmacological studies have proven that *Eucommia ulmoides* Oliv can work on the Wnt/β-catenin signaling pathway, bone morphogenetic protein (BMP)/Smad signaling pathway, and mitogen-activated protein kinase (MAPK) signaling pathway. It can promote bone formation and achieve the purpose of treating OP (13). Many scholars have found that the Wnt/β-catenin signaling pathway can influence the proliferation and differentiation of osteoblasts (OBs) and improve their function and activity, which plays a very important role in bone remodeling (14). Relevant studies have shown that the induction of *Eucommia ulmoides* Oliv alcohol extract can promote the osteogenic differentiation of rat bone marrow mesenchymal stem cells and the expression of β-catenin. Relevant animal studies have shown that *Eucommia ulmoides* Oliv can effectively improve the expression of BMP-2 and achieve bone protection in castrated rats (15). Studies have shown that the extract of *Eucommia ulmoides* Oliv, geniposide, and aucubin can activate extracellular signal-regulated kinase (ERK) signaling pathways and then initiate the BMP-2 signaling pathway, inducing the proliferation and differentiation of OB-MC3T3-E1 cells and stimulating bone formation (16). Relevant studies have shown that the main effective ingredients of *Eucommia ulmoides* Oliv in treating KOA include eugenene, eucommia glucoside, eucommia lipid A, quercetin, etc., which are mainly enriched in the MAPK signaling pathway, NF-κB signaling pathway, and Toll-like receptor signaling pathway. KOA can be treated by inhibiting local inflammation and regulating the cell cycle activities of chondrocytes and synovial cells (17).

Achyranthes bidentata Blume

Achyranthes bidentata Blume (AB) is commonly used by TCM doctors to treat KOA. TCM doctors believe it can nourish the liver and kidney, strengthen muscles and bones, promote blood circulation, and adjust blood distribution. The effective ingredients of AB Blume in treating OP are quercetin, kaempferol, rhein, etc. Related network pharmacological studies have shown that AB can influence the expression of inflammatory factors such as estrogen receptor gene (ESR), MAPK1, and MAPK14 and delay the progression of OP. ESR1 and ESR2 can participate in the metabolic process of bone cells and effectively inhibit the apoptosis of bone cells. MAPK1 and MAPK14 can promote the proliferation and differentiation of OBs and induce the formation of vascular endothelial cells to prevent and treat OP. Therefore, the prevention and treatment of OP by AB plays a role through multiple targets and pathways (18, 19). Total saponins of Achyranthes are one of the effective ingredients of *Achyranthes bidentata* Blume in the treatment of KOA. Many studies have shown that total saponins of Achyranthes can reduce the levels of interleukin-1 (IL-1) and TNF-α to treat KOA. Meanwhile, total saponins of Achyranthes can influence vascular endothelial growth factor (VEGF) antibodies. VEGF can promote the proliferation of blood vessels in subchondral bone, which can remodel subchondral bone structure and then accelerate the progression of KOA. Related animal experiments have shown that total saponins of Achyranthes could reduce VEGF messenger ribonucleic acid (mRNA) levels in KOA model rabbits, so it can inhibit subchondral bone remodeling (20, 21). The hypoxia inducible factor (HIF) signaling pathway may be the common signaling pathway in KOA and OP.

Dipsacus asper Wall.ex Henry

Dipsacus asper Wall.ex Henry's root is a medicine commonly used in orthopedics and traumatology in TCM. Its Chinese name means "continuance of broken bone." Akebia saponin D (ASD) is the main active ingredient in the treatment of OP (22). Related network pharmacological studies have shown that the main effective active ingredients of Dipsacus asper Wall.ex Henry can be used to treat OP by influencing the occurrence and development of OP through multiple targets and genes. Relevant studies have shown that the target genes of OP treatment by Dipsacus asper Wall.ex Henry mainly involve MAPK14, transforming growth factor-beta 1 (TGF-β1), nitric oxide synthase 2 (NOS2), protein tyrosine phosphatase N1 (PTPN1), coagulation factor II (F2), etc. These genes can influence the localization of proteins in the nucleus, active oxygen metabolism, folding protein reaction, and other biological processes, as well as the activities of functional proteins such as aromatase, carboxylesterase hydrolase, and dopamine transmembrane transporters. Dipsacus asper Wall.ex Henry can postpone the process of OP at the gene level. Studies have confirmed that the activation of ESR1 (ERα) can inhibit the growth of osteoclasts (OCs) and promote the proliferation of OBs, which play a role in the treatment of OP. Similarly, as an important candidate gene for OP susceptibility, TGF-β1 plays a significant role in postponing the occurrence and development of OP by regulating the proliferation and differentiation of bone marrow mesenchymal stem cells and OBs (22, 23). Dipsacus asper Wall.ex Henry still plays a significant role in the treatment of KOA, in which the main active ingredient is Dipsacus total saponins, and relevant animal experiments have pointed out that Dipsacus total saponins may play a role in upregulating the autophagy level of knee chondrocytes in KOA rats by inhibiting the overactivation of the phosphatidylinositol-3 kinase (PI3K)/threonine kinase (AKT)/mammalian target of rapamycin (mTOR) signaling pathway (24). The correlation study on the rate of recombinant factor related apoptosis ligand (Fas-L) expressing positive cells has shown that the rate of synovium cell apoptosis protein B-cell Lymphoma-2 (Bcl-2) and Fas-L expressing positive cells was significantly negatively correlated with treatment of Dipsacus asper Wall.ex Henry. Studies on the correlation between different treatment durations of Dipsacus asper Wall.ex Henry and the rate of cells with positive expression of synovial apoptosis proteins Bcl-2 and Fas-L have shown that the rate of cells with positive expression of synovial apoptosis proteins Bcl-2 and Fas-L is significantly positively correlated with the duration of its treatment, which further indicates that Dipsacus asper Wall.ex Henry has a reliable effect in the treatment of KOA (25).

Cuscuta chinensis Lam

Cuscuta chinensis Lam in TCM is considered to nourish both the Yin and Yang of the kidney but also tonify kidney essence. The kidney

masters bone by nourishing the kidney to achieve the purpose of bone disease treatment. The results of network pharmacology showed that there were 14 components of Cuscuta chinensis Lam for preventing OP, such as quercetin, kaempferol, hypericin, and isorhamnetin. Studies have shown that quercetin can reduce bone loss caused by estrogen deficiency by inhibiting cell senescence. The mechanism of kaempferol may be related to inhibiting bone resorption. Hypericin can promote the proliferation and differentiation of OBs through the BMP and Wnt/β-catenin signaling pathways. The mechanism of isorhamnetin in the prevention and treatment of OP is to regulate the receptor activator of NF-κB ligand (RANKL)/receptor activator of NF-κB (RANK)/osteoprotegerin (OPG) signaling pathway, influence the functions of OBs and OCs, improve the damage to bone microstructure, and prevent and treat OP. It can be seen that the active ingredients of Cuscuta chinensis Lam in preventing OP are effective through different mechanisms of action (26). In terms of KOA treatment, dodder polysaccharide may be the main therapeutic effect of dodder extract on rat KOA in the experiment. The relevant experimental results showed that Cuscuta chinensis Lam extract (including dodder polysaccharide) was selected to act on the rat KOA model, and glucosamine was selected as the positive control group. After 4 weeks, according to behavioral manifestations, changes in joint motion, joint gross morphology, imaging, pathological sections, and other indicators of rats, researchers confirmed that its extract can effectively improve the cartilage degeneration of KOA in rats, and it is more effective than glucosamine. The results indicate that Cuscuta chinensis Lam has a clear therapeutic effect on KOA (27).

Treatment of KOA with OP by TCM compounds

TCM compounds are combined with TCM monomeric components in accordance with strict compatibility, in which TCM theory has the distinction of Jun, Chen, Zuo, and Shi. While Jun treats the main symptoms of patients, Chen can help patients relieve other symptoms. For example, TCM compounds can help patients treat sleep disorders, such as poor appetite, or other problems while treating bone diseases. TCM compounds embody the integrity principle of TCM and embody the humanistic thought of TCM.

Zuogui Wan

Modern studies have found that the sensory nervous system regulates bone metabolism at multiple levels. Neuropeptide SP (N-SP) plays an important role in bone cell differentiation, bone metabolism, and bone reconstruction. Both the hypothalamus and bone contain N-SP, and the hypothalamus has the most abundant content, which acts through neurokinin-1 receptor (NK1-R). It has also been found that N-SP can stimulate the proliferation of osteoblast precursor cells, enhance cell activity, and influence bone formation during OB differentiation (28). Runt-related transcription factor 2 (Runx2) is a key gene necessary for bone development that can promote OB and OC differentiation and chondrocyte maturation. Deletion of Runx2 can inhibit OB differentiation and chondrocyte maturation. The expression level of Runx2 in the femur of elderly patients with OP was decreased, and the ability of preosteoblasts to differentiate into OBs was also decreased. The results of studies have shown that compared with the sham operation group, the BMD of the distal femoral subchondral bone of rats in the model group was decreased. Their bone tissue morphology was destroyed, serum inflammatory factors were increased, femur N-SP and Runx2 protein expression was decreased, and hypothalamic neuropeptide N-SP and NK1-R protein and mRNA expression was significantly decreased. This suggests that the morbidity of PMOP combined with OA may be related to the decreased expression of N-SP, NK1-R and Runx2 (29). After intervention with alendronate sodium and Zuogui Wan at different doses, the BMD and bone morphology of rats were improved, the levels of serum inflammatory factors were decreased, the protein expression levels of N-SP and Runx2 in the femur were increased, and the mRNA and protein expression levels of the neuropeptides N-SP and NK1-R in the hypothalamus were increased. The results suggested that sodium alendronate and Zuogui Wan may regulate the expression of N-SP and NK1-R and inhibit the level of serum inflammatory factors, thereby improving the imbalance of bone reconstruction and alleviating cartilage injury. In addition, the Zuogui Wan high-dose group had the best effect on improving BMD and inhibiting serum inflammatory factors. Therefore, the mechanism of Zuogui Wan's prevention and treatment of PMOP combined with OA may be through upregulating the expression of neuropeptide N-SP and NK1-R in the hypothalamus, thus reversing the imbalance of postmenopausal subchondral bone remodeling. However, the mechanism of its regulation of bone metabolism through the neuropeptide network still needs further study (30).

Bushen Huoxue decoction

Relevant studies showed that each dose of Bushen Huoxue decoction downregulated the mRNA expression of the inflammatory factors interleukin 1 (IL-1) and IL-6 in the cells of the 3 groups and upregulated the mRNA expression of the cartilage-repairing cytokines insulin-like growth factor 1 (IGF-1) and TGF-β in chondrocytes, with significant differences compared with the normal group. The changes in the expression of these cytokines in the high-dose Bushen Huoxue decoction group were similar to those in the glucosamine hydrochloride and alendronate sodium combined group. Combined with the relevant experimental results, it can be concluded that the downregulated expression of IL-1 and IL-6 and the elevated expression of IGF-1 and TGF-β may play a role in protecting and repairing chondrocytes and promoting chondrocyte proliferation. This verified the positive correlation between the osteoarthritis (OA) and OP groups at the cytotoxic level and verified the therapeutic mechanism of different doses of Bushen Huoxue Decoction on OA and OP and their comorbidities (31, 32). Another study showed that medium and high doses of Bushen Huoxue decoction can downregulate the expression of NF-κB protein in chondrocytes to a certain extent, promote the proliferation of chondrocytes, and reduce the expression of IL-1 and IL-6 in chondrocytes. The effect of the high-dose group was

most obvious, thus inhibiting the regulation of NF-κB inflammation to a certain extent. It can reduce the pathological reaction of OA and OP and effectively relieve the clinical symptoms of patients with OA and OP (33).

Duhuo Jisheng decoction

Duhuo Jisheng Decoction contains Heracleum hemsleyanum Diels, Euzhong, *Achyranthes bidentata* Blume, Angelica sinensis (Oliv.) Diels, Cynanchum otophyllum Schneid, etc. Duhuo Jisheng Decoction can tonify the liver and kidney, promote blood circulation, dispel wind, and remove dampness. Duhuo Jisheng decoction is a common TCM compound used to treat KOA. For arthritis patients, TNF-α is a cytokine produced by activated macrophages that inhibits OBs and stimulates OCs. As a powerful proinflammatory cytokine, TNF-α, an important regulatory cytokine of inflammation and the immune response, can promote the adhesion and migration of inflammatory cells and stimulate the release of IL-1 and adhesion molecules in the body. Basic studies have shown that the levels of IL-1 and TNF-α in the joint fluid of rabbit-diseased joints are decreased after treatment with Duhuo Jisheng Decoction. It can reduce the knee inflammation of patients mainly by affecting IL-1 and TNF-α. Relevant studies have found that the levels of IL-1 and TNF-α at 1, 3, and 5 weeks after treatment with Duhuo Jisheng Decoction are lower than those of patients treated with conventional treatment, and the levels of IL-1 and TNF-α at each time period after treatment with Duhuo Jisheng Decoction show a gradual downwards trend (34, 35).

In addition, Duhuo Jisheng Decoction can be combined with Western medicine to treat KOA with OP. Blood calcium and blood alkaline phosphatase are both bone metabolic indexes that participate in bone formation and are related to the progression of KOA and OP. In the relevant studies, the total effective rate of the study group after treatment with Duhuo Jisheng Decoction combined with Western medicine was higher than that of the control group. The levels of blood calcium and blood alkaline phosphatase were lower than those of the control group, suggesting that Duhuo Jisheng Decoction combined with Western medicine has a good effect on the treatment of KOA combined with OP. It can also improve the bone metabolism of patients. After treatment, the VAS score of the study group was lower than that of the control group, and the quality of life was better than that of the control group, suggesting that Duhuo Jisheng Decoction can effectively relieve joint pain, facilitate the recovery of arthritis and ensure the quality of life of patients. Duhuo Jisheng decoction combined with Western medicine in the treatment of KOA complicated with OP can relieve joint pain in patients and improve bone metabolism indexes and quality of life. Duhuo Jisheng decoction is worthy of clinical promotion and application (36).

Discussion

In summary, TCM monomers and compounds are effective in treating KOA combined with OP, and the mechanism of TCM in treating OP is realized through systemic, multilink, and multipathway regulation (5). In addition, TCM monomers or compounds can play a therapeutic role in combination with related Western medicines to help expand the scope of treatment. There are various methods for the prevention and treatment of KOA and OP in TCM, which are not limited to the internal administration of TCM monomers and compounds orally but also include Chinese medicine patches, hot compresses, and other means. In addition, acupuncture, massage, traditional exercises, etc., can also be used for preventive health care or treatment (6) or combined rehabilitation training for treatment (37).

However, although the effectiveness of relevant TCM therapies has been confirmed to a certain extent through clinical trials or animal experiments, its main active ingredients and related mechanisms of action are still lacking clear explanations. Meanwhile, the treatment and comprehensive treatment of internal and external TCM treatment lack significant advantages compared with simple Western medicine treatment (6). In addition, there are many separate studies on KOA and OP, but few studies on the combination of the two diseases. Furthermore, relevant TCM studies mainly focus on TCM monomers, TCM compounds, or acupuncture. There is a lack of research on the prevention of traditional exercises, delaying or preventing the progression of KOA combined with OP. Additionally, there are few studies focusing on KOA combined with OP treatment, and most of them mainly focus on the special population of postmenopausal KOA combined with OP. The majority of studies have only focused on patients with KOA or OP patients alone, and KOA combined with OP patients has not received sufficient attention. Furthermore, there are few animal experiments on the relationship between OA and OP (38). Finally, in most trials, the experimental group used Western medicine combined with traditional Chinese medicine, while the control group used Western medicine for comparison, and fewer took placebo or no-treatment control. Although effective decision-making was provided in clinical practice, indicating that the treatment plan of TCM combined with Western medicine was superior to that using Western medicine alone, the efficacy of TCM monomers or compounds alone lacked verification.

In the future, the clinical treatment of KOA or OP should be more balanced because OP is a high-risk factor for the progression of KOA, and KOA can accelerate the progression of OP. More attention can be paid to the treatment from the perspective of regulating bone metabolism (6, 12). In addition, in the clinical treatment of KOA or OP, we should recognize the stage of the disease, stratify patients, make full use of evidence-based medicine, look for higher-level evidence, and consider the stepwise treatment plan (38, 39). In future studies, metabolomics, network pharmacology, and other relevant modern pharmacological research methods can be used to help study the pathogenesis of KOA and OP and clarify the biological characteristics, biological processes, transformation laws, and internal connections of various regulatory mechanisms in the pathological process of KOA combined with OP. They can provide new ideas and a theoretical basis for the prevention and treatment of KOA combined with OP (3). In addition to proving the effectiveness of TCM in the treatment of KOA combined with OP, high-quality clinical experiments and animal experiments should be conducted to study the pathogenesis and pathological changes of KOA and

TABLE 1 TCM monomers and compounds used to treat KOA and OP.

	Name	Main active ingredient	Function chennel
TCM monomers	*Eucommia ulmoides* Oliv	OP: myrcetin, geniposide, and nipinylic acid KOA:eugenene, eucommia glucoside, eucommia lipid A, and quercetin, etc.	OP: Wnt/β-catenin signaling pathway, BMP/Smad signaling pathway, and MAPK signaling pathway KOA: MAPK signaling pathway, NF-κB signaling pathway, and Toll-like receptor signaling pathway
	Achyranthes bidentata Blume	OP: Quercetin, kaempferol, Rhein, etc. KOA: Total saponins of achyranthes abutens	OP: ESR, MAPK1, MAPK14 KOA: IL-1 and TNF-α
	Dipsacus asper Wall.ex Henry	OP: ASD KOA: Dipsacus Total Saponins	OP: MAPK14, TGF-β1, NOS2, PTPN1, F2, etc. KOA: PI3K/AKT/mTOR signaling pathway, Bcl-2 and Fas-L
	Cuscuta chinensis Lam	OP: quercetin, kaempferol, hypericin and isorhamnetin, etc. KOA: dodder polysaccharide	OP: BMP and Wnt/β-catenin signaling pathway, RANKL/RANK/OPG signaling pathway KOA: unclear
TCM compounds	Zuogui Wan	Unclear	N-SP, NK1-R
	Bushen Huoxue Decoction	Unclear	IL-1, IL-6, IGF-1, TGF-β; NF-κB
	Duhuo Jisheng Decoction	Unclear	IL-1 and TNF-α, etc.

OP. Researchers should expand the study patient group, which can provide a higher level of clinical evidence (38, 40).

In brief, we should make full use of modern pharmacological research to help study the mechanism of action of TCM monomers and TCM compounds in treating KOA with OP and study the effective concentration and side effects on this basis, which can better serve clinical practice. In the clinical practice of Western medicine treatment, clinical practitioners could try to participate in TCM therapy to improve the clinical benefit of patients. The relevant TCM monomers and compounds mentioned in this article are shown in Table 1.

Conclusion and perspectives

The combination of KOA and OP is common in middle-aged and elderly people, and the participation of traditional Chinese medicine therapy during treatment can help patients improve their quality of life. Compared with simple Western medicine, the participation of Chinese medicine can make the treatment more diversified, making the treatment more individualized and targeted. Future studies should be more inclusive of patient populations and should not be limited to postmenopausal women with KOA and OP. At the same time, more attention is given to animal experiments to provide effective evidence for clinical use. In addition, with the participation of modern pharmacology, finding the common target or pathway of KOA and OP may provide ideas for the development of KOA or OP disease and the study of the relationship between KOA and OP. In clinical practice, more attention should be given to exploring the treatment plan of characteristic TCM therapy for KOA combined with OP so that significant therapeutic effects can be obtained in clinical treatment and patients can obtain higher clinical benefits. In summary, Chinese medicine and Chinese medicine compounds have certain effectiveness in the treatment of KOA combined with OP and can provide ideas for future research on disease development and pathological changes, but more in-depth exploration is needed in specific clinical services.

Author contributions

GZ: Writing – original draft, Writing – review & editing. XZ: Writing – original draft, Writing – review & editing. ZG: Writing – original draft, Writing – review & editing. JZ: Writing – original draft, Writing – review & editing. ML: Conceptualization, Writing – review & editing. JL: Conceptualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Guidelines for the diagnosis and treatment of knee osteoarthritis with integrative medicine based on traditional Chinese medicine

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Knee osteoarthritis (KOA) is a common geriatric disease in middle-aged and elderly people. Its main pathological characteristics are articular cartilage degeneration, changes in subchondral bone reactivity, osteophyte formation at joint edges, synovial disease, ligament relaxation or contracture, and joint capsular contracture. The prevalence rate of symptomatic KOA in middle-aged and elderly people in China is 8.1%, and this is increasing. The main clinical manifestations of this disease are pain and limited activity of the knee joint, which seriously affect the quality of life of patients and may cause disability, posing a huge burden on society and the economy. Although the pathogenesis of KOA is not clear, the treatment of KOA is diverse, and Chinese medicine, which mainly relies on plant-based natural products, has a relatively stable and reliable curative effect. This guideline aims to emphasize the evidence-based staging and stepped treatment of KOA and the therapeutic effect of integrative medicine based on traditional Chinese medicine on KOA. We make recommendations that include the adoption of manual therapy, acupuncture, external application of herbs, herbal plasters, exercise therapy, and other integrative medicine based on traditional Chinese medicine. Users of the above guidelines are most likely to include clinicians and health managers in healthcare settings.

KEYWORDS

degenerative arthritis, musculoskeletal diseases, bone aging, knee osteoarthritis, plant-based natural products, herbal medicine, evidence-based, traditional Chinese medicine

Highlights

- This document discusses the diagnosis, differentiation, treatment, and health management of knee osteoarthritis.
- This document is applicable to the diagnosis and treatment of knee osteoarthritis.
- This document is suitable for use by clinicians in orthopedics, traditional Chinese medicine, acupuncture, manual therapy, rheumatology and immunology, rehabilitation, and other related departments.

Introduction

Knee osteoarthritis (KOA) is classified into the "bone impediment" and "bi syndrome" categories in traditional Chinese medicine (TCM) (1–3). It is a chronic joint disease characterized by articular cartilage degeneration, subchondral bone disease, and synovial inflammation. In the early stage, the main symptoms are knee pain and tenderness, which are obvious when moving down stairs or performing strenuous activity, and in the late stage, joint movement limitation, muscular atrophy, and knee inversion deformity can occur. The prevalence of KOA is 24.7% in men and 54.6% in women aged over 40 years, and the final disability rate of the disease is 53% (2, 3). With the aging population, the incidence of this disease is on the rise. Recent research suggests that nearly half of people over the age of 60 and more than 80% of people over the age of 75 suffer from KOA (2, 3). Age, obesity, inflammation, trauma, and genetic factors may be related to the onset of KOA, which is characterized by primary or secondary degeneration of knee cartilage and bone hyperplasia.

Although there is currently no treatment that can reverse the progression of osteoarthritis, both pharmacological and non-pharmacological interventions that can alleviate these symptoms are being used (1–5). Clinicians practicing "integrative medicine based on traditional Chinese medicine" pay attention to the needs that are not being met by current interventions. We call these "unmet medical needs," and in fact, it is considered that the purpose of all traditional medicine in modern society is to meet these needs. TCM-based integrative medicine has an advantage in that it can solve some problems in patients with KOA.

Regarding the diagnosis and treatment of KOA, the relevant guidelines issued in China include the *Clinical Diagnosis and Treatment Guide* of the Chinese Medical Association, the *Diagnosis and Treatment Guide of Common Diseases of Orthopedics and Traumatology* of the Traditional Chinese Medicine Association, the *Guidelines for Integrative Diagnosis and Treatment of Knee Osteoarthritis* of the Chinese Integrative Medicine Association, and the *Diagnosis and Treatment Guide of Osteoarthritis* of the Chinese Rheumatology Association. This guide highlights the staged treatment of KOA more than previous versions and previous guidelines. As clinical and basic research advances, recommendations need to be updated. Therefore, the development of clinical practice guidelines for the integration of TCM and Western medicine for KOA based on evidence-based medicine is of great significance, which helps to implement the principles of evidence-based medicine in clinical practice, standardize the clinical diagnosis and treatment techniques of integrative medicine based on TCM, promote the quality of medical services, help clinicians and patients choose the best treatment plan, and achieve better curative effects.

We developed this guideline using a systematic methodology to summarize and evaluate the effects of integrative TCM-based KOA treatment. We hope this guideline provides guidance to prevent and treat KOA in clinical practice. Due to the limitation on the length of this paper, the relevant research methods and procedures are provided in the Supplementary material.

This diagnosis and treatment guide is compiled by referring to the latest international and Chinese guidelines, bringing together the diagnosis and treatment experience and research results of experts in TCM and Western medicine, and it strives to explain the principles of TCM and Western medicine treatment in different periods of the disease in a concise manner. To assist clinicians and TCM doctors, as well as rehabilitative and nursing staff, to better apply the diagnosis and treatment guidelines of integrative medicine based on TCM to the treatment of patients with KOA, its scientific background, practical value, and compliance need to be continuously verified in clinical practice and updated and improved according to feedback from clinical practice.

Diagnosis

Medical history

Patients have a history of strain such as excessive weight bearing of the knee joint, congenital malformations around the knee joint (varus, valgus, etc.), or a history of trauma. Patients are mostly middle-aged and elderly.

Signs and symptoms

1. Pain and tenderness: The incidence of pain and tenderness is 36.8–60.7% (2). The following types of pain occur: ① starting pain: after sitting for a long time or when just getting out of bed, it hurts to start walking, and this pain is slightly relieved after activity; ② activity pain: after walking for a period of time, the pain intensifies; ③ weight-bearing pain: pain occurs when the knee joint is in a weight-bearing state, such as when going up and down stairs; and ④ resting pain: the knee joint in the resting state is painful, especially at night. In addition to pain, local tenderness may appear in the knee joint, which is obvious when the joint is swollen (2, 3).
2. Limited activity: There is joint stiffness and tightness in the morning, which often lasts a few minutes to 10 min, and rarely more than 30 min. Joint lock can gradually appear, late joint activity is obviously limited, and this can eventually disable the patient (3).
3. Joint deformities: In the late stage of the disease, obvious internal, eversion, or rotation deformities can be seen.
4. Bone rub feeling: Joint flexion and extension and bone friction sounds can be heard.
5. Muscle atrophy: Atrophy of the extensor muscle group around the knee joint can occur (3), with extensor atrophy being the most significant (4, 5).

Laboratory examination

In the acute phase, C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) may be slightly elevated, but other blood values, immune complexes, and serum complement factor levels may be normal (1, 2).

Patients with synovitis may have joint effusion. The joint fluid is transparent and light yellow, and its viscosity is normal or slightly

reduced. Joint fluid routine examination can show mild leukocytosis, mainly monocytes. Synovial fluid analysis helps rule out other joint diseases (2, 3).

Imaging examination

Imaging can help to not only diagnose osteoarthritis but also to assess the severity of joint injury, to evaluate disease progression and response to treatment, and to aid in early detection of disease or related complications. This guide describes the grading criteria for X-ray and magnetic resonance imaging (MRI) commonly used in the diagnosis of KOA.

X-ray

Full-length weight-bearing radiographs of the lower limbs of the knee joint, anterior-lateral radiographs of the knee joint, and axial radiographs of the patella are conventionally the preferred images (6). In the early stage, radiographs are usually normal, and in the middle and late stages, asymmetric narrowing of the joint space, subchondral osteosclerosis and/or cystic changes, joint edge hyperplasia and osteophyte formation, and free bodies can be seen. For imaging classification, we refer to the Kellgren–Lawrence image classification method (7), as shown in Table 1.

MRI

MRI helps to detect and assess the extent of lesions in the joint-related tissues, such as cartilage injury, joint synovial effusion, subchondral bone marrow edema, synovitis, and meniscus or ligament injury. It can also be used to exclude tumors and ischemic osteonecrosis. Generally, Recht grading is the standard for MRI (8), as shown in Table 2.

Diagnostic criteria

Diagnostic criteria are mainly based on the patient's symptoms, signs, and imaging results. Here, the diagnostic criteria revised by the Chinese Association of Integrative Medicine Based on Traditional Chinese Medicine in 2018 are adopted (9), as shown in Table 3.

Differential diagnosis of KOA

Knee osteoarthritis should not be confused with the following diseases (10).

Rheumatoid arthritis

Most cases of rheumatoid arthritis are symmetrical microarthritis, mainly involving proximal interphalangeal joints, metacarpophalangeal joints, and wrist joints, with obvious morning stiffness. Subcutaneous nodules may occur, and they may be rheumatoid factor-positive. X-ray examination shows mainly joint erosive change. Other indications of rheumatoid arthritis include the presence of anti-cyclic peptide containing citrulline (anti-CCP) and lung involvement, for example interstitial lung disease. Moreover, RA can begin as monoarthritis in the elderly, often involving knee or other big joints (2, 3).

Gouty arthritis

In middle-aged and elderly people, mostly men, recurrent acute arthritis may occur, which most often involves the first metatarsophalangeal joint and tarsal joint, and sometimes also the knee, ankle, elbow, wrist, and hand joints. It presents with joint redness, swelling, heat, and severe pain. The blood uric acid level is elevated and urate crystals can be found in the synovial fluid. In chronic cases, kidney damage may occur, and gout stones may appear around the joints and in the auricle (3, 6).

Ankylosing spondylitis

Ankylosing spondylitis mostly occurs in young men and mainly affects the sacroiliac joints and spine. Knee, ankle, and hip joints are often involved, morning stiffness is obvious, patients often have inflammatory low back pain, radiological examination often reveals sacroiliarthritis, and patients are often positive for human leukocyte antigen (HLA)-B27 (6, 9).

Psoriatic arthritis

Psoriatic arthritis usually occurs in middle-aged people, and the onset is slow, mainly involving the limbs joints of the distal finger (toe), metacarpal and phalangeal joints, metatarsal joints, and knee and wrist joints. The joint lesions are often asymmetric, and the joints can be deformed. During the course of the disease, psoriasis may cause changes in the skin and nails of the fingers (toes) (9, 10).

Chronic disease management

Knee osteoarthritis should be included in chronic disease management. Chronic disease management includes regular detection of common symptoms, continuous monitoring, evaluation, and comprehensive intervention for KOA and its risk factors, as well as lifestyle management and the evaluation of management effects. Chronic disease management emphasizes health education and

TABLE 1 Kellgren–Lawrence knee osteoarthritis grading method.

Grade	Joint change
Grade 0	No changes
Grade I	Slight joint space narrowing and possible osteophytic lipping
Grade II	Presence of osteophytes and possible narrowing of the joint space
Grade III	Presence of multiple osteophytes, definite narrowing of the joint space, some sclerosis, and possible deformity of the bone ends
Grade IV	Presence of large osteophytes, marked narrowing of the joint space, severe sclerosis, and definite deformity of bone ends

TABLE 2 Recht grading of MRI for knee osteoarthritis.

Grade	Imaging manifestations
Grade 0	No change (normal)
Grade I	Abnormal signals within the cartilage, but the cartilage surface is smooth
Grade II	Mild irregularities on the surface of the cartilage and/or focal defects of less than 50% of the full thickness of the cartilage
Grade III	The surface of the cartilage is severely irregular and/or focal defects of more than 50% of the total thickness of the cartilage but less than the full thickness occur
Grade IV	Full-layer cartilage defect with exposed subchondral bone

non-drug interventions at all stages of disease and development to maximize the self-management ability of patients in daily life.

The *AAOS Clinical Practice Guideline Summary: Management of Osteoarthritis of the Knee (Nonarthroplasty), Third Edition* (11) suggests that health education and lifestyle changes play an important role in the treatment of KOA and that orthopedic surgeons and other skeletal and musculoskeletal health professionals should treat arthritis as a chronic disease.

Clinical stage and Chinese classification of KOA

Clinical stage

Traditional Chinese medicine concepts and imaging results should be combined in KOA staging and classification for chronic disease management and treatment (12, 13). Under the holistic view of TCM and general health concepts, KOA is divided into five stages based on the theory of TCM treatment without disease, the modern concept of knee preservation, and the requirements of patient subdivision in chronic disease management, combined with imaging evaluation, as shown in Table 4 (Highly recommended).

Evidence description

The previous three-stage method was not detailed enough to facilitate the management of chronic diseases. The stages of our guideline are based on the theory of treatment without disease, setting up the early stage (phase 1) to control risk factors, while the rest of the stages are subdivided into four phases according to the development of the disease, in order to achieve the management of the whole life cycle of chronic KOA, delay the degeneration and aging of the joint (including the postoperative period) as much as possible, prolong the service life of the joint, and improve the quality of life. The main consensus for regarding KOA classification is the five-stage clinical classification method, which is mainly based on the theory of Chinese treatment of non-disease, the modern concept of knee protection, and the requirements of chronic disease management in patient population segmentation (Highly recommended).

Chinese classification

The syndrome differentiation methodology based on the *Guiding Principles for Clinical Research of New Chinese Medicine*, the *Classification and codes of diseases and patterns of traditional Chinese medicine*, and the *Guidelines for the Diagnosis and Treatment of Common Diseases of Orthopadics and Traumatology in traditional Chinese medicine* is further improved by the previous literature review, and the following syndrome types are summarized. There may be different syndromes or mixed syndromes, which can be differentiated according to clinical practice (14–18).

TABLE 3 Classification criteria of knee osteoarthritis.

Sequence number	Symptoms or signs
1	Recurring knee pain in the past month
2	Age ≥ 50 years
3	Morning stiffness time ≤ 30 min
4	Bone friction while moving the knee
5	X-ray (standing or weight-bearing position) shows narrowing of the joint space, subchondral bone sclerosis and/or cystic degeneration, and osteophyte formation at the joint margin
6	MRI shows cartilage injury, osteophyte formation, subchondral bone marrow edema and/or cystic change, degenerative meniscal tear, and partial or full cartilage loss

Knee osteoarthritis can be diagnosed if diagnostic criteria 1 + 2 + 3 + 4 or 1 + 5 or 1 + 6 are met.

Dampness-cold obstruction syndrome

Traditional Chinese medicine pathogenesis: Cold and damp qi invade joints.

Main symptoms: The joint pain feels heavy; cold aggravates the feeling, and warmth reduces it.

Secondary symptoms: A heavy pain feeling in the waist, a pale tongue, greasy white fur, and a gentle pulse may occur.

Dampness-heat obstruction syndrome

Traditional Chinese medicine pathogenesis: Heat and damp qi invade joints.

Main symptoms: Joints are inflamed (redness of skin, swelling, heat, pain), flexion and extension are difficult, and walking is difficult.

Secondary symptoms: These include fever, thirst but no desire to drink, and being upset; the tongue is red, the fur is yellow and greasy, and the pulse is moist or slippery.

Qi-blood stagnation syndrome

Traditional Chinese medicine pathogenesis: Poor circulation of qi and blood.

Main symptoms: Joint pain and tingling may occur; after rest, the pain is worse.

Secondary symptoms: These include dark complexion, a dark purple tongue, and ecchymosis; the pulse may be heavy and uncomfortable.

Deficiency of liver and kidney

Traditional Chinese medicine pathogenesis: The function of the Zangfu is degraded, and the body is unable to nourish the bones and muscles.

Main symptom: Dull joint pain.

Secondary symptoms: These include waist and knee weakness and acid pain, which is even worse in case of labor. Moreover, the patient

TABLE 4 Imaging stages and clinical manifestations of knee osteoarthritis.

Stage	Clinical manifestation	Imaging manifestations	Time division
Stage I (pre-stage)	Joints have mild discomfort; patients experience cold intolerance; going up the stairs, squatting, and standing up is difficult; joints may have friction; and joint activity may make noise. Acute synovitis may occur in very few patients after intense exercise, but the diagnostic criteria do not include osteoarthritis or developmental internal and external joint inversion deformity.	Cartilage wear grade 0. The Kellgren–Lawrence imaging grade is 0 or I, and MRI findings are normal.	Pre-disease stage/erotogenic stage
Stage II (early stage)	In the early stage, knee osteoarthritis (KOA) can be confirmed by diagnostic criteria. Non-drug therapy can be given, and acute episodes of pain after excessive exercise or exertion can generally be cured clinically.	Meniscus injury stage. Meniscus degeneration, tear, or exophysis and subchondral bone marrow edema can occur, and symptoms of single ventricular high pressure may be revealed by imaging. MRI shows abnormal signals within the cartilage, but the surface of the cartilage is smooth. The Kellgren–Lawrence imaging grade is I or II.	Stage of attack/remission
Stage III (middle stage)	The number of acute episodes of joint pain and swelling increases, which require painkiller control, and the symptoms are not easy to cure, which require multiple long-term therapies.	Period of partial cartilage wear. Partial cartilage wear is accompanied by subchondral bone edema and meniscus degeneration, tear, or exophysis, and imaging may reveal symptoms of single ventricular hypertension. MRI shows mild irregularities on the surface of the cartilage and/or focal defects of less than 50% of the full thickness of the cartilage. The Kellgren–Lawrence imaging grade is II or III.	Stage of attack/remission
Stage IV (late stage)	The developmental articular inversion angle is increased. The number of acute attacks of joint pain and swelling increases, and the symptoms cannot be completely relieved by medication.	Single compartment contact phase. MRI shows serious irregularities on the surface of the cartilage and/or focal defects of more than 50% of the full thickness of the cartilage, bone marrow edema, and even local exposed and necrotic subchondral bone. The Kellgren–Lawrence imaging grade is III or IV.	Stage of attack/remission
Stage V (surgery stage)	The effect of conservative treatment is poor, the joints are stiff, movement is obviously impaired, swelling pain often occurs, muscle atrophy occurs, and a walker or a crutch is often needed to walk.	Multiple ventricular degeneration stage. The Kellgren–Lawrence imaging grade is IV. MRI shows extensive full-layer cartilage defects, exposed subchondral bone, and even bone necrosis.	Stage of attack/remission

Stage of attack: Knee joint pain is moderate or severe and persistent severe pain may cause sleep difficulties; knee joints are swollen, their function is limited, and patients limp or are even unable to walk. Remission: Mild pain in the knee joint is aggravated by fatigue or weather changes; patients may suffer from acid swelling, fatigue, or limited knee joint activity.

may have a red tongue with a small amount of coating; furthermore, their pulse may be thin and weak.

Qi-blood weakness syndrome

Traditional Chinese medicine pathogenesis: Deficiency of Qi and blood leads to a loss of nourishment of muscles and bones.

Main symptom: Joint pain and discomfort.

Secondary symptoms: These include less sleep and more dreams, uncontrollable sweating or night sweats, dizziness, palpitation, shortness of breath, a seedy look, a thin tongue, a thin white fur, and a thin and weak pulse.

Treatment

Therapeutic principle

Knee osteoarthritis treatment should follow the steps of TCM and Western medicine. KOA is a chronic degenerative joint disease, clinically divided into stage I (pre-stage), stage II (early stage), stage III (middle stage), stage IV (late stage), and stage V (surgery stage). The overall treatment method is a combination of non-drug and drug therapy, including surgical treatment if necessary, and treatment should be individualized. We should not only flexibly choose Chinese medicine, acupuncture, manual therapy, and other therapies according to the TCM system of syndrome differentiation and treatment but also carry out step treatment based on TCM and Western medicine and strictly grasp the indications. In step therapy, the treatment plan needs to be optimized continuously to maximize the curative effect. The comprehensive treatment effect of TCM is good throughout the entire course of treatment. Health education and exercise are important measures for treatment and consolidation of curative effects (12–14) (Highly recommended).

Description of the evidence

According to the clinical manifestations and imaging evaluation of KOA, KOA is divided into five stages for the staged TCM-based integrative medicine treatment. The clinical stages emphasize the

concepts of "treatment without disease" and "prevention before disease" to actively prevent the occurrence of KOA by improving the patient's lifestyle and avoiding risk factors. When KOA occurs, in order to prevent or delay the progression of KOA, the clinical manifestations and imaging data are integrated to provide individualized step therapy, making full use of the advantages of Chinese and Western medicine (19).

Non-drug therapy

Non-drug therapy plays a very important role in the treatment of KOA throughout all clinical stages.

Health education and self-management

Knee osteoarthritis patients should be educated about their health and helped to manage themselves.

Health education (20, 21): Health education can reduce pain and improve the psychosocial status of KOA patients. Doctors should guide patients to: ① recognize the disease and have a clear purpose of treatment (improve symptoms, delay the development of the disease); ② build confidence, eliminate the burden of thought, and relieve anxiety and fear of sports; ③ closely cooperate with doctors in diagnosis and treatment; and ④ adjust their lifestyle and perform reasonable exercise (Evidence level: Level II, highly recommended).

Self-management (22, 23): Overweight and obesity are recognized risk factors for the onset of KOA, which can lead to joint pain and even disability in patients. Diet control combined with exercise can improve the efficacy of weight loss in the treatment of KOA symptoms (Evidence level: Level II, highly recommended).

Exercise therapy

Knee osteoarthritis patients should practice therapy under the guidance of a doctor, such as straight leg elevation, Tai Chi (23), and Baduanjin (24) (Evidence level: Level I, highly recommended).

Description of the evidence

A meta-analysis (23) involving eight randomized controlled trials (RCTs) showed that Tai Chi can improve pain in patients with KOA (ES = −0.75, 95% confidence interval [CI]: −0.99 to −0.51; Q = 8.9, $p = 0.26$; $I^2 = 21\%$), reduce rigidity (ES = −0.70, 95%CI: −0.95 to 0.46; Q = 9.6, $p = 0.21$; $I^2 = 27\%$), and improve activity (ES = −0.91, 95%CI: −1.12 to 0.70; Q = 7.2, $p = 0.40$; $I^2 = 3\%$). A meta-analysis (24) involving seven RCTs showed that Baduanjin could improve KOA patients' pain (mean difference [MD] = 1.69, 95%CI: 2.03–1.35, $p < 0.01$), reduce rigidity (MD = 0.86, 95% CI: 1.13–0.58, $p < 0.01$), and improve functional activity (MD = 2.23, 95%CI: 3.65–0.82, $p < 0.01$).

Manual therapy

Manual therapy is used to reduce pain and restore knee motion in KOA patients. The use of pushing and kneading point pressing, pulling and stretching the knees, shaking the knees, and holding and plucking the knees, can play a role in relaxing the tendons and clearing collaterals, promoting blood circulation and removing blood stasis, releasing adhesion, and smoothing the joints, which can improve joint stiffness and muscle strength, reduce joint pain, and improve joint function (25, 26). Patients with infection, skin lesions, tumors, and cardiovascular and cerebrovascular diseases should be treated with caution (Evidence level: Level I, highly recommended).

Manual therapy can effectively relieve the clinical symptoms of patients with KOA and improve their quality of life, without obvious adverse reactions.

Description of the evidence

A meta-analysis (25) involving eight RCTs showed that manual therapy improved the healing rate (odds ratio [OR] = 1.81, 95%CI: 1.14–2.88, $p < 0.01$) and the apparent rate for KOA (OR = 2.03, 95%CI: 1.43–2.88, $p < 0.01$), and there were no reports of serious adverse reactions in the included studies. A meta-analysis (26) that included 16 studies showed that manual therapy improved the total response rate (OR = 4.53, 95%CI: 3.06–6.69, $p < 0.00001$), reduced pain (MD = −2.72, 95%CI: −4.19 to −1.25, $p < 0.00001$), improved the WOMAC score (MD = −14.21, 95%CI: −14.86 to −13.56, $p < 0.00001$), and improved the hospital knee joint score (MD = 6.32, 95%CI: 4.58–8.06, $p < 0.00001$).

Acupuncture

Acupuncture can reduce pain and restore knee motion in KOA patients. Acupuncture, including millimeter acupuncture therapy, warm acupuncture therapy, and electric acupuncture therapy, has a positive effect on relieving KOA pain and improving joint function (27, 28). Moxibustion, which integrates heat therapy, light therapy, drug stimulation, and specific acupoint stimulation, can effectively reduce the vascular permeability of inflammatory foci, improve hemorheologic and hemodynamic indices, relieve knee pain, improve joint function, and improve patients' quality of life in clinical application (29, 30) (Evidence level: Level I, highly recommended).

Recommended acupoints

Xiyan, Yanglingquan, Zusanli, Xuehai, Liangqiu, internal Xiyan, Yinlingquan, Heding, Xiyangguan, and Pian (31).

Description of the evidence

A meta-analysis (27) involving 11 RCTs showed that acupuncture was effective in reducing pain in patients with KOA [$n = 2{,}387$; standard mean difference (SMD) = −0.12, 95%CI: −0.20 to −0.04; $I^2 = 0\%$] and in improving functional activity [$n = 2{,}408$; MD = −1.25, 95%CI: −1.97 to −0.53; $I^2 = 0\%$]. A meta-analysis (28) that included 14 studies showed that compared with Western medicine, warm acupuncture treatment is more effective in improving the long-term overall effective rate [relative risk (RR) = 1.16, 95%CI: 1.04–1.29, $p = 0.008$] and the short-term cure rate (RR = 2.35, 95%CI: 1.59–3.45, $p < 0.0001$), with fewer adverse reactions than Western drugs (RR = 0.20, 95%CI: 0.05–0.75, $p = 0.02$). A meta-analysis (29) of 13 studies showed that moxibustion was superior to conventional care plus sham moxibustion in reducing WOMAC scores (MD = 7.56, 95%CI: 4.11–11.00, $p = 0.00$). Most of the adverse events caused by moxibustion can be cured without medical treatment. An overview

(involving a re-meta-analysis) (30) that included 10 systematic reviews showed that moxibustion and moxibustion combined therapy improved the overall response rate in KOA patients (RR = 1.17, 95%CI: 1.13–1.21, $p < 0.001$). Four studies reported 10 common discomfort symptoms caused by moxibustion; these adverse events can be resolved naturally, or even avoided, so moxibustion is safe in the treatment of KOA.

Acupotomy

Patients with KOA with confirmed or highly suspected soft tissue adhesion can be treated with acupotomy. Acupotomy can be performed on the suprapatellar bursa, subpatellar fat pad, internal Xiyan-acupoint, external Xiyan-acupoint, tibial collateral ligament, iliotibial bundle, and anseropodium sac. By cutting, separating, spatulating, adjusting, and releasing tendon ligament and other soft tissues, the biomechanical balance of the knee joint can be achieved. It is suitable for KOA patients with knee pain, morning stiffness, muscle adhesion, functional limitation, and obvious contracture flexion deformity. It can relieve knee pain and improve joint function, with good safety (32) (Evidence level: Level II, highly recommended).

Description of the evidence: A meta-analysis (32) including 20 RCTs showed that the effective treatment rate in the acupotomy group was higher than that in the acupuncture group ($\chi^2 = 11.920$, $p = 0.610$, $I^2 = 0\%$, RR = 1.16, 95%CI: 1.11–1.22). The VAS score of the acupotomy group was lower than that of the acupuncture group ($\chi^2 = 94.340$, $p = 0.000$, $I^2 = 89\%$, MD = −1.24, 95%CI: −1.58 to −0.90, $p = 0.000$). In conclusion, the available evidence shows that acupotomy is an effective method for the treatment of KOA, and the efficacy is higher than that of acupuncture therapy.

Physical therapy

Physical therapy is recommended to help patients with KOA with related symptoms. Common methods include thermal therapy (33), magnetic therapy (34), infrared irradiation (35), hydrotherapy (36), wax therapy (37), ultrasound (38), and other physiotherapy methods, which can be combined with acupuncture, manipulation, and other therapies to improve joint activity, relieve pain and muscle tension, promote local blood circulation, and reduce inflammatory (Evidence level: Level I, Highly recommended).

Braces

Braces are recommended for patients with mobility difficulties or KOA patients with patellar arthritis. They have the following purposes:

① Reduce the load on the affected joints: Canes and walkers can be used to assist activities (39).

② Joint protection: Elastic sleeves can be worn to protect joints, such as knee pads. The treatment of patellofemoral compartment osteoarthritis with a patellofemoral medial patellofemoral patch can significantly reduce pain. Medial ventricular osteoarthritis of the knee joint can be assisted by wedge insoles (40–42) (Evidence level: Level I, weak recommendation).

Drug treatment

Sometimes, non-drug treatment is ineffective, and drug treatment can be selected according to the condition of joint pain.

Drugs for external use

Knee osteoarthritis patients can use TCM, proprietary Chinese medicines, or non-steroidal anti-inflammatory drugs (NSAIDs) for topical use:

① The topical use of Chinese herbs mainly includes fumigation, application, and iontophoresis (43–45) (Level of evidence: I, highly recommended).

② The external use of proprietary Chinese medicines mainly includes various pastes, plagues, ointments, and tinctures (46–48) (Level of evidence: I, highly recommended).

③ Non-steroidal anti-inflammatory preparations for local topical use, which may cause minor adverse reactions, can reduce joint pain and tenderness (49) (Evidence level: Level II, highly recommended).

Joint cavity injection therapy

Joint viscoelastic supplement therapy with agents such as sodium hyaluronate and medical chitose (joint cavity injection) should be used according to doctors' clinical experience and patients' specific conditions (50–52). Platelet-rich plasma is rich in a variety of growth factors and inflammatory regulators, which can protect chondrocytes, promote cartilage healing, reduce intra-joint inflammation, relieve pain, and improve joint function (53). Injection of long-acting corticosteroids into the joint can relieve pain and reduce exudation. The curative effect lasts for several weeks to several months, and repeated injection in the same joint is preferred to avoid aggravating articular cartilage; the injection interval should not be shorter than 4–6 months (2, 54).

Description of the evidence: a meta-analysis (50) of 89 studies showed that joint viscoelastic supplements reduced pain symptoms in patients but increased the risk of adverse events. Results of an RCT (51) involving 82 patients showed no difference in Knee Society Score (KSS) function and VAS scores between the sodium hyaluronate group and the steroid group at 4 weeks, and sodium hyaluronate was significantly better than the steroid at 6 months. A meta-analysis (52) of 54 studies showed that intra-articular injection of sodium hyaluronate could provide relief within 4 weeks. Patients with joint pain symptoms reached a peak response within 8 weeks, and the treatment effect was superior to acetaminophen painkillers, NSAIDs, and COX-2 inhibitors. Results of a meta-analysis that included 15 studies (53) showed that in terms of long-term pain relief and functional improvement of the knee joint, platelet-rich plasma (PRP) injection may be more effective than sodium hyaluronate injection, but the optimal dose, the time interval and frequency of injections, and the ideal treatment for the different stages of KOA remain uncertain. In an RCT (54) that included 66 patients, before treatment and at 7 and 28 days after treatment, the WOMAC scores were 99.6 ± 38.9, 44.2 ± 23.5, and 25.4 ± 21.5, respectively; the treatment of KOA

with joint injection of semetasone palmitate can improve the pain symptoms, cystic effusion, and inflammation, with few adverse reactions.

Diagnosis and prescription for Chinese medicine

Chinese medicine

Dampness-cold obstruction syndrome

Treatment is based on the principles of warm channel dispelling cold, nourishing blood, and activating the pulse-beat (14, 55–59).

Main prescription: Modified Juanbi Decoction (*The Golden Mirror of Medicine*; Highly recommended).

Common drugs: *Notopterygium incisum* Ting ex H. T. Chang, *Saposhnikovia divaricata* (Trucz.) Schischk, *Angelica sinensis* (Oliv.) Diels, Glycyrrhizae, Paeonia tacti lora Pall, and *Zingiber officinale* Roscoe.

Dampness-heat obstruction syndrome

Treatment is based on clearing heat dehumidification, clearing collaterals, and relieving pain (14, 60).

Main prescription: Modified Simiao Decoction (*Danxi's Experiential Therapy*) (Weak recommendation).

Common drugs: *Atractylodes lancea*, *Coix lacryma-jobi*, *Achyranthes bidentata* Blume, *Lonicera japonica* Thunb, *Trachelospermum jasminoides* Lindl. Lem, and *Phryma leptostachya* L. subsp. *asiatica* (Hara) Kitamura.

Qi-blood stagnation syndrome

Treatment is based on promoting blood circulation, removing blood stasis, clearing collaterals, and relieving pain (14, 61).

Main prescription: Modified Taohong Siwu Decoction (*YiLei YuanRong*; Highly recommended).

Common drugs: *Rehmannia glutinosa* (Gaetn.) Libosch. ex Fisch. et Mey, *Angelica sinensis* (Oliv.) Diels, Paeonia tacti lora Pall, *Ligusticum chuanxiong* hort, *Prunus persica* (L.) Batsch, and *Carthamus tinctorius* L.

Deficiency of liver and kidney

Treatment is based on nourishing liver and kidney (14, 62).

Main prescription: Modified Duhuo Jisheng Decoction (*Precious Essential Formulary for Emergency*; Weak recommendation).

Common drugs: *Taxillus sutchuenensis* (Lecomte) Danser, *Eucommia ulmoides* Oliver, *Achyranthes bidentata* Blume, *Asarum sieboldii* Miq, *Poria*, and *Gentiana macrophylla*.

Qi-blood weakness syndrome

Treatment is based on supplementing qi and nourishing blood (14, 63).

Main prescription: Modified Bazhen Decoction (Danxi's Experiential Therapy; Highly recommended).

Common drugs: *Panax ginseng* C. A. Mey, *Cinnamomum cassia* Presl, *Ligusticum chuanxiong* hort, *Rehmannia glutinosa* (Gaetn.) Libosch. ex Fisch. et Mey, *Poria*, *Angelica sinensis* (Oliv.) Diels, and Paeonia tacti lora Pall.

Chinese patent medicine

It is recommended that clinicians give proprietary Chinese medicines (PCMs) to KOA patients according to their TCM syndrome differentiation. There are various kinds of PCM for the treatment of KOA. Biqi capsule, Longbii capsule, Huli San capsule, Xianling Gubao capsule, Jintiange capsule, and Zhuanggu Guanjie capsule (61–65) can be selected (Evidence level: Level I, weak recommendation).

Description of the evidence: a meta-analysis (64) involving 12 RCTs showed that Huli San capsule treatment improved the symptom remission rate of KOA (RR = 1.38, 95%CI: 1.13–1.69, $p = 0.02$) and the knee function score (MD = 2.88, 95%CI: 0.81–4.94, $p = 0.006$) compared to conventional treatment. There was no significant difference in VAS score (MD = −0.57, 95%CI: −1.42 to −0.29, $p = 0.19$). The results showed that the use of Huli San capsule or Huli San capsule plus conventional Western medicine treatment could improve the symptom relief rate, Lysholm score, knee joint function score, and VAS score of patients with KOA and alleviate the symptoms of knee joint pain, swelling, and movement restriction; moreover, no serious adverse reactions have been reported. A meta-analysis (65) that included seven studies showed that compared with the control group, the treatment response rate in the experimental group was improved (OR = 4.63, 95%CI: 2.83–7.56, $p < 0.01$). The WOMAC score of the test group was lower than that of the control group [weighted mean difference (WMD) = −13.14, 95%CI: −22.07 to −4.22, $p < 0.05$]. The VAS score of the test group was lower than that of the control group (WMD = −15.49, 95%CI: −18.84 to −12.15, $p < 0.01$). There was no significant difference in the incidence of adverse reactions between the experimental group and the control group (OR = 0.73, 95%CI: 0.30–1.78, $p > 0.05$). In conclusion, Zhuanggu Guangjie capsule can reduce the VAS score and WOMAC score of patients with KOA and improve the effective rate, and the clinical efficacy is good, but the risk of adverse reactions should be paid attention to.

A network meta-analysis (66) involving 58 RCTs showed that the top three interventions with the best total response rate were Jinwu Gutong capsule + Glucosamine (GlcN), Xianling Gubao + aminosaccharide, and Biqi capsule. The top three interventions with the best VAS score were Panlongqi tablet, Xianling Gubao + GlcN, and Xianling Gubao + NSAIDs. The top three interventions with the best reduction of WOMAC total score were Jintiange capsule + NSAIDs, Jinwu Gutong capsule + GlcN, and Biqi capsule + NSAIDs. The three interventions with the best efficacy in reducing the Lequesne index were Xianling Gubao + NSAIDs, Biqi capsule + NSAIDs, and Jintiange capsule + NSAIDs. The three best interventions to reduce the level of TNF-α were Xianling Gubao + GlcN, Jintiange capsule, and Jintiange capsule + GlcN = Jinwu Gutong capsule + GlcN. In terms of safety, the top five interventions with the fewest adverse reactions were Biqi capsule, Jinwu Gutong capsule, Biqi capsule + NSAIDs, Xianling Gubao + NSAIDs, and Jintiange capsule. The results showed that the combination of PCM and NSAIDs or GlcN could improve the clinical therapeutic effect and reduce the adverse reactions in patients with KOA.

A meta-analysis (67) of 21 RCTs including 16 papers using the effective rate of continuous variables as the index showed that the Xianling Gubao group was superior to the control group in terms of effective rate (RR = 1.21 95%CI: 1.16–1.26, $p < 0.00001$). A total of five studies were included with bicategorical variables as the index of pain

relief time. Xianling Gubao was superior to the control in shortening pain relief time (MD = −1.51, 95%CI: −1.81 to −1.21, $p < 0.00001$). A total of five studies were included using bicategorical variables to improve the Lysholm knee function score, and Xianling Gubao could significantly improve knee function compared with the control group (MD = 17.21 95%CI: 10.02–24.39, $p < 0.00001$). In addition, adverse reactions were reported in five of the 21 included studies, all of which resolved spontaneously without specific treatment. In conclusion, Xianling Gubao capsule can effectively improve several indices of patients with KOA and is worthy of clinical promotion and use.

A meta-analysis (68) including six RCTs showed that Biqi capsule combined with Western medicine resulted in a better overall response rate in the treatment of KOA than Western medicine alone (RR = 1.22, 95%CI: 1.15–1.29, $p < 0.00001$), and the WOMAC score was lower than that of the Western medicine group (MD = −10.57, 95%CI: −12.17 to −8.97, $p < 0.00001$). Biqi capsule combined with Western medicine was better than Western medicine alone in reducing the VAS score of KOA patients and improving their quality of life, and no serious adverse events occurred. In conclusion, Biqi capsule combined with Western medicine has good clinical effects and safety in the treatment of KOA.

Pain relief drugs

Medications can be taken orally to relieve pain in KOA patients:

① Acetaminophen: Because the elderly are prone to adverse reactions to NSAIDs and synovitis is not a major factor in the early stage of KOA, short-term acetaminophen can be used in mild cases (69).

② NSAIDs: These have both analgesic and anti-inflammatory effects and are the most commonly used drugs to control osteoarthritis symptoms (70). The main adverse effects include gastrointestinal symptoms, kidney or liver functional impairment, impaired platelet function, and an increased risk of cardiovascular adverse events. They should be used with caution if the patient is at risk for cardiovascular adverse events.

③ Opioids: For patients with acute pain attacks, when acetaminophen and NSAIDs cannot fully relieve pain or there is a drug contraindication, weak opioids can be considered, which are better tolerated and less addictive, such as oral tramadol. Such treatments should be started at a low dose and slowly increased every few days to reduce adverse reactions (71) (Evidence level: Level I, highly recommended).

Disease-modifying OA drugs and cartilage protectants

Such drugs tend to work slowly, taking weeks of treatment to exert effects. Therefore, they are called slow acting drugs. They can reduce the activity of matrix metalloproteinase and collagenase, and they can not only exert anti-inflammatory and analgesic effects but can also protect articular cartilage and delay the development of KOA. However, there is no recognized ideal drug at present, and commonly used drugs such as GlcN, Diacerein, and chondroitin sulfate may have certain effects (72–74) (Evidence level: Level I, highly recommended).

Surgery

For patients with recurrent knee swelling pain and joint effusion, the effect of non-surgical treatment is not good, the pain is progressive, the joint is obviously dysfunctional, and surgical treatment can be considered to correct the deformity and improve joint function. Surgical treatment is recommended after assessment of the condition and surgical indications.

Arthroscopic surgery (level of evidence: I)

Arthroscopic surgery is recommended for early and middle stage patients (75–78). Arthroscopy is both diagnostic and therapeutic, mainly for patients with mechanical locking or meniscal tears and other symptoms. Through arthroscopic free body cleaning and meniscus molding, the symptoms of some patients in the early and middle stages can be alleviated, the internal joint environment can be improved, and the synovial inflammatory reaction can be alleviated. For advanced patients with abnormal force lines and obvious osteophytic hyperplasia, arthroscopic irrigation or clearance alone has poor results.

Description of the evidence: a meta-analysis (75) of nine studies showed that pain symptoms in patients with KOA could be improved at 3 and 6 months compared with the control group, but there was no significant improvement in motor function.

An RCT (76) that included 107 patients showed that the average WOMAC scores of 56 patients in the operation group were 624 ± 98 and 865 ± 589 at 1- and 2-year follow-up, respectively. The average WOMAC scores of 51 patients in the control group were 902 ± 521 and 914 ± 605 at 1- and 2-year follow-up, respectively. At 1-year follow-up, the WOMAC score of the operation group was significantly different from that of the control group ($p < 0.05$), but there was no significant difference in WOMAC score between the two groups at 2 years after surgery ($p > 0.05$). In conclusion, limited arthroscopic surgery can provide short-term (≤1 year) symptom relief in patients with KOA.

An RCT (77) involving 70 patients showed that the VAS score decreased by 24.45 ± 9.09, 18.45 ± 11.60, and 8.29 ± 14.19% in the PRP treatment group at 3, 6, and 9 months, respectively. In the arthroscopic treatment group, the VAS score decreased by 18.96 ± 5.85, 7.33 ± 8.60, and 3.20 ± 7.39% at 3, 6, and 9 months, respectively. A statistically significant difference was observed between the two groups only at 3 and 6 months ($p < 0.05$). The WOMAC score decreased by 24.03 ± 11.41, 17.45 ± 9.24, and 9.49 ± 9.80% in the PRP group and by 11.27 ± 5.73, 5.70 ± 4.78, and 0.13 ± 5.06% in the arthroscopic group at 3, 6, and 9 months, respectively. At all three time points, the difference between the two groups was statistically significant ($p < 0.001$). In conclusion, this study suggests that both PRP and arthroscopy can reduce the WOMAC and VAS pain scores in patients with KOA.

Periknee osteotomy (evidence level: level I)

Periknee osteotomy is suitable for younger (generally <65 years old), relatively active patients with good bone mass, external joint deformity, and meniscus protrusion. The knee joint structure is preserved to the maximum extent, and KOA symptoms are relieved

by changing the lower limb force line, improving function and effectively relieving joint pain in patients.

1. Proximal tibial osteotomy: The tibia is the main varus deformity, with MPTA <85°. Medial KOA meets the following characteristics: ① grade 0 cartilage wear: young active patients with obvious varus deformity, symptoms after exercise, and a strong desire for surgery; ② meniscus injury period and partial cartilage wear period: it is a strong indication period, especially for patients over 45 years old with imaging manifestations of internal and lateral ventricular hypertension and ineffective conservative treatment; ③ bone contact stage: patients with active exercise and significant proximal varus deformity or patients who refuse to undergo joint replacement and have good mobility and are not obese (79–83).
2. Distal femoral osteotomy: Distal femoral osteotomy is performed in patients with distal femoral malformations. It is mainly used in patients with lateral interventricular KOA with valgus deformity (84, 85) or medial interventricular KOA with distal femoral deformity.

Description of the evidence: a meta-analysis (79) involving 13 studies showed that medial tibial open wedge osteotomy and lateral tibial closed wedge osteotomy were mainly associated with postoperative posterior inclination of the tibial plateau (MD = 2.82, 95%CI: 1.31–4.33, $p = 0.0002$). Significant differences in patellar height BPI (MD = −0.09, 95%CI: −0.11 to −0.07, $p < 0.00001$) and operative time (MD = −19.48, 95%CI: −31.02 to −7.94, $p = 0.0009$) were also found. Both surgical methods had similar effects on the postoperative mechanical axis angle (MD = −0.01, 95%CI: −0.51 to 0.48, $p = 0.96$), correction angle (MD = −0.16, 95%CI: −0.75 to 0.43, $p = 0.60$), HSS score (MD = −0.46, 95%CI: −1.47 to 0.55, $p = 0.37$), VAS score (MD = 0.12, 95%CI: −0.24 to 0.48, $p = 0.51$), Lysholm score (MD = −0.17, 95%CI: −2.53 to 2.19, $p = 0.89$), and complications (OR = 0.68, 95%CI: 0.25–1.82, $p = 0.44$). In conclusion, the overall clinical efficacy of medial open tibia and lateral closed wedge osteotomy in the treatment of single compartment KOA is similar, but the medial open wedge osteotomy is easier to perform, and it is easy to increase the posterior inclination of the tibial plateau and the patella decline after surgery. The clinician should conduct adequate preoperative imaging evaluation and individual selection of the appropriate surgical procedure for patients with single compartment KOA.

A meta-analysis (80) that included five studies showed that there was no significant difference between the experimental group and the control group in terms of bone non-union.

Alternative materials combined with locking plate technology provide a safe and effective alternative to open wedge high tibial osteotomy (HTO) with an osteotomy gap greater than 10 mm, but there is a possibility of bone non-union.

A meta-analysis (81) that included 19 studies and a meta-analysis (82) that included 11 studies showed that HTO was superior to monocondylar replacement in terms of range of motion.

The results of a clinical study (84) that included 22 patients showed that the femoral tibial angle, negative gravity line ratio, and distal lateral femur angle were significantly improved 1 year after surgery compared with those before surgery ($p < 0.05$). The pain VAS score and knee HSS score were significantly improved at 1 and 2 years after surgery ($p < 0.05$), but there was no significant difference between these scores at 1 year after surgery and these scores at 2 years after surgery ($p > 0.05$). In conclusion, distal medial wedge osteotomy of the femur is an ideal knee-saving treatment method for patients with osteoarthritis combined with knee valvaration. It can achieve satisfactory medium-term clinical results and is beneficial to the repair of lateral interventricular cartilage of the knee joint.

A clinical study (85) that included 33 patients showed that the HSS score was increased after surgery ($p < 0.05$), while the VAS score was decreased ($p < 0.05$). Imaging examinations showed that the positions of the femoral tibial angle (aFTA), distal lateral angle of the femur (aLDFA), and mechanical axis in the tibial plateau were significantly improved after operation ($p < 0.05$). All the bones healed. In conclusion, distal medial osteotomy of the femur can effectively correct the line of force of lower limbs, reduce the pressure load of the lateral compartment, and preserve the original motion of the knee joint, with accurate clinical effects.

A clinical study (86) involving 15 patients showed that there were 14 cases of bone healing at the osteotomy 3 months after surgery, and one case of bone healing was delayed until 6 months after surgery due to fracture of the bone cortex at the osteotomy hinge during surgery. There was no significant difference in knee flexion motion and Kellgren–Lawrence grade of osteoarthritis 2 years after surgery ($p > 0.05$). The knee valgus angle was significantly corrected 3 months after surgery, and the KOOS score of knee function was significantly increased 2 years after surgery ($p < 0.05$). In conclusion, distal medial femoral closure osteotomy is effective in the treatment of lateral interventricular osteoarthritis of the knee. Postoperative complications of osteotomy non-union and internal fixation stimulation are less common, and the operation does not affect knee flexion mobility. Patients can exercise with early weight bearing function.

Unicompartmental knee arthroplasty (level of evidence: level I)

Patients with severe unilateral knee wear or abnormal alignment can be treated with unicompartmental knee arthroplasty (UKA) (87–89). UKA includes medial and lateral monocondylic replacement. Medial single condylar replacement is suitable for patients with single compartment osteoarthritis of the knee whose medial joint wear is dominant, the force line is changed by 5–10°, the ligament is intact, and the flexion contracture is not more than 15°. Lateral single condylar replacement is suitable for bone-to-bone knee lateral compartment osteoarthritis with normal medial compartment cartilage, flexion and valgus deformity <15°, flexion and extension motion >90°, intact anterior cruciate ligament, normal function of the posterior cruciate ligament, and stable and correctable valgus deformity of the knee joint. UKA preserves the normal structure of the knee as much as possible in order to obtain better proprioception and functional recovery. Description of the evidence: A meta-analysis that included 17 studies (87) showed that the postoperative complications (OR = 4.52, 95%CI: 2.30–8.90, $p < 0.001$), Lysholm score (MD = −5.53, 95%CI: −11.11 to 0.05, $p = 0.05$), and revision rate (OR = 1.67, 95%CI: 1.01–2.76, $p = 0.05$) were significantly better in the UKA group than in the HTO group. There were no significant differences between the two groups in operation time, blood loss, other knee function scores, range of

joint motion, rate of clinical outcome, force of lower limb line, and cartilage degeneration ($p > 0.05$). In conclusion, HTO and UKA can achieve similar and satisfactory clinical outcomes in the treatment of medial ventricular osteoarthritis of the knee. In contrast, UKA is superior to HTO in terms of postoperative complications, Lysholm score, and revision rate.

A meta-analysis of 24 studies showed that (88) the intraoperative blood loss ($p < 0.05$), drainage volume ($p < 0.05$), blood transfusion rate ($p < 0.05$), operation time ($p < 0.05$), KSS score ($p < 0.05$), HSS score ($p < 0.05$), and knee motion ($p < 0.05$) in the UKA group were better than those in the total knee replacement group, but the renovation rate of the former was significantly higher than that of the latter ($p < 0.05$). There was no significant difference in postoperative complications or in the improvement in curative effect ($p > 0.05$). In conclusion, UKA is beneficial to reduce intraoperative blood loss, drainage volume, blood transfusion rate, and operation time, and it improves the knee joint score and range of motion. The advantage of total knee replacement is a lower revision rate. Clinical planning for KOA patients should pay more attention to their own conditions and needs.

Total knee arthroplasty (level of evidence: I)

Total knee arthroplasty (TKA) is suitable for the treatment of severe multi-ventricular osteoarthritis of the knee joint, especially with severe joint pain and deformity, and the daily life of patients with severe symptoms is strongly affected after non-surgical treatment; it is also suitable for KOA patients after receiving a failed orthopedic osteotomy (90–93). TKA is effective in relieving pain and improving joint function.

Description of the evidence: a meta-analysis of 191 studies (90) showed that patients receiving primary TKA experienced rapid improvements in pain and function during the first year after surgery. After 10 years, pain might not be alleviated, but function has improved.

A meta-analysis of 19 studies (91) showed that most patients were satisfied with the procedure and their daily functional activities had benefited significantly. TKA resulted in significant medium- and long-term outcomes in terms of pain and function, resulting in high patient satisfaction.

Results of a clinical study (92) involving 95 patients showed that in patients with KOA eligible for unilateral TKA, non-surgical treatment after TKA provided better pain relief and improved function after 12 months than non-surgical treatment alone.

Prevention and care

Prevention (highly recommended)

Patients with KOA should pay attention to appropriate prevention and care. The main prevention methods are as follows:

① Strict control of weight and diet. Weight reduction is very beneficial to reduce the joint burden, improve joint function, reduce pain, and so on (2, 9).

② Reducing the trauma of the knee joint. Patients should reduce the trauma of the knee joint and avoid repeated stress.

③ Preventing osteoporosis, often participating in outdoor activities, getting more sunshine, and so on. Patients with severe osteoporosis should be given anti-osteoporosis therapy.

④ Doing correct exercises and avoiding strenuous activities, such as long-distance running, repeated squatting, kneeling, and lifting heavy objects.

Care (highly recommended)

① Patients should pay attention to the changes of the four solar terms, including the wind, cold, and summer humidity.

② Patients should avoid standing and walking for a long time and pay attention to knee joint protection.

③ Proper rest and the use of canes can reduce the load on the affected joints.

④ Aerobic activities such as lifting legs and stretching knees in bed, walking, swimming, and cycling help maintain joint function (2, 10).

⑤ Suitable shoes and insoles can absorb shocks.

Summary and conclusion

Patients with KOA should be treated in stages and steps, and patients at different stages of the disease may be treated in roughly the same way, but the focus should be different. For patients with stage I (pre-stage) symptoms, a clear diagnosis should be made, attention should be paid to the health education of the diagnosed patients, and the awareness of prevention and care should be strengthened. Risk factors should be controlled, such as controlling blood sugar, choosing appropriate shoes, reducing excessive weight bearing of the knee, and performing exercise. Patients whose lives are seriously impacted can consider therapy and TCM treatment, such as acupuncture, manual therapy, or electroacupuncture. Premature drug treatment is not recommended.

In stage II (early stage) and III (middle stage) KOA patients, the focus is on avoiding artificial joint replacement surgery and using the patient's original joint as much as possible, but surgery can be considered to improve the patient's knee deformity. For patients diagnosed with stage II (early stage) KOA, osteotomy and orthosis can be selected according to the patient's knee condition on the basis of health education, strengthening prevention and care, and non-drug treatment (exercise, therapy, and TCM). For patients with stage III (middle stage) KOA, drugs can be used to control pain and other symptoms. For example, TCM compounds can be used in combination with Western medicine to help patients reduce the frequency of taking Western medicine and regulate the patients' Zangfu function. Meanwhile, acupotomy and other means can be used to help restore knee motion. Orthopedic osteotomy may be considered for patients with significant deformities. However, patients should also be properly informed that the relevant symptoms are not easy to control, and psychological counseling should be given.

For patients with stage IV (late stage) KOA, TKA surgery should be avoided as far as possible. If the combination of drug therapy and non-drug therapy does not achieve satisfactory clinical results, knee saving surgery (arthroscopic debridement, HTO, UKA) can be considered.

TABLE 5 TCM literature basis and recommendation level.

TCM literature classification		TCM literature recommended grade	
Level I	Large sample randomized controlled trials with clear results and low false positive or false negative rates	Grade I	Supported by at least two Level I studies
Level II	Small sample randomized controlled trials with uncertain results and high false positive or false negative rates	Grade II	Supported by only one Level I study
Level III	Non-randomized, contemporaneous controlled trials based on expert consensus from ancient literature	Grade III	Supported only by Level II studies
Level IV	Historical comparative study, contemporary expert consensus	Grade IV	Supported by at least one Level III study
Level V	X-ray (standing or weight-bearing position), case reports, uncontrolled studies, expert opinions, and papers on osteophyte formation at the joint margin	Grade V	Supported only by Level IV or Level V studies

For patients with stage V (surgery stage) KOA, it is recommended to perform TKA, focusing on the prevention of relevant complications while actively carrying out rehabilitation exercise, and after surgery, drug therapy or non-drug therapy can be given to actively help patients recover, so that patients can return to society faster.

This study has some limitations. First, the most important primary screening information in the diagnosis of KOA is the physical examination data, radiographic features, and the patient's complaints. For example, the combination of osteophytes and knee pain is known to be a very important clue for the diagnosis of KOA, with a likelihood ratio of more than 10. It may be helpful for clinicians to make an accurate diagnosis if the guideline provides information on the relationship between specific diagnostic test results and the patient's physical examination, sensitivity, and specificity, along with citations to previous studies. It should provide more detailed information about valuable physical examinations that function better as high-quality information for screening diagnosis. Second, there are three internationally recognized clinical classification criteria for KOA: the European League Against Rheumatism (EULAR), the American College of Rheumatology (ACR), and the National Institute for Health and Care Excellence (NICE) criteria. Although the references for the criteria presented in the Diagnostic criteria section of this manuscript are from China, they are considered to be based on the ACR criteria in terms of content. For the completeness of the guideline, it should further present and compare at least two of the above criteria from outside China, such as EULAR and NICE, and the current guideline based on the references mentioned in this manuscript in the next step. Third, for the clinical staging and Chinese classification of KOA, we think that the "theory of Chinese treatment of non-disease" is a concept that should be considered in modern KOA treatment. However, unfortunately, it seems difficult to find previous epidemiological studies that support the classification. As the latest KOA-related research supports the concept of prevention before the onset of disease, more studies are needed, for example, actively incorporating relevant pathophysiological information into this guideline. It would be helpful if the guidelines provide as much updated evidence as possible to support the staged treatment of KOA. For KOA-related interventions such as acupuncture and Tai Chi, further research on standardized practices is needed. Additional information about the dosage and duration of each remedy needs to be obtained. There may be interaction or safety issues when TCMs/PCMs are used together with Western synthetic drugs, which need to be further explored. Finally, the manner in which the KOA diagnosis is made requires greater detail. For instance, is there an initial screening questionnaire? How were radiographic findings standardized? Were there any other tools or assessment methods, such as gait analysis, that were used to support the diagnosis? Theoretically, the proposed criteria seem robust, but their true utility in KOA diagnosis will only be clarified in real-world settings. These criteria should be tested in various clinical environments, including primary care settings. These issues show that more in-depth research is necessary.

To sum up, for patients with confirmed KOA, we should consider the evidence-based staging and stepped treatment of KOA and provide TCM treatment to improve clinical outcomes and extend the service life of the joint. Clinical treatment should be based on multiple approaches, and attention should be paid to the physical and mental health of patients.

Evidence quality classification

First, according to the type of study, the quality of the included studies was evaluated using a scale. Studies that meet the requirements (score $\geq$ 5 on the AMSTAR scale for meta-analyses, score $\geq$ 3 on the modified Jadad scale for RCTs, score $\geq$ 13 for non-randomized clinical trials) were classified according to the criteria in Table 5. The final quality of evidence was graded according to the recommendation grade standard of TCM literature (Table 5).

Recommended strength classification

The nominal group method was used to grade the recommendation strength, and experts voted based on factors such as evidence level, efficacy, safety, economy, and patient acceptance. The recommended direction includes recommended and not recommended, and the recommended strength includes strong and weak.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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Glossary

TCM	Traditional Chinese medicine
KOA	Knee osteoarthritis
Knee osteoarthritis	"Bone impediment (骨痹)" and "bi syndrome"
Knee osteoarthritis	A chronic joint disease characterized by articular cartilage degeneration, changes in subchondral bone reactivity, osteophyte formation at joint edges, synovial disease, ligament relaxation or contracture, capsular contracture, and muscle weakness (1–3)

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Longbie capsules reduce bone loss in the subchondral bone of rats with comorbid osteoporosis and osteoarthritis by regulating metabolite alterations

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Background and objective: With the development of global population aging, comorbidity (≥2 diseases) is a common health problem among elderly people. Osteoarthritis (OA) and osteoporosis (OP) are common in elderly individuals. There is a lack of drug therapy for OA and OP comorbidities. The purpose of this study was to explore the efficacy and mechanism of Longbie capsule (LBJN), which contains various plant herbs, in treating OA and OP comorbidities (OA + OP) in rats using metabolomics techniques.

Methods: We created an OA+OP rat model through bilateral oophorectomy combined with meniscus instability surgery. Thirty SD rats were randomly divided into five groups (six in each group), namely, the sham group, OA group, OA+OP group, LBJN low-dose group (0.625g/kg, OA+OP+LB-L group) and LBJN high-dose group (1.25g/kg, OA+OP+LB-H group). After 8 weeks of intervention, we used micro-CT to detect bone microstructure status, ELISA to measure bone metabolism indicators, and UPLC–MS technology for metabolomics analysis. Finally, the screened differentially expressed metabolites were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and functional enrichment analysis.

Results: The micro-CT results showed that LBJN significantly improved the bone mineral density (BMD) and bone quality of subchondral bone in OA + OP rats, and LBJN regulated the expression of bone alkaline phosphatase (BALP), osteoprotegerin (OPG), and tartrate-resistant acid phosphatase (TRACP) in serum to maintain bone metabolism balance. Metabolomics analysis showed that the metabolic trajectory of OA + OP rats after intervention in the OA + OP+LB-H group showed significant changes, and 107 potential biomarkers could be identified. Among them, 50 metabolites were upregulated (such as *zeranol*) and 57 were downregulated (such as *vanillactic acid*). The KEGG functional enrichment results indicated that the differentially expressed metabolites are mainly involved in amino acid metabolism, lipid metabolism, and carbohydrate metabolism. The KEGG pathway enrichment results indicated that LBJN may exert therapeutic effects on OA + OP rats by regulating the cAMP signaling pathway, and the FoxO signaling pathway.

Conclusion: LBJN can maintain bone metabolism balance by regulating serum lipid metabolism, amino acid metabolism, carbohydrate metabolism, and estrogen, thereby reducing bone loss in subchondral bone, which may be a potential mechanism through which LBJN treats OA + OP.

KEYWORDS

geriatric medicine, bone aging, osteoarthritis, osteoporosis, Longbie capsule, plant-based natural products, metabonomics, bone metabolism

1. Introduction

Osteoporosis (OP) is a metabolic bone disease that results in a decrease in bone mass per unit volume due to multiple factors, leading to changes in bone microstructure and susceptibility to brittle fractures (1, 2). Osteoarthritis (OA) is a joint degenerative disease with degenerative articular cartilage, hypertrophic synovium and osteophytes as the main pathological changes (3, 4). The International Osteoporosis Foundation survey found that among the over 200 million people with OP worldwide, the majority are over 60 years old, and 9.6% of men and 18% of women over 60 years old worldwide may suffer from symptomatic OA. Age, sex, genetics, chronic inflammation, endocrine factors, and body mass index (BMI) are all considered common risk factors for OA and OP, and there may be complex correlations between the two (5, 6). A study reported that the proportion of the combined occurrence of OA and OP in postmenopausal women is as high as 56.5% (7), indicating a possible causal relationship between OA and OP. Al Saleh et al. (8) conducted a sampling survey of 3,985 adults and found that patients who had already developed knee osteoarthritis (KOA) were more likely to develop OP and that KOA accelerated the progression of OP. Dequeker et al. (9) found that OP can affect changes in overall bone mass, and abnormalities in the microstructure of subchondral bone tissue may lead to uneven stress on articular cartilage, which may lead to secondary cartilage damage and osteophyte proliferation, thereby promoting the occurrence and progression of OA. A case study found that a higher level of bone mineral density (BMD) can delay the progression of KOA (10). In this context, the merger of OA and OP (OA + OP) may be a serious challenge for future public health. However, there is no drug for targeted therapy of OA + OP. At present, the treatment of OA + OP mainly involves the combination of anti-inflammatory and analgesic drugs, anti-OP drugs, and cartilage-protective drugs (11–13), but this is clearly not the optimal treatment plan. Therefore, exploring drug therapies for OA + OP is an important task at present.

Traditional Chinese medicine has great potential advantages in treating OA + OP, and it has more possibilities for drug conversion. Our previous evidence-based research has confirmed that kidney-tonifying and blood-activating traditional Chinese medicine has a definite therapeutic effect on treating KOA (14). In addition, the kidney-tonifying and blood-activating method is also recommended in the treatment of OP (15). The kidney-tonifying and blood-activating pharmacological effects of traditional Chinese medicine on bones are multifaceted (15–17). These medicines can directly increase the activity of osteoblasts to promote osteoblast regeneration and correct the dysfunction of the immune system to protect gonadal tissue while maintaining sex hormone levels and bone metabolic balance. Kidney-tonifying and blood-activating herbs can also affect the bone remodeling cycle and bone resorption cycle (18, 19). Under the kidney-tonifying and blood-activating theory in traditional Chinese medicine, Guangdong Provincial Hospital of Chinese Medicine has developed the Longbie capsule (LBJN), which contains various plant herbs and has achieved good effects in treating KOA. The main components of LBJN are Xianmao (*Curculigo orchioides Gaertn.*), Bajitian (*Morinda officinalis How.*), Wugong (*Scolopendra subspinipes*), Qishe (Agkistrodon), Quanxie (Scorpio), Tusizi (*Cuscuta chinensis*), Dansen (*Salvia miltiorrhiza Bunge*), Tubiechong (*D.dispar Chanisso et Eysenhard*), Chuanwu (*Aconitum carmichaelii*), and Huangqi (*Astragalus membranaceus (Fisch.) Bunge*). However, the efficacy and mechanism of LBJN in treating OA + OP are still unclear.

Metabonomics is a systematic study of the changing levels of metabolites and is widely used to evaluate the efficacy and potential pharmacological mechanisms of natural products (20). The composition, concentration and structure of metabolites can be obtained through metabonomic detection, and more information can be provided for systems biology research on the basis of further supplementing gene, transcriptional and protein information. The comprehensive, systematic and dynamic characteristics of metabonomics are very similar to the holistic theory of traditional Chinese medicine, which provides a new dimension for the study of the holistic effect of traditional Chinese medicine (21). The purpose of this study was to explore the efficacy and mechanism of LBJN in the treatment of OA + OP using metabolomics methods.

2. Materials and methods

2.1. Animals and ethical approval

A total of 30 SPF-grade female SD rats aged 1 month and weighing 100–120 g were included in this experiment. These rats were purchased from the Guangdong Medical Experimental Animal Center (certificate number: 44007200103590). These SD rats were raised in an SPF environment at the Experimental Animal Center of Guangdong University of Chinese Medicine. The experimental plan was approved by the Animal Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine (ethical approval number: 2021028). The procedures for this experiment were performed in accordance with the regulations on the administration of experimental animals approved by the State Council of the People's Republic of China.

2.2. Animal model construction and intervention measures

All 30 SD rats were divided into five groups, with six rats in each group: the sham group, OA group, OA + OP group, LBJN low-dose group (0.625 g/kg, OA + OP+LB-L group), and LBJN high-dose group (1.25 g/kg, OA + OP+LB-H group). LBJN (batch number: YueYaoZhiZi Z20071030, 0.5 g/capsule) was produced and provided by the Pharmacy Center of Guangdong Provincial Hospital of Chinese Medicine. In addition, the LBJN dose in this study follows the human mouse dose conversion formula, and low- and high-dose groups were formed.

We used isoflurane to anaesthetize SD rats. Except for the sham group, all four groups of rats underwent surgical resection of the medial meniscus and tibial collateral ligament of the knee joint to construct an OA model of meniscus instability. In the sham group, the knee joint capsule was separated and then sutured. Except for the sham group and the OA group, all rats in the other groups underwent surgical resection of both ovaries to construct an OP model. In the sham group and OA group, both ovaries were separated and resutured after removing some adipose tissue around both ovaries. Starting 4 weeks after surgery, medication was administered by gavage. LBJN was dissolved in pure water and made into a mixture of traditional Chinese medicines. The medication program for the OA + OP+LB-L and OA + OP+LB-H groups was administration once a day by gavage and continuous gavage for 8 weeks. The other three groups were given the same dose of pure water by gavage. After 8 weeks of gavage, the rats were euthanized under anesthesia, and the serum and knee joints of each group of rats were collected for further analysis.

2.3. Chemical composition analysis of LBJN

We used high-performance liquid chromatography quadrupole/electrostatic field orbital trap high-resolution mass spectrometry (HPLC-Q-Orbitrap-MS) technology to identify the chemical components in LBJN aqueous solution. The main instruments used for component identification included a Q Exactive high-resolution mass spectrometer (Thermo Fisher Scientific), Ultimate 3000RS ultra-high-performance liquid chromatograph (Thermo Fisher Scientific), and Welch AQ-C18 chromatography columns (2.1 mm × 150 mm, 1.8 μm).

Sample processing was performed as follows: 200 μL of LBJN aqueous solution was added to 1,000 μL of 80% methanol and vortexed for 10 min. The samples were centrifuged for 10 min at a temperature of 4°C with a centrifugal force of 20,000 × g, and the supernatant was filtered for analysis.

The mass spectrum (MS) conditions were as follows: the ion source was an electric spray ionization source (ESI); positive and negative ion switching scanning; full mass/dd-MS2 detection method for detection; resolution of 70,000 (full mass), 17,500 (dd-MS2); scanning range of 100.0–15,000.0 m/z; electric spray voltage of 3.2 kV; capillary temperature of 300°C; collision gas of high-purity argon gas (purity ≥99.999%); sheath gas of nitrogen (purity ≥99.999%), 40 Arb; auxiliary gas of nitrogen (purity ≥99.999%), 15 Arb, 350°C; and data collection time of 30.0 min.

The chromatographic conditions were as follows: a Welch AQ-C18 column (2.1 mm × 150 mm, 1.8 μm) was used; the flow rate was 0.30 mL/min; the aqueous phase was 0.1% formic acid aqueous solution; the organic phase was methanol; the temperature of the column temperature box was 35°C; the temperature of the automatic sampler was 10.0°C; and the injection volume of the automatic sampler was 5.00 μL.

All data collected through high-resolution liquid quality methods was preliminarily organized using Compound Discoverer 3.3 software (Thermo Fisher Scientific) and compared and analyzed in the mzCloud database. Finally, we analyzed the various spectral peaks and further inferred the compound structure based on the ion fragments and retention information provided by references and databases.

2.4. Micro-CT analysis

After fixing the knee joint sample with 4% paraformaldehyde, we used a micro-CT scanner (ZKKS-MCT-Sharp, Guangzhou, China) to scan and analyze the imaging morphology of the femur. During scanning, the knee joint was fixed on the fixator along the long axis. The scanning voltage was set to 70 kV, and the current was 100 μA. The power was 7 W, 4 frames were stacked, the angle gain was 0.72 degrees, scanning was completed by rotating one cycle, and the scanning layer thickness was 15 μM. ZKKS-MicroCT 4.1 software was used to analyze the bone morphology-related parameters of the tibial subchondral bone, including the BMD, bone volume fraction (BV/TV), trabecular number (Tb.N), bone surface area to bone volume ratio (BS/BV), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp).

2.5. Analysis of bone metabolic factors

All blood samples from SD rats were collected and centrifuged at 1500 rpm for 15 min. These serum samples were stored in an environment of −80°C. We used the enzyme-linked immunosorbent assay (ELISA) method to detect the expression levels of serum bone alkaline phosphatase (BALP), osteoprotegerin (OPG), bone gla protein (BGP) and tartrate-resistant acid phosphatase (TRACP). The measurement process was carried out according to the instructions of the ELISA kit (Jiangsu Meimian Industrial Co., Ltd., Jiangsu, China).

2.6. Metabolite analysis and data processing

Ultra-high-performance liquid chromatography–mass spectrometry (UPLC–MS) technology was applied for the analysis of serum metabolites. The instrument platform for LC–MS analysis was the UHPLC-Q Exactive system manufactured by Thermo Fisher Scientific.

For the MS, the optimal conditions were set as follows: heater temperature, 400°C; capillary temperature, 320°C; sheath gas flow rate, 40 arb; Aux gas flow rate, 10 arb; ion-spray voltage floating (ISVF), −2,800 V in negative mode and 3,500 V in positive mode; and normalized collision energy, 20–40-60 V rolling for MS/MS. Data acquisition was performed in data-dependent acquisition (DDA)

mode. The detection was carried out over a mass range of 70–1,050 m/z.

The chromatographic conditions were as follows: 2 μL of sample was separated by an HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm) and then subjected to mass spectrometry detection. The mobile phases consisted of 0.1% formic acid in water:acetonitrile (95:5, v/v) (solvent A) and 0.1% formic acid in acetonitrile:isopropanol:water (47.5:47.5:5, v/v) (solvent B). The sample injection volume was 2 μL, and the flow rate was set to 0.4 mL/min. The column temperature was maintained at 40°C. During the period of analysis, all samples were stored at 4°C.

All collected data were first preprocessed, which included missing value recoding and normalization of original data. The processed data were imported into SIMCA-P 11.5 software for principal component analysis (PCA), supervised partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA), and ions that met the criteria of variable importance in projection (VIP) values ≥1 and $p < 0.05$ were screened as differentially expressed metabolites. The score plot results were used to obtain sample classification information. A loading plot was used to screen for differential metabolic molecules. We classified the differentially expressed metabolites obtained through the internationally recognized Human Metabolome Database (HMDB).[1] SciPy (Python, version 1.0.0) software was applied to draw differentially expressed metabolite heatmaps and to screen for differentially expressed metabolites based on $p < 0.05$. Finally, we imported the obtained differentially expressed metabolites into the Kyoto Encyclopedia of Genes and Genomes (KEGG) Compound (Release 2017-05-01) and KEGG Pathway (Release 2017-05-01) for metabolic pathway analysis.

2.7. Data analysis

SPSS 17.0 software and GraphPad Prism 5.0 were applied for data analysis and result visualization. The measurement data are expressed as the mean ± standard deviation. The *t*-test was used for intergroup comparisons, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Chemical composition of LBJN

We used HPLC-Q-Orbitrap-MS technology to collect MS data for LBJN. The fundamental peak chromatogram of LBJN in positive and negative ion modes is shown in Figure 1. The data collected based on HPLC-Q-Orbitrap-MS technology were processed using Compound Discoverer 3.3 software (Thermo Fisher Scientific) and the mzCloud database, and a total of 515 compounds were matched (Supplementary material 1). The results showed that potential compounds within LBJN that could play pharmacological roles include tanshinone IIA, oleanolic acid, albiflorin, rubiadin, trigonelline, cryptotanshinone, berberine, catechin, and neochlorogenic acid. Table 1 shows the common compounds through which LBJN exerts pharmacological effects.

1 www.hmdb.ca

3.2. The effect of LBJN on the subchondral bone structure

The micro-CT images of the bone structure can be found in Figure 2A. The micro-CT results showed that at 12 weeks after surgery, the BV/TV, BMD, and Tb.N in the OA + OP group were significantly lower than those in the sham and OA groups (Figure 2B), while the Tb.Sp was significantly higher than that in the sham group, indicating significant bone loss in the subchondral bone of the knee joint in OA + OP rats. The BV/TV, BMD, and Tb.N of the OA + OP+LB-L group and the OA + OP+LB-H group were higher than those of the OA and OA + OP groups; the Tb.Sp values of the OA + OP+LB-L group and the OA + OP+LB-H group were lower than those of the OA and OA + OP groups. The above results indicate that the decreases in BMD and bone mass of the subchondral bone of the tibia in the disease state of OA combined with OP are significantly greater than those in OA alone. LBJN can improve the BMD and bone mass of subchondral bone (Figure 2B), indicating that LBJN may have potential drug therapeutic value for OA + OP.

3.3. The effect of LBJN on bone metabolic factors

We used ELISA to detect serum bone metabolism indicators in the rats in each group. The results showed that the BALP expression level in the OA + OP group was significantly higher than that in the sham and OA groups, while that in the OA + OP+LB-H group was significantly lower than that in the OA + OP group (Figure 3). During bone formation, BALP is released by osteoblasts and plays a role in promoting bone formation and bone matrix mineralization (22). When bone volume decreases, meaning Tb.N decreases, the body experiences compensatory bone formation and an increase in serum BALP levels (23). The increase in serum BALP levels reflects an increase in bone formation and also implies higher bone turnover, depletion of the body's ability to regenerate osteoblasts, accelerated apoptosis of osteoblasts, and exacerbation of the condition, leading to a continuous decrease in bone volume (24). Therefore, we can observe that the expression level of BALP in the OA + OP group is higher than that in the sham group. The expression level of OPG in the OA + OP group was significantly lower than that in the OA group, while that in the OA + OP+LB-H group was significantly higher than that in the OA + OP group (Figure 3). There was no statistically significant difference in the BGP expression levels among the groups ($p > 0.05$). The expression levels of TRACP in the OA and OA + OP groups were significantly higher than those in the sham group, while those in the OA + OP+LB-L and OA + OP+LB-H groups were significantly lower than those in the OA + OP group (Figure 3). The above results indicate that LBJN can affect bone metabolism in OA + OP rats by regulating the expression of serum BALP, OPG, and TRACP.

3.4. The effect of LBJN on serum metabolites

We used a nontargeted metabolomics strategy to investigate the effect of high-dose LBJN (1.25 g/kg) on endogenous serum metabolites in OA + OP rats. Using multivariate statistical analysis methods, PCA

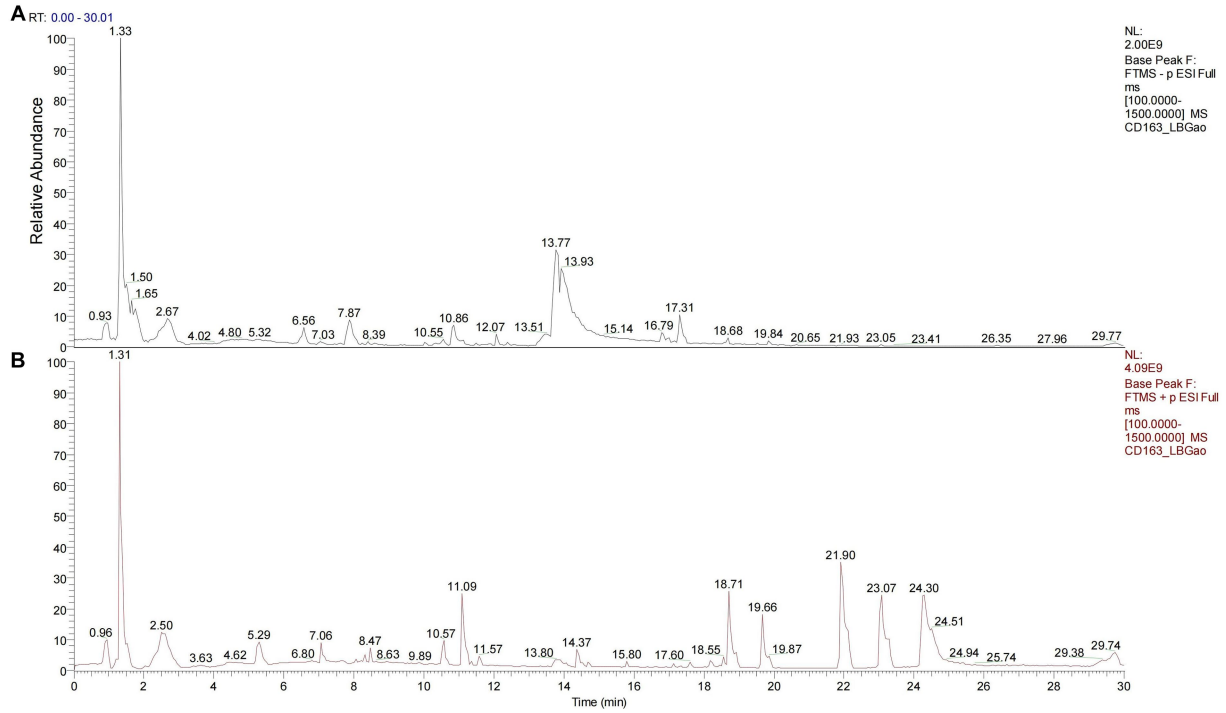

FIGURE 1
Chromatogram of LBJN based on Q-Orbitrap high-resolution liquid mass spectrometry. **(A)** Negative ion mode. **(B)** Positive ion mode.

TABLE 1 Partial results of compound identification in LBJN.

Name	Formula	Annot. Source: Predicted Compositions	Annot. Source: mzCloud Search	Annot. Source: MassList Search	Annot. DeltaMass [Da]	Calc. MW	RT [min]	mzCloud Results	mzCloud Best Match
Oleanolic acid	C30 H48 O3	Not the top hit	Full match	Partial match	−0.0005	456.3599	22.112	3	99.9
Tanshinone IIA	C19 H18 O3	Full match	Full match	Partial match	−0.0005	294.1251	19.671	7	99.7
Rubiadin	C15 H10 O4	Full match	Full match	Partial match	−0.00006	254.0578	18.509	4	99.6
Trigonelline	C7 H7 N O2	Full match	Full match	Partial match	−0.00008	137.0476	1.45	9	99.4
Cryptotanshinone	C19 H20 O3	Full match	Full match	Partial match	−0.00074	296.1405	18.719	3	99.3
Berberine	C20 H17 N O4	Not the top hit	Full match	Partial match	−0.00048	335.1153	11.685	9	97.9
Neochlorogenic acid	C16 H18 O9	Not the top hit	Full match	Partial match	−0.00013	354.095	8.596	2	97.2

and OPLS-DA were performed on the data from the OA + OP and OA + OP+LB-H groups, and corresponding identification models were established. Based on this information, differentially expressed metabolites were identified. The PCA results showed that there were no significant differences in the metabolic profiles among the OA + OP, OA + OP+LB-H, and control groups (Figure 4). The prediction rates of PC1 and PC2 did not reach 50%, so the difference between two groups could not be identified. The PLS-DA results showed that the OA + OP, OA + OP+LB-H, and control groups were completely separated (Figure 5). There were differences in the distribution of metabolites in the body of OA + OP rats compared to the other two groups (OA + OP group and OA + OP+LB-H group, OA + OP group and control group).

The results of the permutation test evaluation of the OPLS-DA model are shown in Figure 6. The Q2 value of the permutation test random model is less than the Q2 value of the original model, and the

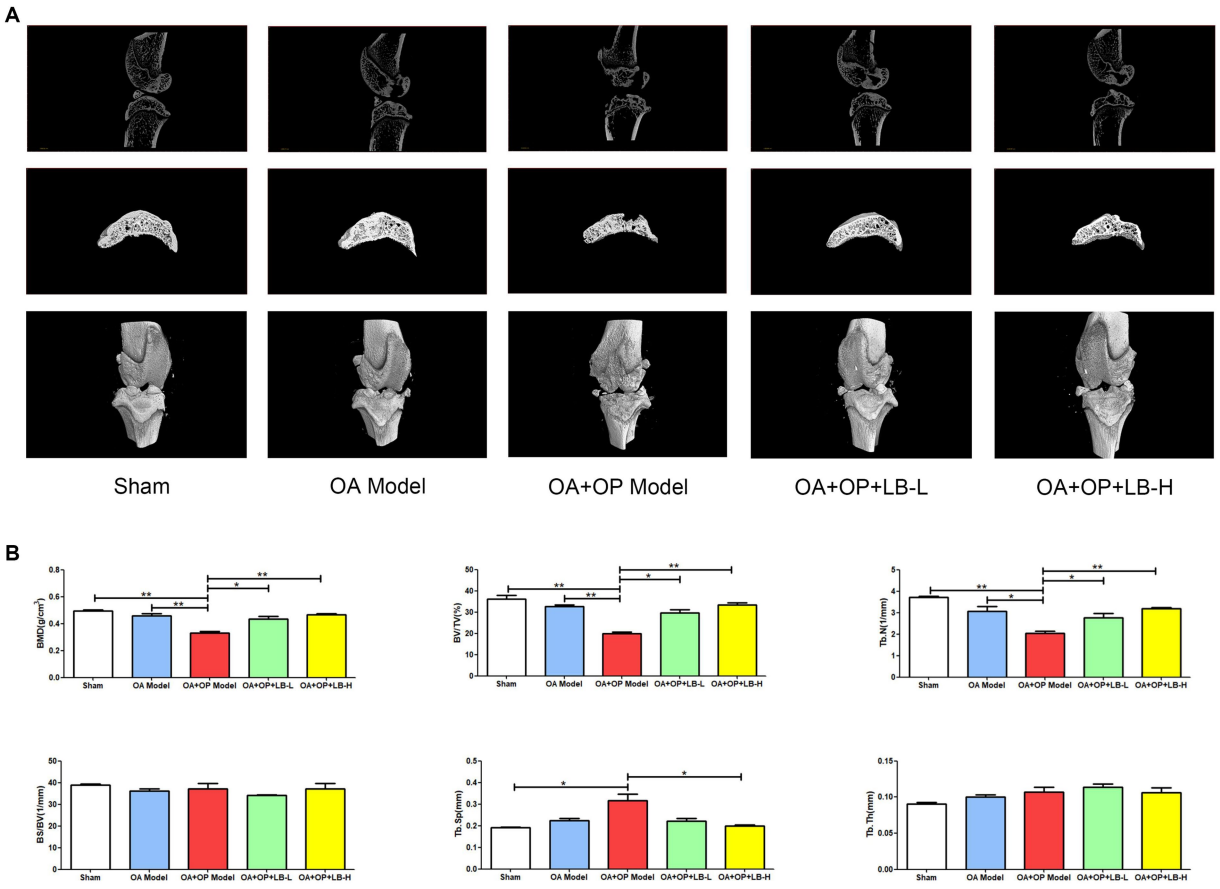

FIGURE 2
Therapeutic effects of LBJN on the bone histomorphometry of subchondral bone in the OA and OP model. **(A)** Micro-CT images of the bone structure in the sham, OA model, OA + OP model, OA + OP+LB-L, and OA + OP+LB-H groups ($n = 4$). **(B)** Morphometric analysis of BMD, BV/TV, Tb.N, BS/BV, Tb. Th, and Tb.Sp in subchondral bone of the knee joint. *$p < 0.05$, **$p < 0.01$.

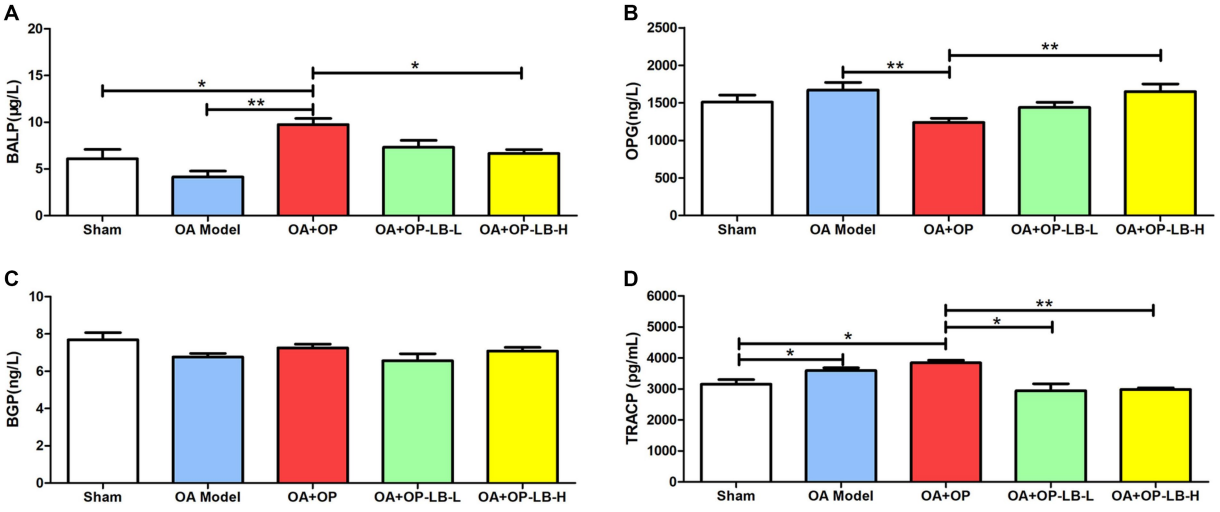

FIGURE 3
Therapeutic effects of LBJN on the serum concentrations of **(A)** BALP, **(B)** OPG, **(C)** BGP, and **(D)** TRACP in the OA and OP model ($n = 6$). *$p < 0.05$, **$p < 0.01$.

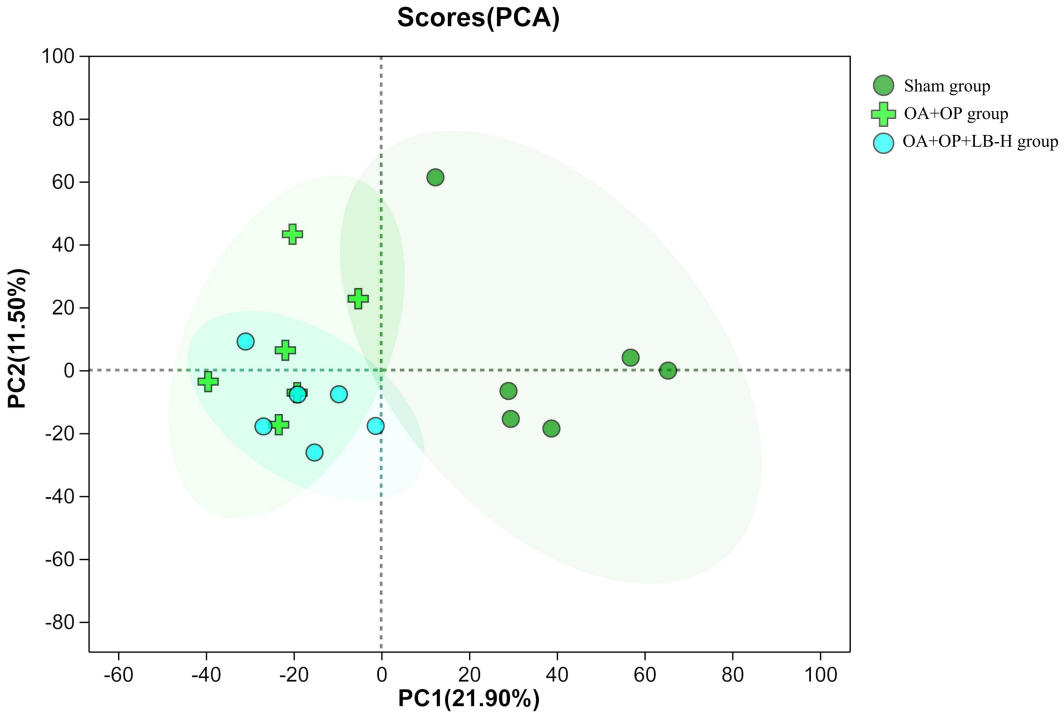

FIGURE 4
Principal component analysis score chart for the OA + OP, OA + OP+LB-H, and sham groups.

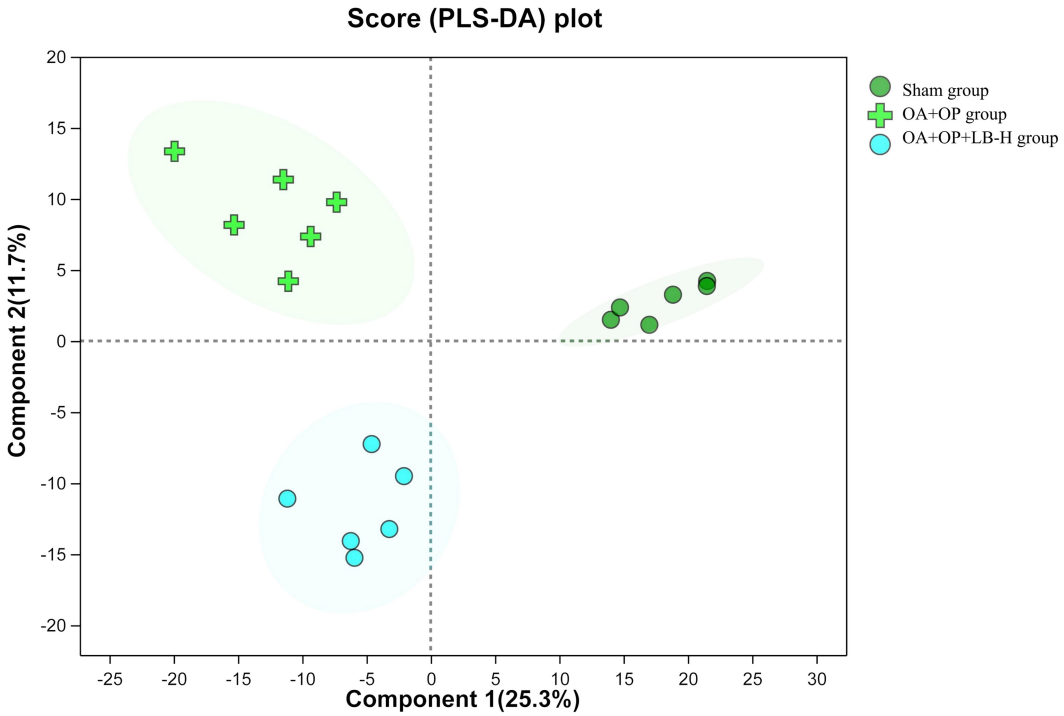

FIGURE 5
Partial least squares discriminant analysis scores for the OA + OP, OA + OP+LB-H, and sham groups.

intercept between the regression line of Q2 and the vertical axis is less than zero, which indicates that the original model has good robustness and no overfitting phenomenon. Therefore, based on a reliable evaluation model, we found significant differences in metabolites between the OA + OP group and the control group, and there were also significant changes in metabolites after LBJN intervention.

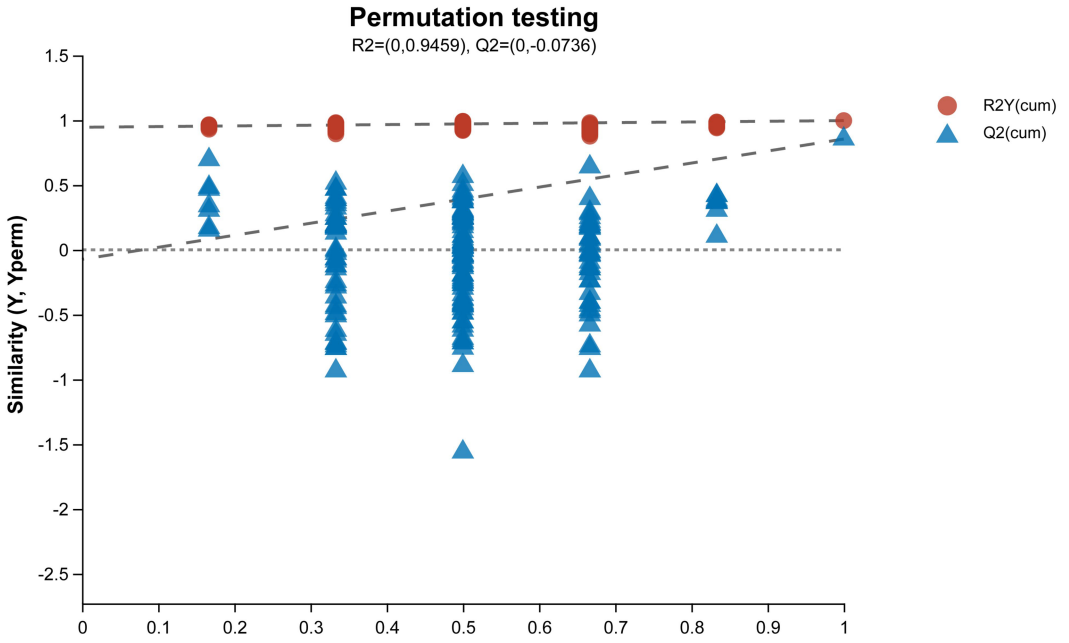

FIGURE 6
Permutation test diagram of the OPLS-DA model. The abscissa represents the replacement retention of the permutation test (the proportion is consistent with the order of the Y variable of the original model, and the point with a replacement retention of 1 is the R2 and Q2 value of the original model); the ordinate represents the R2 (red dot) and Q2 (blue triangle) values obtained by the permutation test, and the two dotted lines represent the regression lines of R2 and Q2, respectively.

In positive ion mode, a total of 107 differentially expressed metabolites were screened (Figure 7), and detailed information on these 107 metabolites is shown in Supplementary material 2. Compared with the OA + OP group, the OA + OP+LB-H group had a total of 50 differentially expressed metabolites that were upregulated and 57 metabolite levels that were downregulated. According to the magnitude of the VIP values, Table 2 shows a total of 10 metabolites with the most significant upregulation and downregulation. Through differentially expressed metabolite volcano maps, it can be found that points farther away from the center are more likely to become potential biological targets (Figure 7). The VIP value analysis results showed that compared with the OA + OP group (Figure 8), the OA + OP+LB-H group showed significant upregulation of metabolites such as eranol, 7,3′-dihydroxyflavone, and 5-hydroxyindoleacetic acid ($p < 0.001$), while vanillactic acid, octanoylcarnitine, and TXB2 were significantly downregulated ($p < 0.001$).

By comparing to the HMDB, we found that the differentially expressed metabolites were mainly classified as amino acids, peptides, and analogs (15.17%); fatty acids and conjugates (8.97%); and fatty acid esters (6.21%) (Figure 9).

3.5. KEGG analysis of the differentially expressed metabolites

The classification results of the KEGG compounds for the differentially expressed metabolites are shown in Figure 10. The differentially expressed metabolites between the model group and the OA + OP+LB-H group were mainly classified as amino acids, oligosaccharides, carboxylic acids, and fatty acids. The results of the KEGG functional enrichment analysis indicate that the differentially expressed metabolites are mainly involved in amino acid metabolism, lipid metabolism, and carbohydrate metabolism (Figure 11). The KEGG pathway enrichment results are shown in Figure 12. The KEGG pathway enrichment results indicate that there are 34 entries for the differentially expressed metabolites involved in body metabolism (Supplementary material 3), such as butanoate metabolism; taurine and hypotaurine metabolism; pyrimidine metabolism; lysine degradation; and alanine, aspartate and glutamate metabolism. In terms of systemic organ systems, a total of 16 differentially expressed metabolites were involved (Supplementary material 3), such as in protein digestion and absorption, cholesterol metabolism, and mineral absorption. In terms of cellular processes, gap junction and ferroptosis were involved. The functional annotation results revealing environmental information processing indicate that the differentially expressed metabolites are mainly involved in pathways such as ABC transporters, the phospholipase D signaling pathway, the FoxO signaling pathway, and the cAMP signaling pathway.

4. Discussion

This study created a rat model of OA and OP comorbidity through bilateral oophorectomy combined with meniscus instability surgery and intervention with LBJN. We found that the main active ingredients of LBJN are tanshinone IIA, oleanolic acid, albiflorin, etc. The research results show that LBJN can improve the bone density and quality of subchondral bone in OA + OP rats, and LBJN can regulate the expression of BALP, OPG, and TRACP in serum, thereby affecting bone metabolism in OA + OP rats. Based on nontargeted

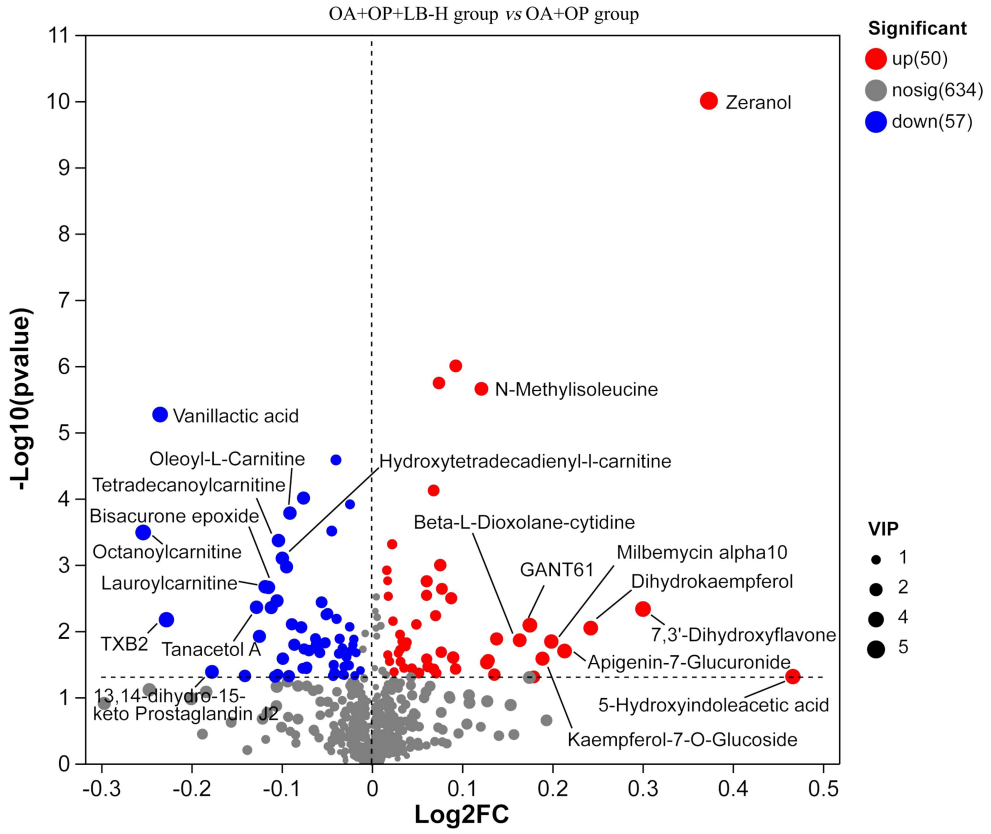

FIGURE 7
Volcanic maps of the metabolic differences between the OA + OP and OA + OP+LB-H groups. The size of the circle represents the variable importance in projection (VIP) value, the scatter color represents the final screening result, significantly upregulated metabolites are represented in red, significantly downregulated metabolites are represented in blue, and non-significantly different metabolites are gray.

metabolomics techniques and multivariate statistical analysis, 107 differentially expressed metabolites were found in the OA + OP+LB-H group compared to the OA + OP group. Among them, zeranol, 7,3′-dihydroxyflavone, 5-hydroxyindoleacetic acid, vanillactic acid, octanoylcarnitine, and TXB2 may be key targets through which LBJN exerts pharmacological effects. The enrichment analysis of KEGG pathways based on these differentially expressed metabolites suggests that the metabolic regulation pathway of LBJN treatment in OA + OP rats may be related to the cAMP signaling pathway and the FoxO signaling pathway.

OA and OP have multiple similar pathogenic factors, such as lipid metabolism disorders and high BMI (25). Under high BMI conditions, excessive release of adipokines and metabolic disorders can lead to an increase in OP and fracture risk. A higher BMI not only increases the mechanical load on the knee joint but also increases the risk of OA through adipose factor-induced aseptic inflammation (26). Therefore, abnormal lipid metabolism accompanied by pathological progression of OP may induce the occurrence and development of OA. This experimental study found that LBJN can regulate lipid metabolism and maintain the balance of bone metabolism. Inflammation and adipocytokines related to obesity may exacerbate the occurrence and progression of OP (27). Adipose tissue is considered an endocrine organ for bone tissue metabolism (28), but it can secrete various inflammatory cytokines and can have negative effects on bone tissue. LBJN may reduce bone loss in the subchondral bone of the knee by improving lipid metabolism disorders, which, to some extent, alleviates the progression of KOA. The regulation of amino acid metabolism is also a key pathway by which LBJN exerts therapeutic effects on OA + OP rats, among which the metabolite 5-hydroxyandoleacetic acid (5-HIAA) may be an important pharmacological target. 5-HIAA is a metabolite of 5-hydroxytryptamine (5-HT) that can represent the level of 5-HT. Research has shown that 5-HT is a key molecule in bone tissue dynamics (29, 30) and can regulate bone metabolism. In addition to the intestinal 5-HT contained in bone tissue, the osteoblasts, osteoclasts and osteocytes can also synthesize 5-HT (29). Yadav et al. showed that 5-HT can inhibit the proliferation of osteoblasts through the 5-HTR1B receptor on the surface of osteoblasts (31). Under normal physiological conditions, FoxO1 protein interacts with activating transcription factor 4 (ATF4) and cAMP responsive element binding protein (CREB) in the nucleus of osteoblasts to maintain normal proliferation. The binding of FoxO1 protein with ATF4 promotes the expression of transcription targets regulated by FoxO1, while binding with CREB inhibits the expression of transcription targets regulated by FoxO1. An increase in 5-HT levels in the blood circulation disrupts the interaction between FoxO1 and CREB (31, 32), which leads to a decrease in osteoblast proliferation activity. Therefore, we believe that LBJN may exert its anti-OP effect by regulating the cAMP/FoxO signaling pathway and acting on 5-HT.

Kidney-tonifying and blood-activating traditional Chinese medicine is a commonly used method for treating OA + OP (14, 33), and LBJN is a representative compound of this type of traditional Chinese medicine. LBJN has good clinical efficacy in the treatment of OA and has good efficacy in the treatment of OA in postmenopausal women. Postmenopausal women are at high risk of bone loss and OP due to a decrease in estrogen levels (34, 35). The imbalance in the activities of osteoblasts and osteoclasts may directly lead to the destruction of bone tissue structure and bone loss (36), and estrogen plays a key role in the balance of the activity of these two types of osteoblasts. The balance of bone metabolism in subchondral bone is also a major factor in maintaining cartilage stability (37). This study also found that LBJN can regulate estrogen metabolism and thereby play a role in improving cartilage quality, with the discovery of a key metabolite, zeranol. Zeranol is a new type of phytoestrogen that can bind to estrogen receptors and has estrogen and estrogen receptor antagonistic effects (38, 39). Estrogen can regulate various cytokines (such as IL-1 and IL-6) and can affect bone metabolism (40, 41). Research has shown that multiple subtypes of interleukin can be highly expressed in osteoporotic tissues, with IL-1 and IL-6 receiving significant attention (42, 43). IL-6 is a cytokine with extensive biological activity that is secreted by osteoclasts, osteoblasts, bone stromal cells and monocytes/macrophages (44) and plays a key role in the occurrence and progression of OP. IL-6 can stimulate the proliferation of osteoclasts and improve their functional expression (42), thereby promoting the occurrence of OP. IL-1 is an activator of osteoclasts and stimulates bone resorption (45). Osteoblasts can produce IL-6 and tumor necrosis factor (43, 45) under the stimulation of IL-1, and these three cytokines can jointly promote bone resorption. One study showed that a decrease in estrogen causes an increase in the IL-6 content (46) and then inhibits the apoptosis of osteoclasts. The FoxO/cAMP signaling pathway is also closely related to inflammation (47–49) and plays an important role in regulating the inflammatory microenvironment in obesity, OP, and OA. These findings indicate that the intervention effect of LBJN on OA + OP rats is characterized by impacting multiple pathways and targets.

TABLE 2 Main differential metabolic product information.

Metabolite	Regulate	VIP value
Zeranol	Up	5.4731
7,3′-Dihydroxyflavone	Up	4.0761
5-Hydroxyindoleacetic acid	Up	3.8894
GANT61	Up	3.6068
Apigenin-7-Glucuronide	Up	3.5811
Dihydrokaempferol	Up	3.5093
Milbemycin alpha10	Up	3.4012
Kaempferol-7-O-Glucoside	Up	3.2909
N-Methylisoleucine	Up	3.0862
Beta-L-Dioxolane-cytidine	Up	3.0578
Vanillactic acid	Down	4.071
Octanoylcarnitine	Down	4.0155
TXB2	Down	3.8165
13,14-dihydro-15-keto Prostaglandin J2	Down	3.1308
Tetradecanoylcarnitine	Down	2.9628
Tanacetol A	Down	2.9463
Lauroylcarnitine	Down	2.9399
Oleoyl-L-Carnitine	Down	2.9321
Bisacurone epoxide	Down	2.8844
Hydroxytetradecadienyl-l-carnitine	Down	2.8801

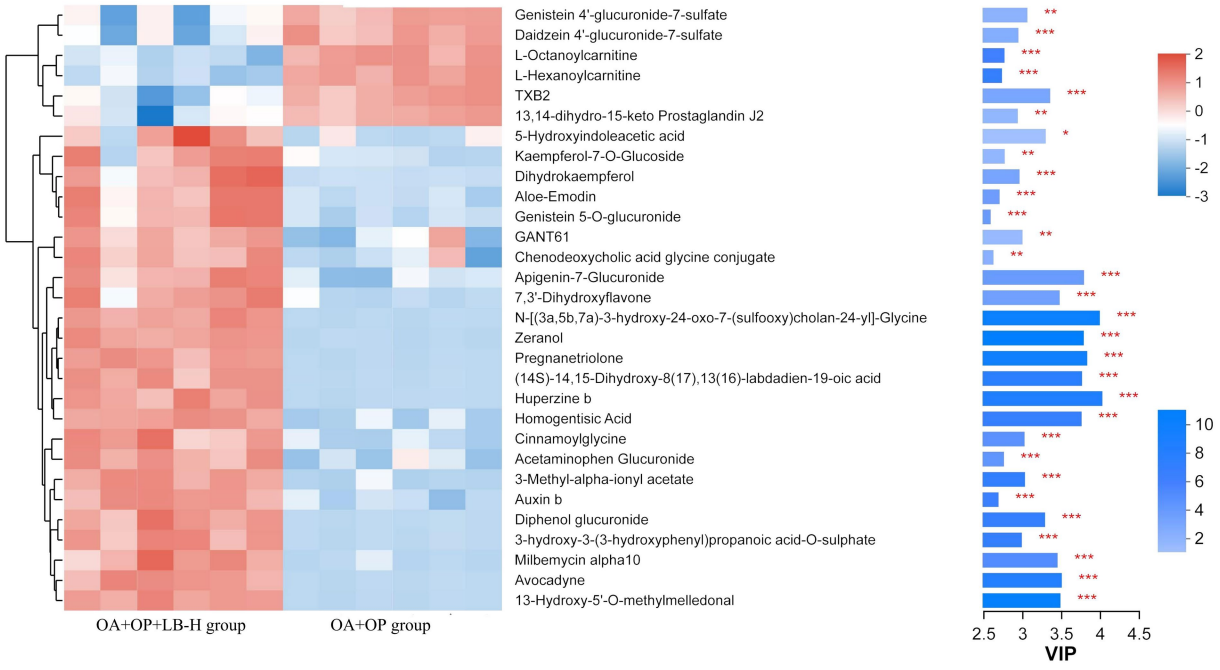

FIGURE 8
VIP value analysis of metabolites in the OA + OP and OA + OP+LB-H groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

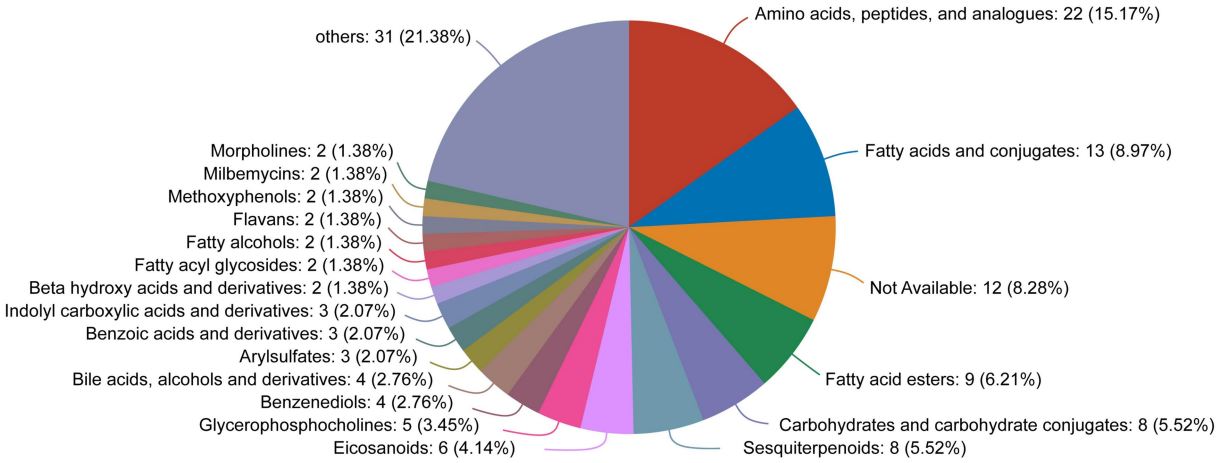

FIGURE 9
HMDB compound classification diagram for the differentially expressed metabolites between the OA + OP and OA + OP+LB-H groups.

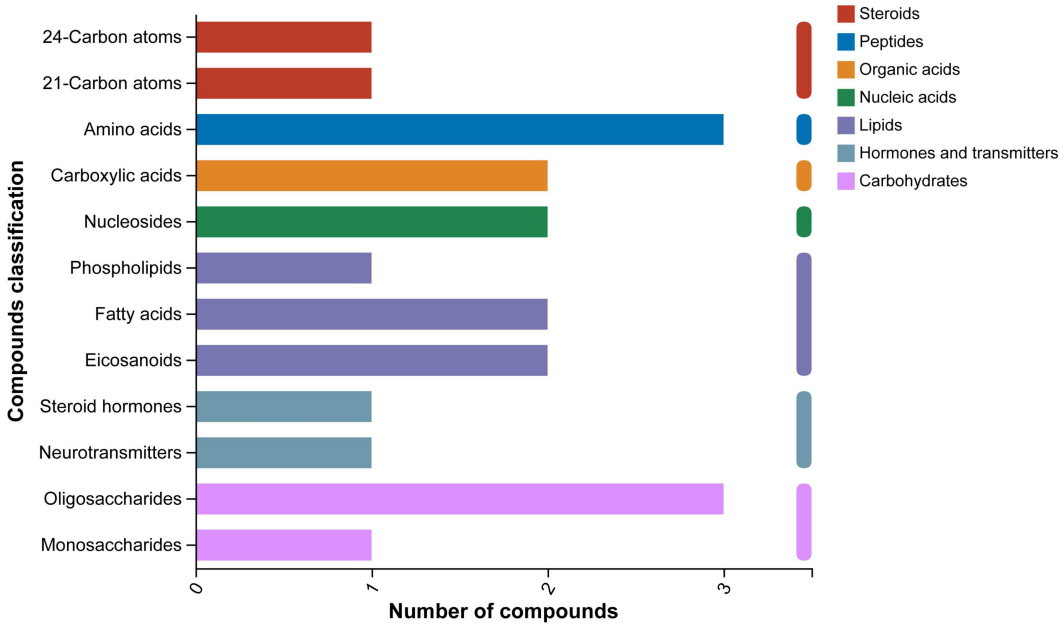

FIGURE 10
KEGG compound classification diagram of the differentially expressed metabolites between the OA + OP and OA + OP+LB-H groups.

This study has the following limitations. First, although we have identified the differentially expressed metabolites in the treatment of OA + OP with LBJN, the specific mechanism of action of LBJN has not yet been revealed. This will be the next step in this research and will be further explored in depth. Second, it is difficult to separate the components of LBJN and their related metabolites from metabolic samples containing a large amount of biological matrix, which may affect the accuracy of the conclusions of this study. Third, LBJN contains many potentially effective chemical components, but we cannot determine which components play the main pharmacological role, which represents an area for further exploration in future research. Finally, this study did not include an OP group, which did not allow us to compare the outcomes of LBJN treatment in an OP group versus the OA + OP group.

5. Conclusion

LBJN can maintain bone metabolism balance by regulating serum lipid metabolism, amino acid metabolism, carbohydrate metabolism and estrogen, further reducing bone loss in subchondral bone, which may be a potential mechanism of action for LBJN in treating OA + OP. The findings of this study provide evidence for the efficacy

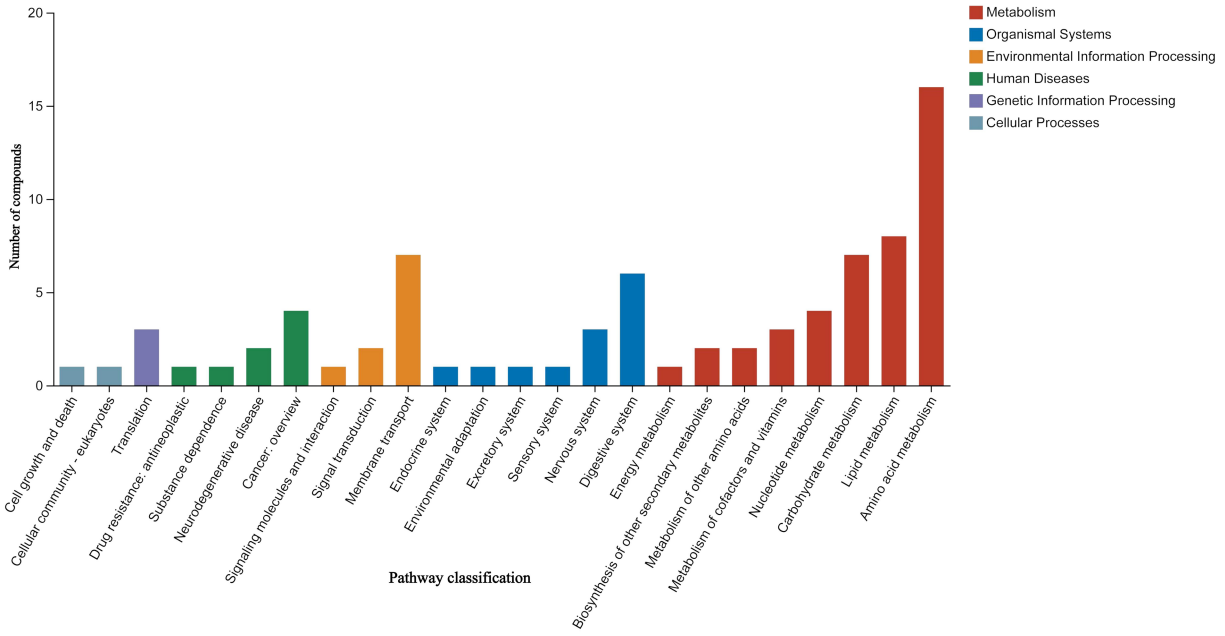

FIGURE 11
KEGG functional enrichment map of the differential expressed metabolites between the OA + OP and OA + OP+LB-H groups.

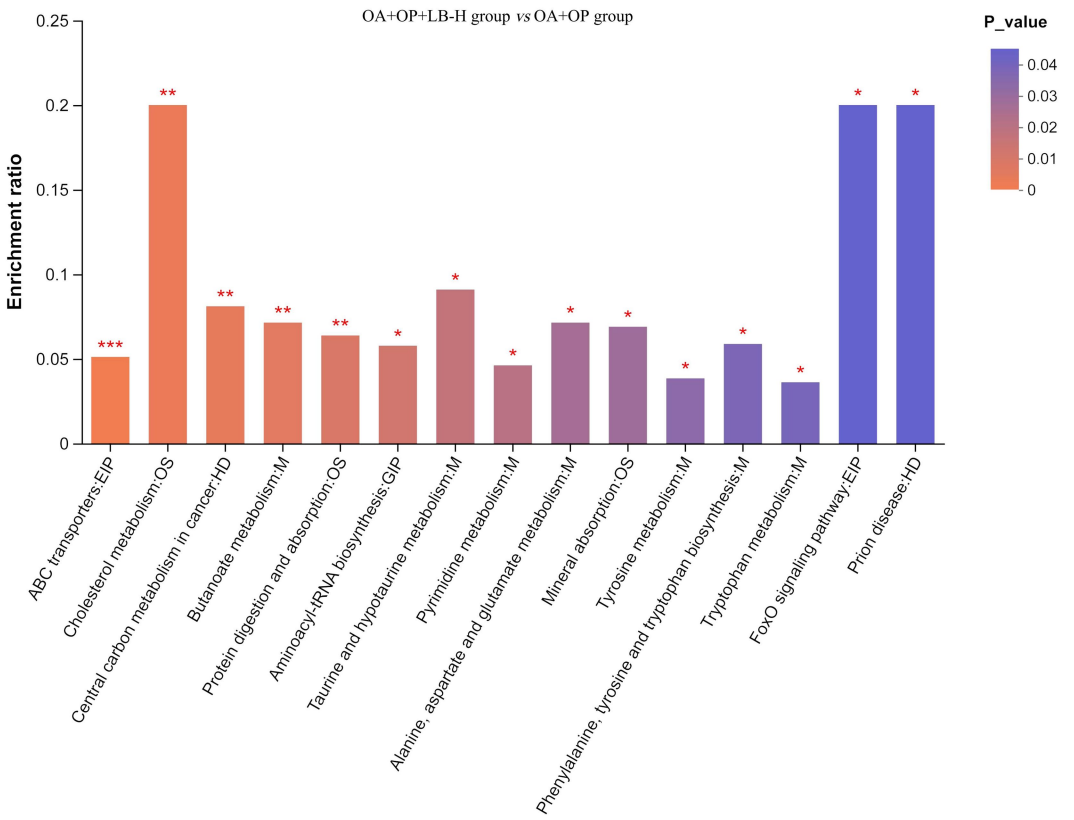

FIGURE 12
Enrichment map of the KEGG pathways for the differentially expressed metabolites between the OA + OP and OA + OP+LB-H groups. The color gradient of the column indicates the significance of enrichment. ***p-value or FDR < 0.001. **p-value or FDR < 0.01. *p-value or FDR < 0.05.

and mechanism of kidney-tonifying and blood-activating herbs in treating OA and OP comorbidities, but these findings depend on the future implementation of clinical randomized controlled trials for verification.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the Experimental Animal Center of Guangdong Provincial Academy of Chinese Medical Sciences. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

GL: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. JZ: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. DZ: Data curation, Methodology, Writing – original draft, Writing – review & editing. YD: Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. HH: Formal analysis, Investigation, Software, Writing – original draft, Writing – review & editing. WY: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. GZ: Data curation, Investigation, Writing – original draft, Writing – review & editing. ZG: Data curation, Formal analysis, Software, Writing – original draft, Writing – review & editing. JP: Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing. JL: Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1256238/full#supplementary-material

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Glossary

OA	Osteoarthritis
OP	Osteoporosis
LBJN	Longbie capsule
OA + OP	OA and OP comorbidities
BALP	Bone alkaline phosphatase
OPG	Osteoprotegerin
TRACP	Tartrate-resistant acid phosphatase
KOA	Knee osteoarthritis
BMD	Bone mineral density
HPLC-Q-Orbitrap-MS	High-performance liquid chromatography quadrupole/electrostatic field orbital trap high-resolution mass spectrometry
MS	Mass spectrum
ESI	Electric spray ionization
BV/TV	Bone volume fraction
Tb.N	Trabecular number
BS/BV	Bone surface area to bone volume ratio
Tb.Th	Trabecular thickness
Tb.Sp	Trabecular separation
ELISA	Enzyme-linked immunosorbent assay
UPLC–MS	Ultra-high-performance liquid chromatography-mass spectrometry
ISVF	Ion-spray voltage floating
DDA	Data-dependent acquisition
PCA	Principal component analysis
PLS-DA	Partial least squares discriminant analysis
OPLS-DA	Orthogonal partial least squares discriminant analysis
VIP	Variable importance in projection
HMDB	Human Metabolome Database
KEGG	Kyoto Encyclopedia of Genes and Genomes

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Effect of pilose antler polypeptide on the mechanism of bone homeostasis in osteoporosis

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Osteoporosis stands out as a prevalent metabolic disorder, bearing significant repercussions on human well-being and overall quality of life. It remains an urgent concern within the global public health framework due to its widespread occurrence. Osteoporosis arises from an abnormal metabolism in osteoblasts and osteoclasts, resulting in a disruption of the delicate equilibrium between bone formation and bone resorption. Within this context, deer antler peptides emerge as natural active compounds, wielding a pivotal role in governing the differentiation, proliferation, and mineralization of osteoblasts, as well as influencing the activity of osteoclasts. This article aims to consolidate our comprehension of the mechanisms underpinning the dynamic balance between bone formation and resorption, meticulously orchestrated by osteoblasts and osteoclasts in osteoporosis. Furthermore, it offers a comprehensive overview of how deer antler peptides, through their modulation of relevant signaling pathways, contribute to the enhancement of bone homeostasis. These insights deepen our understanding of the pathological processes through which deer antler peptides ameliorate bone homeostasis, while also presenting novel strategies for osteoporosis management.

KEYWORDS

osteoporosis, bone homeostasis, pilose antler polypeptide, osteoblasts, osteoclast

Introduction

Osteoporosis (OP) manifests as a condition marked by a reduction in bone density, resulting in heightened bone fragility and vulnerability to fractures (1). It is reported by the National Osteoporosis Foundation in the United States, the persistent aging of the global populace is poised to trigger a substantial surge in osteoporosis cases (2). Projections indicate that by 2030, the global tally of adults grappling with osteoporosis and diminished bone density will surpass 200 million (2). Moreover, a daunting economic impact anticipated in the United States by 2040, the financial strain attributed to osteoporosis-linked fractures is estimated to soar to an astounding 50 billion USD, presenting a formidable challenge to public health (2, 3).

The fundamental pathological mechanism underlying osteoporosis (OP) is an aberration in bone metabolism and disruption of bone homeostasis, notably characterized by a substantial reduction in bone formation capacity and heightened bone resorption (3). At the cellular level, this manifests as a diminution in the expression of osteoblasts (OBs) and an escalation in osteoclasts' (OCs) activity (4). Ultimately, these alterations culminate in a thinning of cortical bone, a decrease in trabecular numbers, and an increase in trabecular spacing, collectively rendering the bones fragile (4). Therefore, upholding a dynamic equilibrium between OBs and OCs emerges as pivotal for both the structure and development of bones.

Pilose antler polypeptide/velvet antler polypeptide (PAP/VAP), a potent extract derived from traditional Chinese medicine, deer antler, effectively promotes the proliferation of osteoblasts (OBs) and enhances bone cell mineralization ability (5), which amplifies the expression and activity of essential factors like BMP-2, ALP, estrogen, and bone-protective agents, consequently augmenting calcium and phosphorus levels within the human body (5). Moreover, it demonstrates the capability to suppress inflammatory factors and osteoclast activity. By multiple pathways such as MAKP, EGF, NF-κB, BMP-2, insulin, ERK, and PI3K/Akt, PAP significantly contributes to maintaining a balanced bone homeostasis (5). So the article commencing with an exploration of the pathological mechanisms of osteoporosis (OP) comprehensively outlines the pertinent effects of PAP on bone metabolism, offering fresh insights into the mechanisms by which PAP intervenes in OP.

Dynamic balance between osteoblasts and osteoclasts

At present, bone mineral density remains the principal diagnostic parameter for osteoporosis (OP) employing dual-energy X-ray absorptiometry (DXA) to calculate the T-score. A T-score of <−2.5 is indicative of osteoporosis (3). This diagnostic approach stems from the primary pathological feature of OP, which is the decline in bone mass. Bone mineral density assessment offers valuable insights into the abundance of bone mass within the human body (6). The quantity of bone mass is contingent on the population of bone cells, while an aspect intimately tied to the activity of osteoblasts (OBs) and osteoclasts (OCs) (7). Thus, maintaining a dynamic equilibrium between OBs and OCs emerges as pivotal in the onset and progression of OP.

Osteoblasts (OBs) represent a subset of undifferentiated monocytes originating from mesenchymal stem cells (MSCs) within the bone marrow. The differentiation of mesenchymal stem cells into osteoblasts predominantly hinges on signaling pathways such as BMP (bone morphogenetic protein) and Wnt/β-catenin, although pathways like NF-κB also play a contributory role (8). The differentiation sequence initiates as mesenchymal stem cells undergo transformation into osteo-chondroprogenitor cells. These cells, when activated by osteogenic transcription factors like runt-related transcription factor 2 (Runx2), drosophila distal less 5 (DLX5), and osterix (OSX), subsequently progress into pre-osteoblasts (9). Early osteogenic genes, inclusive of bone-derived alkaline phosphatase (BALP) and collagen1α1 (COL1A1), along with typical osteoblast markers like bone sialoprotein II (BSP II), osteocalcin (OCN), and osteopontin (OPN), govern the transcriptional pathway guiding the maturation of pre-osteoblasts into fully mature osteoblasts (8, 9).

During the proliferation phase of osteoblasts, notable alterations take place within the Golgi apparatus and endoplasmic reticulum of their organelles (10). Vesicle transport becomes highly coordinated at the plasma membrane, facilitating close interactions between neighboring osteoblasts (11). As osteoblasts progress towards differentiation, they initiate the production of bone matrix within their cells. This matrix encompasses adjacent osteoblasts, progressively giving rise to bone tissue (12). Subsequently, through the deposition of hydroxyapatite calcium, the bone tissue undergoes mineralization alongside the extracellular matrix, ultimately contributing to the development of human bones (10–12).

A healthy skeleton does not maintain continuous generation and necessitates the bone resorption process, in which osteoclasts (OCs) play a vital role in clearing aged and damaged bone. OCs are multinucleated cells formed by the fusion of mononuclear precursor cells and are considered terminally differentiated cells. These cells originate from mononuclear hematopoietic myeloid lineage cells, where myeloid progenitor cells transform into pre-monocytes under the stimulation of PU.1 and MITF (13). Subsequently, under the influence of M-CSF, pre-monocytes progress into osteoclast precursors (13). Various cell types, including bone marrow stromal cells, T cells, osteoblasts, and B cells, have the capacity to upregulate the expression of receptor activator of nuclear factor-kappa B ligand (RANKL), which serves as an NF-κB ligand receptor (14). Then RANKL activates the RANK receptor on osteoclasts (OCs) initiating a cascade that transforms osteoclast precursors into osteoclasts (14). This activation stimulates the proliferation, differentiation, and multinucleation of osteoclasts (14). Mature osteoclasts manifest as multinucleated cells with adhesive molecules and a dynamic cell skeleton. On the other hand, osteoblasts can secrete acid proteases and modify the microenvironment of adhesion sites, leading to a reduction in collagen within bones and the breakdown of aged and damaged bone tissue (15, 16). Therefore, the harmonious coordination and equilibrium between osteoblasts and osteoclasts are pivotal, ensuring normal bone growth and representing critical factors in maintaining bone mass within the human body.

Influenced by factors such as aging, hormone levels, and other diseases, the expression levels of osteoblasts (OBs) and osteoclasts (OCs) experience shifts. This disruption upsets the delicate balance between them, culminating in suppressed bone formation and heightened bone turnover and resorption. As a result, there is an imbalance in bone homeostasis, weakening not only the material properties of bone, such as mineral size and collagen, but also triggering adverse alterations in bone shape and structure. These changes include a decrease in trabecular thickness, alterations in connectivity, reduction in cortical bone thickness, and enlargement of pores (17, 18). Ultimately, these alterations contribute to the pathological structure characteristic of osteoporosis.

As individuals age, a plethora of aging factors manifest within the body, encompassing DNA damage, heightened oxidative stress, telomere shortening, and chromatin deformation, among others. These factors not only diminish the differentiation capacity of bone marrow mesenchymal stem cells into osteoblasts but also impair their functionality and regenerative potential (19, 20). Moreover, they result in a reduced expression of crucial transcription factors like RUNX2, osterix, and nuclear factor erythroid 2-related factor 2 (Nrf2), thereby exacerbating bone resorption and disrupting bone homeostasis (21, 22).

The decline in estrogen levels is recognized as a significant contributor to this imbalance in bone homeostasis. Postmenopausal women, experiencing reduced estrogen levels, undergo a shorter lifespan of osteoblasts (OBs) and a relatively prolonged lifespan of osteoclasts (OCs) (23, 24). This discrepancy leads to diminished bone formation compared to bone resorption, ultimately resulting in an imbalance in bone homeostasis. Concurrently, an increase in RANKL expression can also provoke this imbalance. Research indicates that reduced estrogen levels can decrease osteoprotegerin (an antagonist

to RANKL) and encourage elevated RANKL expression; RANKL, in turn, activates osteoclasts (OCs), heightening bone resorption rates (25, 26). Additionally, certain malignant bone diseases or immune disorders such as multiple myeloma and rheumatoid arthritis, or hormonal suppression in patients (e.g., females with breast cancer, males with prostate cancer), can augment the expression of RANKL (27–29). This heightened osteoclast activity contributes to an imbalance in bone homeostasis (4).

Inhibiting the principal pathways by which mesenchymal stem cells differentiate into osteoblasts is also a pivotal factor in the disruption of bone homeostasis. Research has uncovered abnormal expression of Forkhead Box F2 (FOXF2) in postmenopausal women with low bone mass; FOXF2 can hinder osteoblast formation via the Wnt 2b/β-catenin signaling pathway. Notably, when FOXF2 was knocked out in mice, an increase in bone mass was observed (30).

In specific cases, patients with osteoporosis (OP) and concurrent degenerative or hematological disorders may accumulate an excess of iron in their bodies. Excess iron can impede the Wnt signaling pathway, inducing morphological changes such as mitochondrial membrane shrinkage, condensation, loss, and outer membrane rupture. Additionally, this excess iron generates lipid peroxidation products (LPO) and reactive oxygen species (ROS), disrupting the differentiation process of osteoblasts (31).

Pilose antler polypeptide/velvet antler polypeptide

As a prominent component of traditional Chinese medicine, Deer antler is sourced from the antlers of young, non-calcified, velvet-covered male sika deer or red deer, and it encompasses various active substances and amino acids (32). One of its significant constituents is PAP, a short peptide comprising several amino acids including glycine, glutamic acid, leucine, valine, and alanine (33). PAP, a natural active component extracted from deer antler, accounts for 50 to 60% of the total wet weight of deer antler (34). PAP plays a pivotal role in enhancing bone metabolism and addressing the imbalance in bone homeostasis (33–35).

Some studies have elucidated the beneficial role of PAP in regulating bone formation and resorption (Figure 1):

Promotion of bone formation

PAP has demonstrated significant potential in augmenting bone formation. In ovariectomized female rats, PAP administration led to increased levels of estrogen, BMP-2, and ALP, effectively promoting bone formation (36). Notably, when compared to the estradiol group (estradiol being a drug utilized to counter osteoporosis stemming from diminishing estrogen levels), PAP exhibited superior efficacy in enhancing bone mineral density (36). Moreover, when co-cultured with human osteosarcoma cells in isolated and purified forms, (OS-732), PAP displayed the ability to enhance ALP expression, further encouraging bone formation (37). *In vitro* culture studies involving rat cells revealed that varying concentrations of PAP resulted in increased BMP-2 levels, with the 400 μg/mL concentration of PAP demonstrating the most notable impact (38). Further *in vitro* cell culture investigations indicated that PAP augments the expression of the key transcription factor Runx2, crucial for osteogenesis. It also enhances the content of OCN, ALP, OPN, and BSP, thereby promoting bone formation (39). Long-term gavage administration of PAP in a rat model of ovariectomy-induced osteoporosis exhibited notable benefits. It elevated estrogen and ALP levels, and improved calcium and phosphorus levels, as well as enhanced bone mineral density (40, 41). PAP was also shown to increase bone calcium content in a rat model of retinoic acid-induced osteoporosis, effectively improving the quantity, width, and spacing of bone trabeculae. Additionally, PAP significantly stimulated bone cell proliferation, enhancing bone mass, density, and formation more effectively than calcium gluconate (42). Moreover, polylactide glycolide (PLGA) microspheres loaded with PAP demonstrated enhanced efficacy by not only improving trabecular area and cortical average thickness in ovariectomy-induced osteoporotic rats but also by enhancing the bioavailability of PAP, addressing the challenge of PAP's short half-life (43).

Inhibition of bone resorption

PAP effectively inhibits bone resorption through its anti-inflammatory and antioxidative effects. PAP displays the capability to diminish bone resorption by mitigating inflammation, which accomplishes this by reducing the levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), (44–46). By doing so, it curtails the inflammatory

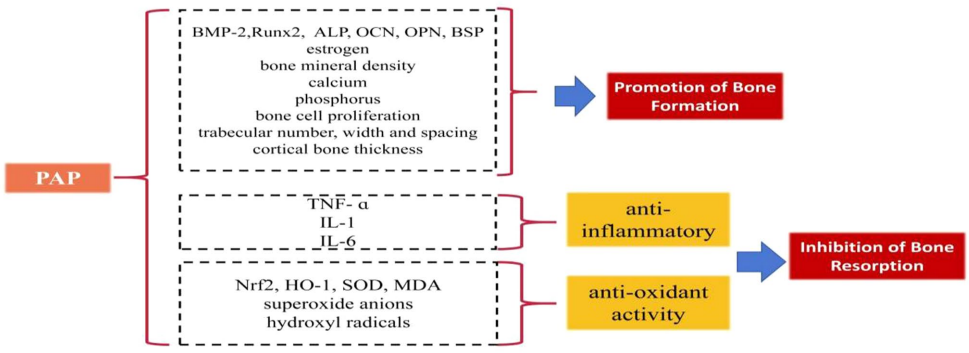

FIGURE 1
The beneficial role of PAP in regulating bone formation and resorption.

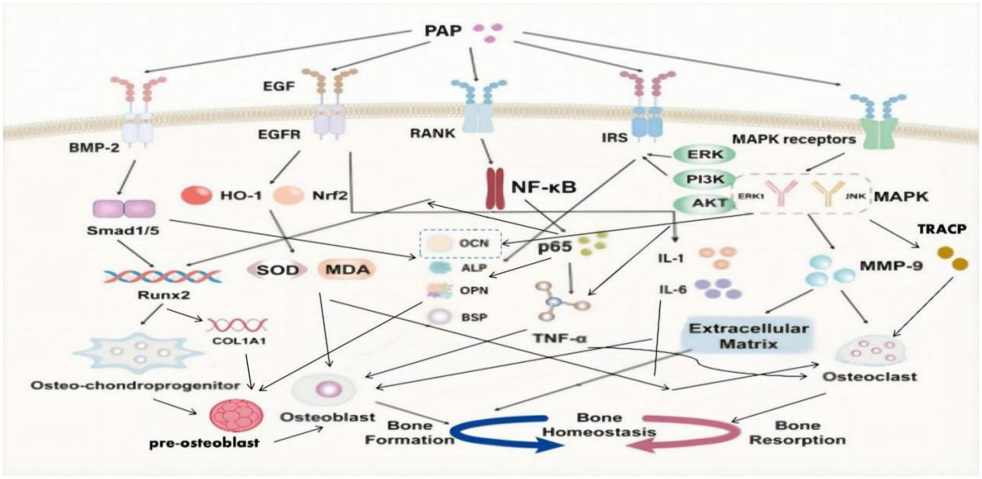

FIGURE 2
Signal pathway of deer antler peptides improving bone homeostasis.

response, safeguarding osteoblasts, and thwarting the induction of bone resorption prompted by pro-inflammatory cytokines (44–46). Furthermore, PAP exerts an antioxidative effect that contributes to the inhibition of bone resorption. It induces the production of superoxide dismutase (SOD), nuclear factor erythroid 2-related factor 2 (Nrf2), and heme oxygenase-1 (HO-1), while inhibiting malondialdehyde (MDA). This orchestrated response combats oxidative reactions, diminishing the proliferation of osteoclasts stimulated by oxidative factors and subsequently reducing the rate of bone resorption (47, 48). Additionally, enzymatic digestion and further purification of PAP yield a component of PAPs that exhibits highly potent antioxidant activity. This component showcases significant abilities in scavenging superoxide anions and hydroxyl radicals (49).

In addition to the mentioned effects, PAP holds promising potential in improving bone homeostasis by enhancing the immune system. Several *in vitro* experiments conducted on mice have shown that PAP can elevate the number of CD4(+)/CD8(+) cell subsets, enhance the cytotoxicity of NK cells, and boost the overall immune response in mice (50, 51). The immune system plays a significant role in bone resorption, as evidenced by multiple studies. The activation of the immune system often requires calcium and phosphate obtained from bone absorption induced by inflammatory factors (52–54). However, it's important to note that while the potential link between PAP and immune system modulation for maintaining bone homeostasis is intriguing, there's currently no confirmed animal research in this area, leaving it as an uncharted field. Further investigations are warranted to fully understand and validate this aspect.

Signal pathway of pilose antler polypeptide improving bone homeostasis

BMP-2/Smad1, 5/Runx2 signaling pathway

Bone morphogenetic protein-2 (BMP-2) stands as a pivotal member within the BMP family, widely acclaimed for its robust osteoinductive properties. BMP-2 plays several critical roles in bone regeneration and repair (55, 56):

1. Recruitment and angiogenesis: BMP-2 plays a crucial role in enhancing the recruitment of osteochondral progenitor cells to specific bone formation sites. Additionally, it stimulates angiogenesis, promoting the formation of new blood vessels to support the burgeoning bone growth.
2. Osteoblast differentiation: BMP-2 exhibits impressive capabilities as an inducer of osteoblast differentiation. It sets in motion the differentiation process of mesenchymal stem cells into osteoblasts, which are responsible for bone formation and subsequent mineralization.
3. Bone regeneration: Notably, BMP-2 significantly contributes to bone regeneration by boosting the activity of osteoblasts and aiding in the formation of new bone tissue whose presence markedly stimulates bone regeneration processes to a pivotal aspect of overall bone health and healing (Figure 2).

SMADs represent pivotal regulatory proteins and downstream effectors within numerous signaling pathways. Among them, Smad1 and Smad5 are prominently associated with the BMP signaling pathway (57). Upon BMP activation of SMADs, these proteins augment their own gene expression by chromatin remodeling. Moreover, they recruit specific transcription factors, thereby regulating factors associated with bone development (58). Central to the regulation of osteoblast differentiation is Runx2, a critical transcription factor within the Runx family (59). Runx2 plays an essential role throughout all stages of mesenchymal stem cell differentiation into osteoblasts (60). Additionally, Runx2 regulates the expression of type I collagen alpha 1 (COL1A1) in osteoblasts and exerts influence over the proliferation of osteoprogenitor cells (59, 60).

PAP has shown the ability to activate essential signaling pathways, including BMP-2/Smad1 and Smad5/Runx2 (61). PAP's activation leads to the upregulation of Smad1 and Smad5 expression, enhancement of the key transcription factor Runx2, elevation of bone-related ALP and OCN levels, and stimulation of the differentiation and mineralization of bone marrow mesenchymal stem cells into osteoblasts (62–64). This comprehensive action promotes bone formation, effectively addressing the problem of decreased bone mass seen in osteoporosis (61–64).

These findings underscore the potential of PAP in promoting bone regeneration and repair by modulating key signaling pathways involved in osteoblast differentiation and bone formation.

MAPK/MMP-9 signaling pathway

The MAPK family encompasses four subtypes: extracellular signal-regulated kinase 1/2 (ERK1/2), p38, extracellular signal-regulated kinase 5 (ERK5), and c-Jun N-terminal kinase (JNK) (65). Notably, JNK and ERK1 are implicated in enhancing osteoclast differentiation and proliferation, ultimately amplifying bone resorption efficiency (66, 67).

Matrix metalloproteinases (MMPs) represent a vital protease family responsible for degrading the extracellular matrix, including collagen (68). This family comprises 23 different members, each characterized by distinct structural domains and functions (67). Specifically, MMP-9 demonstrates high expression and activity in osteoporotic bone tissue. Aside from its role in degrading the extracellular matrix, MMP-9 can regulate osteoclast gene expression, thereby compromising bone strength and resilience (69, 70).

PAP demonstrates the ability to diminish the activity of ERK1, JNK, and MMP-9 induced by retinoic acid in osteoporotic rats (71). Through this inhibition, PAP effectively reduces MMP-9-mediated degradation of the extracellular matrix and the consequent stimulation of osteoclasts (71). Additionally, PAP exerts inhibitory effects by reducing the release of osteocalcin (OCN) from bones into the blood, lowering the levels of tartrate-resistant acid phosphatase (TRACP) in the serum (71). These actions collectively counter bone resorption in individuals affected by osteoporosis.

Therefore, PAP may modulate the MAPK/MMP-9 signaling pathway, playing a role in maintaining bone mineral density and strength, ultimately contributing to maintaining a healthy bone structure and function.

NF-κB signaling pathway

In fact, TNF-α has multiple regulatory effects in bone metabolism. Not only does negatively impacts bone formation by osteoblasts but can also independently induce osteoclast differentiation with the involvement of p50, p52, and NF-κB (72, 73). NF-κB ligand coupling with RANKL leads to activation and differentiation of osteoclasts derived from mononuclear hematopoietic myeloid lineage cells, promoting bone resorption (74).

PAP can inhibit the NF-κB signaling pathway, downregulate the expression of p65 protein, improve osteoblast differentiation inhibited by TNF-α, enhance the expression of the key transcription factor Runx2, increase the levels of osteoblast markers (such as OCN, ALP, OPN, BSP), promote osteoblast differentiation and mineralization, inhibit TNF-α-induced osteoclast differentiation, resist bone resorption, and address the bone homeostasis imbalance in osteoporosis (39).

These findings highlight the potential therapeutic role of PAP in alleviating the detrimental effects of TNF-α on bone health by modulating key signaling pathways to enhance osteoblast functionality and suppress osteoclast activity.

EGF/EGFR signaling pathway

Epidermal Growth Factor (EGF) protein family is widely expressed in the skeletal system, prominently present in osteoblasts and osteoclasts (75). EGF, as a peptide composed of 53 amino acid residues, plays a crucial role in bone health (76, 77):

1. Promotion of osteoblast maturation: EGF and its receptor can increase the levels of osteocalcin (OCN) and alkaline phosphatase (ALP), both important markers of osteoblast activity, which promotes the maturation and functionality of osteoblasts.
2. Regulation of bone deposition: Ligands of the EGF receptor (EGFR) enhance bone deposition and bone matrix formation, achieving this by regulating the proliferation of osteoblasts and growing chondrocytes, which are critical for bone growth and modeling.

PAP has a significant anti-inflammatory and antioxidant effect through the EGF/EGFR signaling pathway, not only protecting osteoblasts and promoting bone formation but also inhibiting the induction of inflammatory and oxidative factors on osteoclasts, thereby reducing bone resorption (48):

1. Anti-inflammatory effect: PAP exhibits a notable anti-inflammatory effect by modulating the EGF/EGFR signaling pathway. This modulation leads to a reduction in the levels of pro-inflammatory cytokines in the serum, including TNF-α, IL-1, and IL-6. By this mechanism, PAP effectively alleviates inflammation and helps in maintaining a balanced inflammatory response within the body.
2. Anti-oxidant effect: PAP demonstrates a remarkable ability to enhance the antioxidant defense mechanism. It achieves this by upregulating the expression of crucial factors such as nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1). Additionally, PAP boosts the activity of superoxide dismutase (SOD), a vital antioxidant enzyme. Concurrently, it reduces the levels of malondialdehyde (MDA), a reliable marker of oxidative stress. These concerted actions contribute to PAP's robust antioxidant properties, reinforcing the body's defense against oxidative damage and stress.

In summary, EGF and its receptor play a significant role in bone biology. PAP utilizes the EGF/EGFR signaling pathway to exert anti-inflammatory and antioxidant effects, which can have a positive impact on bone homeostasis and overall health in osteoporosis.

Insulin signaling pathway

Currently, there are four subtypes of insulin receptor substrates (IRS): IRS-1, IRS-2, IRS-3, and IRS-4 (78, 79). However, only IRS-1 and IRS-2 play crucial roles in regulating osteoblasts by the insulin signaling pathway in the growth and development of bones (78, 79). Insulin has been confirmed to induce osteoblast differentiation and proliferation, and regulate the synthesis of collagen and ALP through the ERK and PI3K pathways (80, 81).

PAP binds to IRS (insulin receptor substrate) and activates the insulin signaling pathway. This activation involves downstream insulin

signaling molecules, including ERK and partial PI3K/Akt pathways. When osteoblasts co-cultured with PAP were exposed to ERK and PI3K inhibitors, it significantly reduced the mineralization of osteoblasts and ALP activity (82). Hence, PAP plays a crucial role in enhancing the vitality of osteoblasts and elevating the levels of ALP, OPN, and OCN through the insulin signaling pathway mediated by ERK and partial PI3K/Akt signaling (82). Ultimately, this modulation affects osteoblast differentiation, proliferation, maturation, and mineralization.

These discoveries underscore the vital involvement of the insulin signaling pathway in regulating osteoblast function and bone formation. By modulating this pathway, PAP demonstrates its potential to enhance bone formation in osteoporosis, presenting a promising avenue for therapeutic intervention.

Conclusion and perspectives

In recent years, the aging of the global population has brought osteoporosis (OP) to the forefront as a critical global health concern. Due to the fact that OP has been steadily rising, the incidence of fractures imposes a significant burden on public health systems worldwide (83–85). It is imperative to comprehend the underlying mechanisms of OP to formulate effective strategies for its prevention and treatment. Bone homeostasis, characterized by a delicate balance between bone formation led by osteoblasts and bone resorption led by osteoclasts, is at the core of OP pathophysiology. However, the specific mechanisms that disrupt this balance have not been fully elucidated. Further research is essential to gain insights into the etiology, pathology, and physiology of OP. Exploring the intricate molecular and cellular processes that govern bone remodeling, the role of various signaling pathways, the impact of hormonal changes (such as those related to aging and hormone levels), and the contributions of genetic and environmental factors are all crucial areas of study in the quest to better understand and ultimately address OP.

PAP is a natural active ingredient extracted from the young antlers of Male Sika deer or red deer that have not yet ossified and are covered in hair. Research on PAP has been conducted in many Asian countries, including China, South Korea, and Japan, focusing on its molecular and cellular mechanisms. PAP has shown promise in regulating a variety of diseases, including those related to the cardiovascular system, skeletal system, and immune system (5, 86, 87).

In the context of bone metabolism, PAP has become a significant research focus, particularly in the field of osteoporosis. Studies have shown that PAP has various beneficial effects (36–49):

1. Regulating osteoblasts and promoting bone formation: PAP can regulate the proliferation, differentiation, maturation, and mineralization of osteoblasts, supporting the formation of new bone tissue.
2. Inhibiting osteoclast activity and resisting bone resorption: PAP can also inhibit the activity of osteoclasts by its anti-inflammatory and antioxidant propertiesn and increase the lifespan of osteoblasts as well as resist bone resorptionm, which helps maintain a healthy balance between bone formation and bone resorption.

As osteoporosis is characterized by imbalanced bone homeostasis with decreased bone formation and increased bone resorption, the ability of PAP to positively impact both osteoblasts and osteoclasts makes it a promising area of research in the field of osteoporosis, which offers potential as a therapeutic agent for improving bone health and addressing bone-related conditions.

PAP stands as a promising intervention to address compromised bone formation in osteoporosis. It achieves this by not only inhibiting bone resorption but also effectively maintaining bone homeostasis. PAP exerts its effects by multifaceted signaling pathways, including MAPK, EGF, NF-κB, BMP-2, insulin, ERK, and PI3K/Akt (38, 48, 61–64, 71, 82). These pathways collectively contribute to PAP's capacity to enhance bone health and offer potential therapeutic benefits for individuals dealing with osteoporosis.

PAP does present certain limitations. Primarily, much of the research on PAP is derived from animal experiments, indicating a need for more extensive high-quality clinical randomized controlled trials specifically centered on PAP as the primary component in medications. Additionally, economically, the sourcing of deer antler, a key ingredient for PAP, can be relatively expensive, and the process to prepare PAP is associated with high costs. Pharmacologically, PAP faces challenges such as low bioavailability, a short half-life, and vulnerability to proteolytic degradation. However, comprehensive analysis regarding its pharmacokinetics and pharmacology is lacking, highlighting the need for further in-depth research and evaluation (5, 43). These limitations underscore the necessity for continued research and improvement in the development and application of PAP for potential clinical use.

Efforts are actively underway to enhance and advance PAP. Promising strategies to address its limitations are being explored. For instance, approaches such as utilizing PLGA microspheres or nano TCP/gelatin/PAP composite materials can significantly improve PAP's bioavailability (43, 88). Moreover, optimizing the extraction process of PAP holds the potential to enhance its antioxidant capacity and overall safety (89). In terms of cost-effectiveness, techniques like ice acetic acid and ultrasonic fragmentation offer avenues to streamline production and reduce costs (90).

Considering these advancements and ongoing research endeavors, it is plausible to envision PAP and its derived products playing a pivotal role in the future management of osteoporosis. Continued research and innovative approaches will further shape the potential of PAP as a valuable component in the comprehensive management and treatment of osteoporosis.

Author contributions

GW: Writing – original draft. YM: Writing – review & editing. WO: Writing – review & editing. CZ: Writing – review & editing, Conceptualization. WZ: Writing – review & editing, Conceptualization.

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Conflict of interest

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Glossary

OP	Osteoporosis
OBs	Osteoblasts
OCs	Osteoclasts
PAP	Pilose antler polypeptide
Runx2	Runt-related transcription factor 2
ALP	Alkaline phosphatase
BSP	Bone sialoprotein
OCN	Osteocalcin
OPN	Osteopontin
BMP	Bone morphogenic proteins
Wnt	Wingless-related integration site
ERK	Extracellular signal regulated kinase
MAPK	Mitogen-activated protein kinase
JNK	C-Jun N-terminal kinase
MMP	Matrix metalloproteinase
RANKL	Receptor activator of NF-κB ligand
TNF	Tumor necrosis factor
IL	Interleukin

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Aconine attenuates osteoclast-mediated bone resorption and ferroptosis to improve osteoporosis via inhibiting NF-κB signaling

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Osteoporosis (OP), a prevalent public health concern primarily caused by osteoclast-induced bone resorption, requires potential therapeutic interventions. Natural compounds show potential as therapeutics for postmenopausal OP. Emerging evidence from *in vitro* osteoclastogenesis assay suggests that aconine (AC) serves as an osteoclast differentiation regulator without causing cytotoxicity. However, the *in vivo* functions of AC in various OP models need clarification. To address this, we administered intraperitoneal injections of AC to ovariectomy (OVX)-induced OP mice for 8 weeks and found that AC effectively reversed the OP phenotype of OVX mice, leading to a reduction in vertebral bone loss and restoration of high bone turnover markers. Specifically, AC significantly suppressed osteoclastogenesis *in vivo* and *in vitro* by decreasing the expression of osteoclast-specific genes such as *NFATc1*, *c-Fos*, *Cathepsin K*, *and Mmp9*. Importantly, AC can regulate osteoclast ferroptosis by suppressing Gpx4 and upregulating Acsl4, which is achieved through inhibition of the phosphorylation of I-κB and p65 in the NF-κB signaling pathway. These findings suggest that AC is a potential therapeutic option for managing OP by suppressing NF-κB signaling-mediated osteoclast ferroptosis and formation.

KEYWORDS

aconine, osteoporosis, osteoclast, ferroptosis, NF-κB signaling

Introduction

Osteoporosis (OP), a common metabolic skeleton disorder characterized by low peak bone mass and increased susceptibility to fracture, is a major public healthy burden for elderly population and postmenopausal women (1). According to estimations, a 50-year-old white woman has 50% lifetime risk of osteoporotic fracture, while all postmenopausal women over the age of 65 have a history of fractures (2, 3). For healthy bone density, the organ of bone often undergoes continuous remodeling to control bone mass, which involves two essential processes: osteoclast-mediated bone resorption (breakdown) and osteoblast-mediated bone formation (build-up) (4). When the activity of osteoclasts is increased, osteoblastic bone formation does not keep pace with bone resorption, excessive bone resorption results in bone loss (5, 6). Therefore, compounds with anti-osteoclastogenic properties may represent promising therapeutic agents for osteoprotection.

Osteoclasts, derived from hematopoietic precursor cells in the bone marrow, whose differentiation and maturation are regulated by a variety of systemic cytokines and signaling (7, 8). Mature osteoclasts with multinuclear features can tightly attach to the bone surface, leading to bone resorption (9). During osteoclast differentiation, bone marrow-derived macrophages (BMMs) are elevated after nuclear factor(NF)-kappa B ligand (RANKL) stimulation, followed by NF-κB and MAPK signaling activation, and subsequently stimulate osteoclastogenesis by promoting the specific genes expression that typify the osteoclast lineage, including c-Fos, Mmp9, Cathepsin K and T cell nuclear factor cytoplasmic 1 (NFATc1) (10, 11).

Growing evidence reveals that ferroptosis, a new form of cell death, contributes to several degenerative disorders, including age-related OP (12, 13). It features iron overload and accumulation of lipid peroxides, and the specific biological characteristics presents downregulation of glutathione peroxidase 4 (Gpx4) expression and upregulation of acyl-CoA synthetase long-chain family member 4 (Acsl4) (14). Emerging reports indicate that ferroptosis plays critical role in the development of RANKL-induced osteoclasts differentiation (15), and the higher level of ferroptosis, the stronger osteoclast activities, indicating that the potential benefit of regulating ferroptosis in osteoclast to prevent bone loss.

Aconine (AC), a diester alkaloid isolated from a traditional Chinese medicine *Aconiti Lateralis Radix Preparata* (Fuzi), has been proven to be safe for human consumption (16), It mainly functions to recess inflammation in arthritis and heart-protective effects (17, 18). Furthermore, another study has demonstrated that AC suppressed RANKL-induced osteoclast differentiation in RAW264.7 cells by inhibiting NF-κB and NFATc1 activation (19). However, whether AC regulates *in vivo* osteoclast formation and activity as well as OP progression has not yet been explored.

Therefore, in the present study, the efficacy of AC in preventing bone loss in ovariectomy (OVX)-induced OP mice, as well as the inhibitory effect on osteoclast activities *in vivo* and *in vitro* were investigated. Moreover, we sought to decipher the potential working mechanisms of AC on osteoclast ferroptosis through the NF-κB signaling pathway. These findings will provide comprehensive insight into the potential therapeutic implications and underlying mechanisms of AC for treating OP.

Materials and methods

Animals

Female C57/BL6 mice at the age of 8 weeks were purchased from Shanghai JSJ Laboratory Animal Co., Ltd. (Shanghai, China) and housed at the animal facility of Shanghai Municipal Hospital of Traditional Chinese Medicine (TCM) under standard conditions. 8-10-week-old male *Opg* knockout mice and C57/BL6 mice were purchased from the Shanghai Research Center of Model Organisms. All animal experiments were approved by the Animal Experiments Ethical Committee of Shanghai Municipal Hospital of TCM (No. 2022022).

Ovariectomy -induced OP mouse model and treatments

Twenty-four mice were randomly divided into three groups (n = 6 per group): sham group, OVX group, and OVX + AC group. Bilateral ovariectomies were performed sodium pentobarbital (40mg/kg) anesthesia to induce OP in the OVX and OVX+AC groups. For the sham group, a portion of the adipose tissue surrounding the ovaries was removed without resecting them. On day 7 after the operation, mice in OVX + AC group were intraperitoneally injected with AC (5 mg/kg) once daily for 8 weeks, while the sham and OVX group mice were received a vehicle control injection.

Micro-CT analysis

The L5 lumbar vertebra were harvested from the sacrificed mice. All data were acquired using a Skyscan 1172 μCT scanner (Bruker, Kontich, Belgium) at a source voltage of 49 kV and a source current of 179 uA with a voxel size of 10 μm. The region of interest (ROI) pertaining to the L5 lumbar vertebra can be described as a cylindrical volume. This volume is defined by a circular base with a radius of 2 mm, located at the bottom of the L5 vertebral body, and extending upwards for the height of the L5 vertebra. The parameters of ROI were analyzed as follows: bone mineral density (BMD), bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp), which were measured from 3D images of cancellous bone reconstructed from the bitmap dataset.

Histology and staining

Samples were fixed in 10% neutral-buffered formalin, decalcified in ethylenediaminetetraacetic acid (EDTA) solution (pH 7.4), dehydrated, and embedded in paraffin. Serial midsagittal

sections (4 μm thick) were then cut and stained with hematoxylin and eosin (H&E) staining for morphometric analysis.

The immunohistochemistry (IHC) staining was performed according to the manufacturer's instructions for SP Link Detection Kits (ZSGB-BIO, PV-9001/PV9002) as we previously described (20). Briefly, paraffin sections were rehydrated and digested with proteinase K solution (10 mg/ml) for 20 minutes at 37°C. Then the sections were respectively incubated with primary antibodies of Runx2 (Abcam, ab192256, 1:400), Osterix (Abcam, ab 209484, 1:400), Mmp9 (Abcam, ab38898, 1:1000), Cathepsin K (Abcam, ab19027, 1:500), NFATc1 (Abcam, ab2796, 1:500), c-Fos (Abcam, ab222699, 1:500), Gpx4 (Abcam, ab125066, 1:1000), Acsl4 (Abcam, ab155282, 1:1000), p-p65 (CST, #3033, 1:500) and p-I-κB (CST, #2859, 1:500) overnight at 4°C. Followed by coloration with 3,3'-Diaminobenzidine (DAB) solution and hematoxylin counterstaining, dehydration, clearance, and mounting. The stained sections were captured with a light microscope (Leica, DM6).

For the immunofluorescence (IF) staining, the expression of p65 in the L5 lumbar vertebra was determined by IF staining as previously described (20). Paraffin sections (4 μm thick) of the lumbar vertebra among groups were treated with a primary antibody anti-rabbit p65 (CST, #8242, 1:1000) overnight at 4°C, then incubated with fluorescent-labeled secondary antibodies for 1 hour, and counterstained with DAPI. Finally, the sections were scanned using a microscope (Leica, DM6).

Tartrate-resistant acid phosphatase staining

In this study, the L5 vertebra among groups was performed TRAP staining (Wako, #294-67001) according to the manufacturer's recommendations. In brief, paraffin sections (4 μm thick) were rehydrated and applied with a sufficient TRAP staining solution for 30 minutes at room temperature (RT). Distilled water to soak the sections and sufficient of nuclear staining solution was applied for 4 ~ 5 seconds, then immediately wash one section by moving them up and down in distilled water. Dry the sections on a heater plate at 37°C and mounting them with xylene. In the middle of the L5 vertebral adjacent to the intervertebral disc, the number of osteoclasts to bone surface ratios (20x field) was quantified.

Enzyme-linked immunosorbent assay

Serum markers of bone turnover were detected using the appropriate ELISA kits in strict accordance with the manufacturer's instructions. The cleaved-off of type I collagen (PINP) (Sangon Biotech, D721053) and bone-specific alkaline phosphatase (BALP) (Sangon Biotech, D721049) were performed for bone formation, while carboxy-terminal cross-linking telopeptide of type I collagen (β-CTX) (Sangon Biotech, D721187) was detected for bone resorption.

Serum aspartate transaminase (AST), alanine transaminase (ALT) and blood urea nitrogen (BUN), creatinine (Cr) were detected by Laboratory Department of Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine.

In vitro osteoclastogenesis assay

Bone marrow cells were isolated from 10-week-old WT or *Opg* KO mice by flushing the marrow space of femora and tibiae. The cells were then plated at a density of 8×10^3 cells/mL in 96-well plates and treated with M-CSF (50 ng/mL) for 3-4 days to induce macrophage enrichment. After 3-4 days, the cells were treated with various concentrations of AC (0, 10, or 20 μM) followed by M-CSF (200 ng/mL) and RANKL (200 ng/mL) stimulation until osteoclasts differentiated in the control group. When fully mature multinucleated osteoclasts were detected, TRAP activities were performed using a TRAP assay kit (Solarbio, G1492). If TRAP-positive multinucleated cells had three or more nuclei, they were classified as osteoclast-like cells.

RNA sequencing

To further explore the molecular mechanisms underlying AC treatment against osteoclast formation, we performed bioinformatics analysis of mRNA transcriptomes. BMMs from WT mice were seeded in a 12-well plate and cultured with osteoclast-stimulating medium (200 ng/mL M-CSF and 200 ng/mL RANKL) in the presence or absence of 20 μM AC subjected to 48 h incubations. Cells then were collected for RNA sequencing. Total RNA was isolated using the Trizol Reagent (Invitrogen Life Technologies), after which the concentration, quality and integrity were determined using a NanoDrop spectrophotometer (Thermo Scientific). Three micrograms of RNA were used as input material for the RNA sample preparations. Sequencing libraries were generated according to the following steps. Firstly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in an Illumina proprietary fragmentation buffer. First strand cDNA was synthesized using random oligonucleotides and Super Script II. Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and the enzymes were removed. After adenylation of the 3′ends of the DNA fragments, Illumina PE adapter oligonucleotides were ligated to prepare for hybridization. To select cDNA fragments of the preferred 400-500 bp in length, the library fragments were purified using the AMPure XP system (Beckman Coulter, Beverly, CA, USA). DNA fragments with ligated adaptor molecules on both ends were selectively enriched using Illumina PCR Primer Cocktail in a 15-cycle PCR reaction. Products were purified (AMPure XP system) and quantified using the Agilent high sensitivity DNA assay on a Bioanalyzer

2100 system (Agilent). The sequencing library was then sequenced on NovaSeq6000 platform (Illumina) Genekinder Medicaltech (Shanghai) Co., Ltd, China.

RNA extraction and RT-PCR assay

BMMs from WT or *Opg* KO mice were seeded in a 24-well plate and cultured with osteoclast-stimulating medium (200 ng/mL M-CSF and 200 ng/mL RANKL) in the presence or absence of AC for 5 days to form osteoclasts. Cells were collected for examination into the expression of osteoclast-specific genes using RT-PCR. Total RNA was extracted using EZ-press RNA purification (EZBioscience, B0004D) and was reverse transcribed by 1 μg using the Reverse Transcription Kit (TaKaRa, RR037A). cDNA was amplified by RT-PCR using an SYBR Green qPCR Kit (TaKaRa, RR420A) with sequence-specific primers (Supplemental Table 1). Each sample was repeated with 3 independent RT-PCR amplifications. Fold changes of genes of interest were calculated using control samples as 1.

Western blot

To determine the effect of AC on osteoclast function or ferroptosis levels, BMMs were seeded in 6 cm plates and cultured with osteoclast-stimulating medium in the presence or absence of 20 μM AC subjected to 48 h incubations. Total cellular proteins were collected to detect the osteoclast or ferroptosis-specific makers.

Protein levels were determined using a BCA protein assay kit (Beyotime) and Chemiluminescence reagent (Beyotime) was used to visualize protein bands. All data were acquired using the ChemiDOC Imaging System (BIO-RAD). The antibodies used for Western blot were Cathepsin K (Abcam, ab19027, 1:1000), c-Fos (CST, #2250, 1:1000), Gpx4 (Abcam, ab125066, 1:1000), Acsl4 (Abcam, ab155282, 1:1000), and GAPDH (Beyotime, A0208, 1:3000). GAPDH was used as an internal control.

To confirm the connection between NF-κB signaling and osteoclast formation/ferroptosis. BMM cells were pretreated with 10 ng/mL recombinant NF-κB (Novoprotein, #CR72) for 2 h followed by osteoclast-stimulating medium in the presence or absence of 20 μM AC for 24 h. Total proteins were harvested to assess the changes in key markers associated with osteoclast or ferroptosis proteins.

Statistics analysis

Data were presented as mean ± standard deviation (SD) and were analyzed using the GraphPad Prism software (4.0). One-way analysis of variance (*ANOVA*) followed by least significant difference (*LSD*) or Tamhane's T2 was used for multiple comparisons. P values < 0.05 were considered statistically significant.

Results

AC prevents bone mass loss in OVX-induced OP mice

To determine the therapeutic potential of AC on OP progression *in vivo*, mice were subjected to OVX modeling and subsequently treated with AC via intraperitoneal injection once a day for 8 weeks. The L5 vertebrae of OVX mice were harvested and subjected to evaluate bone microstructure by μCT. The results of μCT showed that OVX mice exhibited obvious reductions in trabecular bone mass compared with the sham group, while AC treatment could effectively prevent bone loss in OVX mice (Figure 1A). Similarly, OVX mice demonstrated significantly decrease in BMD in the L5 vertebrae, versus the sham group (0.184 ± 0.009, vs. 0.115 ± 0.013, respectively; P<0.001), whereas AC therapy enhanced BMD (0.143 ± 0.009) accompanied by improved bone microstructural parameters, as indicated by increases in BV/TV, Tb.Th and Tb.N, and a decrease in Tb.Sp of the OVX mice (Figures 1C–G). Subsequently, histological examination using H&E staining was conducted in order to provide a more comprehensive understanding of the pathological alterations occurring in the bone structure. As expected, the trabecular bone of the OVX mice in L5 vertebrae exhibited a decrease in thickness, quantity, and areas. However, the administration of AC markedly ameliorated these alterations (Figures 1B, H). These data suggest that AC treatment has the potential to effectively prevent extensive bone loss in OP modeling.

AC maintains bone homeostasis by blocking the high bone turnover and remodeling the OVX-induced osteoporotic microenvironment

The maintenance of healthy bone mass is a multifaceted process that is governed by the removal of mineralized bone by osteoclasts and the subsequent replacement of new bone by osteoblasts (21, 22). Measurement of serum β-CTX levels present the degree of bone resorption, while serum PINP and BALP levels provide evidence of bone formation (23–25). Postmenopausal women with OP frequently have accelerated bone turnover activity, characterised by elevated serum levels of osteogenic and osteoclastic metabolic markers (26). Our study revealed that mice with OP produced by OVX displayed elevated levels of bone formation markers, including PINP and BALP, as well as the bone resorption marker β-CTX. However, these effects were greatly attenuated following injection of AC therapy (Figures 2A–C). Then, we conducted an IHC experiment to assess the expression of osteogenic-related proteins, Runx2 and Osterix. Notably, the results of IHC staining showed that the number of Runx2-expressing cells and Osterix-expressing cells located on the trabecular bone surface arranged in a shuttle shape was drastically increased in the OVX group relative to that in the sham group, the treatment of AC could reduce the excessive expression of Runx2 and Osterix (Figures 2D, E, G, H). In order to evaluate the effects of AC on

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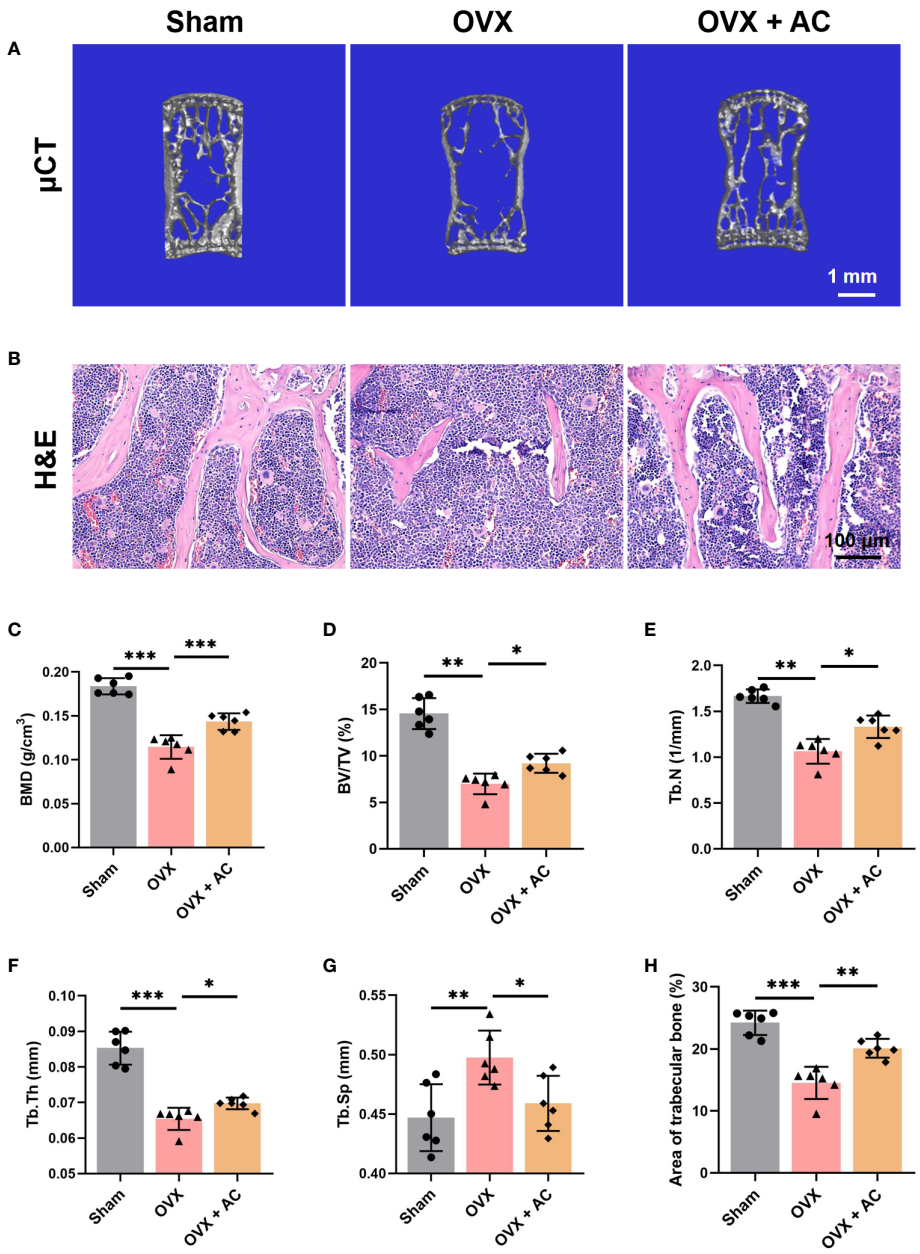

FIGURE 1
AC protects against bone loss in OVX-induced OP mice. **(A)** Representative μCT images of 3D of L5 lumbar vertebrae. Scale bar: 1 mm. **(B)** Representative images of H&E staining of L5 lumbar vertebrae in each group. Scale bar: 100 μm. Quantitative analyses of parameters regarding the bone architecture of L5 lumbar vertebrae, including **(C)** bone mass density (BMD), **(D)** bone volume/tissue volume (BV/TV), **(E)** trabecular thickness (Tb. Th), **(F)** trabecular number (Tb. N), and **(G)** trabecular separation (Tb. Sp). **(H)** The ratio of the area of trabecular bone. Data presented as means ± s.d. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. n = 6.

osteoblast differentiation, we employed osteoblastic MC3T3-E1 cell line induced osteogenic induction media for 21 days to analyze ALP activities and mineralization ability by ALP staining and Alizarin Red-s (AR-S) staining. However, AC treatment showed no obvious inhibition of ALP activities and mineralization ability, compared to the DMSO group without AC treatment (Supplemental Figure 1). Next, TRAP staining was employed to evaluate the osteoclastogenesis. The results revealed that OVX modeling caused a notable increase in positive osteoclast staining within the trabecular bone of L5 vertebrae. Furthermore, the number of osteoclasts within the trabecular bone area were significantly increased by ~30% compared to the sham group, whereas AC has the ability to impede the osteoclastogenesis with reduction by ~50% of osteoclasts number in the trabecular bone area in OVX mice (Figures 2F, I). Thus, AC administration reduces the formation of osteoclasts and inhibits osteoblast related key regulators, as well as reinstates a state of elevated bone turnover in mice subjected to OVX operation. Importantly, the inhibitory effect of AC on osteoclastogenesis may be more pronounced than its anti-osteogenesis effect, hence providing a protective mechanism against bone loss.

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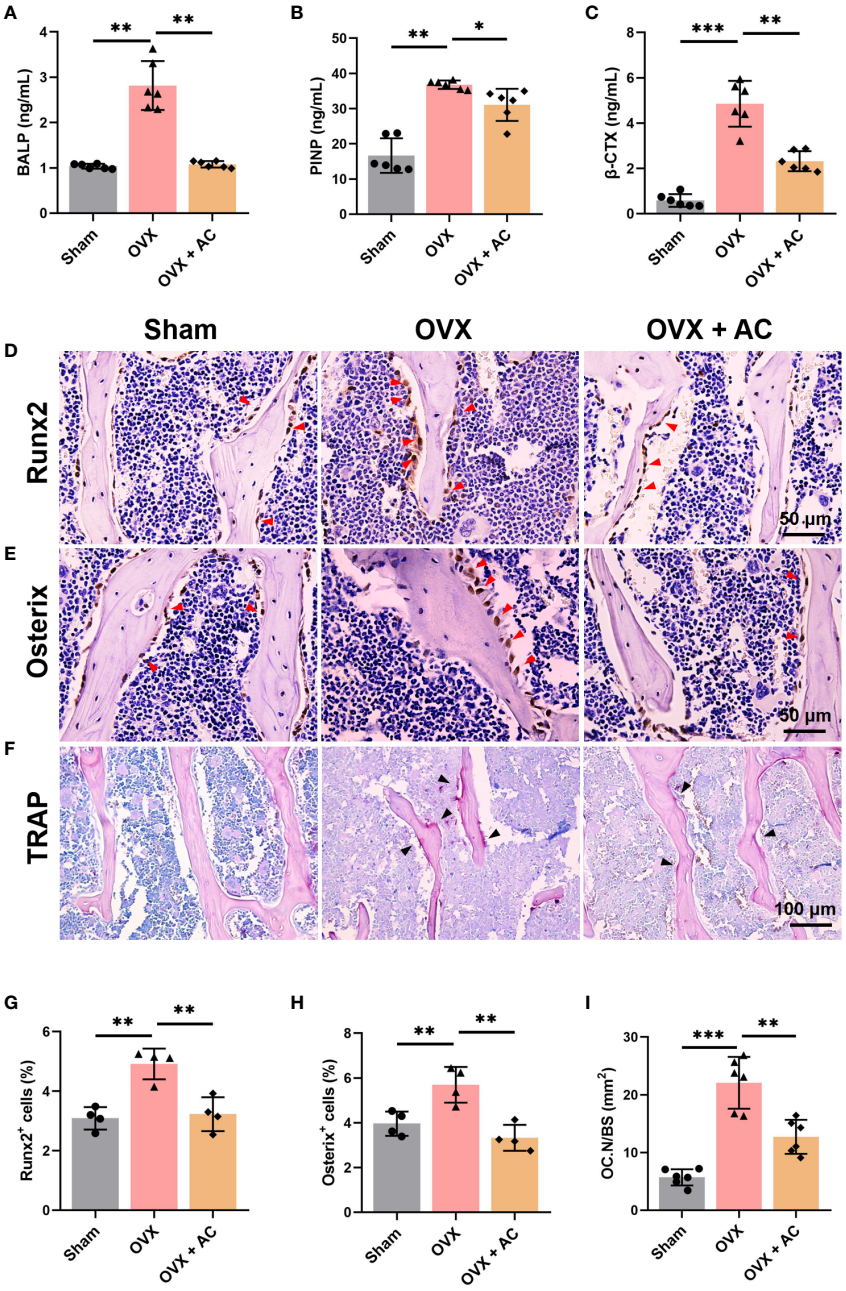

FIGURE 2
AC reverses the high bone turnover makers and remodels the osteoporotic pathophysiological microenvironment in OVX mice. Serum levels of bone formation markers of **(A)** bone-specific alkaline (BALP) and **(B)** N-terminal propeptide of type I collagen (PINP). **(C)** Serum β-CTX levels of the bone resorption marker. n = 6. IHC staining of **(D)** Runx2 and **(E)** Osterix in the lumbar vertebrae of each group. The red arrows represented the positive expression. **(F)** Representative of TRAP staining among groups. The black arrows represented the TRAP-positive cells. **(G, H)** Quantification of Runx2$^+$ and Osterix$^+$ cells. n = 4. **(I)** The ratio of the number of osteoclasts to per unit bone surface (OC.N/BS). n = 6. Data presented as means ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001.

AC represses osteoclast-specific gene expressions

Postmenopausal OP is caused by osteoclast-mediated bone resorbed surpassing osteoblast-mediated bone formed, leading to an imbalance in bone remodeling (27). Thus, we further assessed the changes in osteoclast function by determining the expression levels of osteoclast differentiation markers, including c-Fos, NFATc1, Cathepsin K, and Mmp9, using IHC staining. We found that OVX modeling upregulates the expression of c-Fos, NFATc1, Cathepsin K, and Mmp9 in the L5 vertebrae, and these increases were partially suppressed by treating OVX mice with AC (Figures 3A–H). There is evidence that osteoprotegerin (OPG) acts as a negative regulator of osteoclast formation by competing

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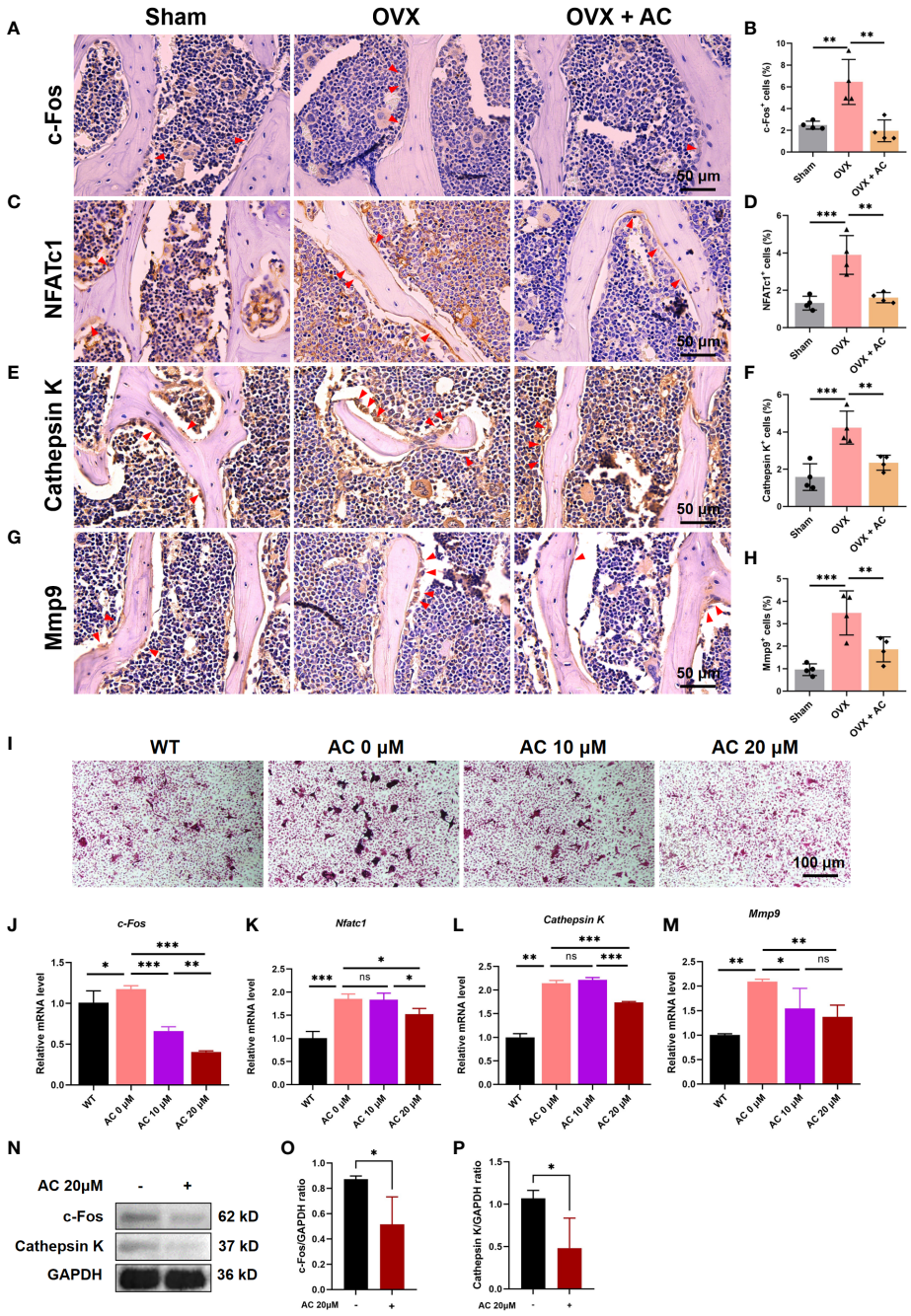

FIGURE 3
AC inhibits osteoclast formation and represses the expressions of osteoclast-specific genes. **(A–H)** IHC staining and quantitative analysis of c-Fos, NFATc1, Cathepsin K, and Mmp9 in the lumbar vertebrae among groups. Scale bar: 50 μm. n = 4. **(I)** BMMs were isolated from WT or *Opg* KO mice and were treated with different concentrations of AC (0, 10, or 20 μM) followed by osteoclast-inducing media with the M-CSF (200 ng/mL) and RANKL (200 ng/mL) stimulation until the osteoclasts differentiated in the control group. The osteoclast formation among groups was detected by TRAP staining. n = 3. **(J–M)** Expression levels of the osteoclastic-specific gene of *c-Fos*, *Nfatc1*, *Cathepsin K*, and *Mmp9*, respectively. Data presented as means ± s.d. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. n = 3. **(N)** BMMs were isolated from WT mouse tibiae and femora and were cultured with osteoclast-stimulating medium (200 ng/mL M-CSF and 200 ng/mL RANKL) to stimulate osteoclast formation in the presence or absence of 20 μM AC subjected to 48 h incubations. The protein expression levels of c-Fos and Cathepsin K were determined by Western blot. GAPDH was used as an internal control. **(O)** The levels of c-Fos protein were normalized to that of GAPDH. **(P)** The levels of Cathepsin K protein were normalized to that of GAPDH.

for the binding between the receptor activator of NF-κB (RANK) and its ligand (RANKL) (28, 29). Our and others' research found that knockout of *Opg* resulted in severe OP phenotype with higher RANKL and osteoclast generation (30, 31). Thus, we cultured the bone marrow cells from *Opg* KO mice treated with different AC (0, 10 or 20 μM) to observe the effect of AC on osteoclast formation. Expectedly, AC treatment markedly inhibited osteoclastogenesis in a dose-dependent manner (Figure 3I), and AC at a high dose (20

μM) effectively downregulate their overexpression of *c-Fos*, *Nfatc1*, *Cathepsin K* and *Mmp9* in osteoclasts (Figures 3J–M). To further investigate the effect of AC on osteoclast formation, BMMs were isolated from WT mouse tibiae and femora and were cultured with osteoclast-stimulating medium in the presence or absence of 20 μM AC subjected to 48 h incubations. As expected, AC demonstrated a significant inhibitory effect on the key regulators of osteoclast formation such as c-Fos and Cathepsin K (Figures 3N–P). Moreover, to exclude the possibility that the observed inhibitory effect of AC on osteoclastogenesis might be due to cytotoxicity, the viability of mouse leukemic monocyte/macrophage cell line RAW264.7 cells were treated with or without AC concentrations of 10 and 20 μM for 24 h. The CCK assay results revealed no significant variations in cell activity between AC 0, 10 and 20 μM (Supplemental Figure 2), demonstrating that AC had no obvious cytotoxic effect on osteoclast precursor cells at the concentrations used in this study. These findings reveal that AC treatment inhibits the key regulators of osteoclastogenesis, hence preventing excessive bone absorption.

AC inhibits ferroptosis of osteoclasts in OVX mice

Emerging evidence has demonstrated that ferroptosis was involved in RANKL-mediated osteoclastogenesis *in vitro* and *in vivo* (15). Therefore, we evaluated the effect of AC on osteoclast ferroptosis by detecting the key anti-ferroptosis protein, Gpx4, and a critical pro-ferroptosis marker, Acsl4. As determined *in vitro*, AC exhibited a promotive effect on the expression of critical anti-ferroptosis factors Gpx4, while concurrently suppressing the expression of the pro-ferroptosis protein Acsl4 in osteoclast (Figures 4A–C). Consistent with the effect of AC against ferroptosis-related key factors observed in BMMs, IHC staining showed that AC reversed the decreased expression of Gpx4 and enhanced expression of Acsl4 of osteoclasts in OVX mice (Figures 4D–G). These data imply that AC may have an effect on regulation for key genes of ferroptosis in osteoclast.

AC suppresses the activation of the NF-κB signaling pathway in osteoclasts

It has been reported that osteoclast formation and function are dependent on the NF-κB signaling pathways (32). To further explore the molecular mechanisms underlying AC treatment against osteoclast formation, we performed bioinformatics analysis of mRNA transcriptomes. As shown in Figure 5A, the heatmap illustrated the top 50 differentially expressed genes within up-and-down gene expressions in the AC group as compared to the DMSO group. KEGG analysis found that NF-κB signaling pathway were enriched in terms of differentially expressed genes within AC and DMSO groups (Figure 5B). To confirm the effect of NF-κB signaling on osteoclast formation or ferroptosis subsequent to AC therapy, BMM cells were pretreated with 10 ng/mL recombinant NF-κB for 2 h followed by osteoclast-stimulating medium in the presence of 20 μM AC for another 24 h. We found that recombinant NF-κB protein treatment not only reversed the effect of AC on the expression of Gpx4 and Acsl4, but also increased the c-Fos expression, which had been inhibited by AC treatment (Figures 5C–G). Next, to gain a more comprehensive understanding of AC on regulation of NF-κB signaling transduction, the expression of p65 nuclei translocation in the L5 lumbar vertebra of each group was detected by IF staining. It can be seen that the nuclei levels of p65 were markedly elevated in OVX mice, however, the upregulation of p65 levels could be well inhibited by AC (Figures 5H, I). We then further investigated the expression of p-p65 and p-I-κB in L5 vertebrae using IHC staining. The results showed that the expression of p-p65 and p-I-κB in the osteoclast of OVX mice were both increased as compared to the sham group, whereas AC-treated OVX mice exhibited a decrease in the levels of p-p65 and p-I-κB (Figures 5J–M). Collectively, these results suggest that the inhibitory effects of AC on osteoclast formation and osteoclast-mediated ferroptosis are dependent on the activity of NF-κB signaling.

AC treatment present a good biosafety for OVX-induced OP modeling

At the endpoint, the *in vivo* toxicity of AC was investigated by measuring the levels of hepatotoxicity markers (AST, ALT) and nephrotoxicity markers (BUN, Cr) in each group. Relative to the sham group, there was no discernible changes in the serum of AST, ALT levels and BUN, Cr levels after treatment with AC versus the OVX without AC treatment group (Figures 6A–D). Therefore, AC treatment presents a good biosafety for OP therapy.

Discussion

During the development of OP, the activation of osteoclasts has the capacity to induce excessive bone resorption, leading to the deterioration of bone density and alterations in the microarchitecture of bone (33). Therefore, targeting the inhibition of osteoclasts activity is a promising therapeutic approach for addressing OP. AC, a non-toxic component of *Aconiti Lateralis Radix Preparata*, has been found to inhibit the RANKL-induced osteoclast formation and function in pre-osteoclastic RAW264.7 cells (19). However, there is limited knowledge regarding the impact of AC on bone loss and OP progression *in vivo*. In this study, we have successfully shown, for the first time, AC could reduce bone loss and inhibit the elevation of bone turnover markers in mice subjected to ovariectomy. Additionally, AC treatment was found to effectively lower both the numbers and activity of osteoclasts. Specifically, AC could prevent ferroptosis in osteoclasts by increasing Gpx4 and decreasing Ascl4, which is mediated by the inhibition of NF-κB activity (Figure 7). These findings elucidate a new potential perspective to the understanding of the mechanisms by which AC contributes to preventing bone loss in OP modeling.

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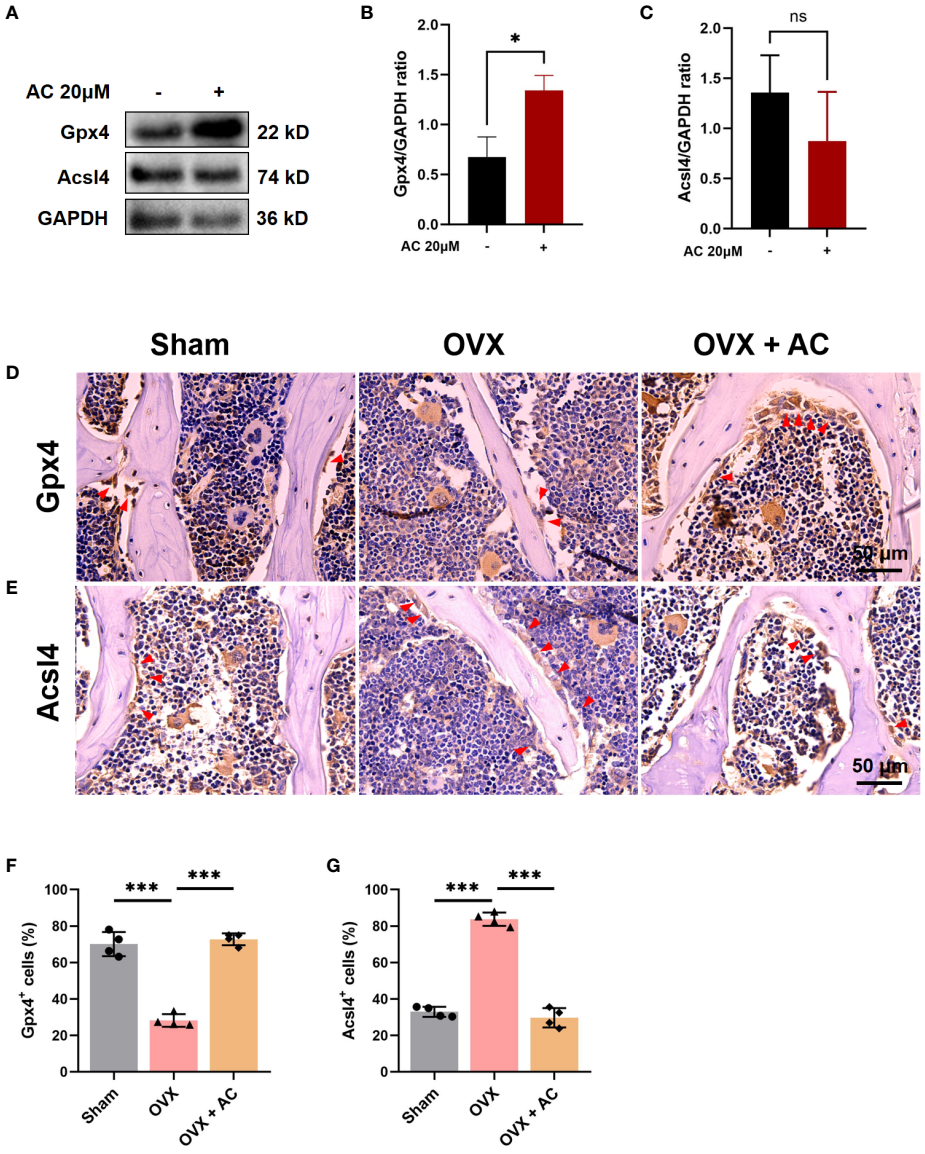

FIGURE 4
AC reverses the osteoclast ferroptosis in OVX mice. **(A)** BMMs, isolated from WT, were cultured with osteoclast-stimulating medium (200 ng/mL M-CSF and 200 ng/mL RANKL) to induce osteoclast formation in the presence or absence of 20 μM AC subjected to 48 h incubations. The protein expression levels of Gpx4 and Acsl4 were determined by Western blot. GAPDH was used as an internal control. **(B)** The levels of Gpx4 protein were normalized to that of GAPDH. **(C)** The levels of Acsl4 protein were normalized to that of GAPDH. **(D)** IHC staining of Gpx4 in the lumbar vertebrae of each group. Scale bar: 50 μm. **(E)** IHC staining of Acsl4 in the lumbar vertebrae among groups. Scale bar: 50 μm. **(F)** Quantitative analysis of Gpx4-positive cells in each group. **(G)** Quantitative analysis of Acsl4-positive cells in each group. The red arrows represented the positive cells. Data presented as means ± s.d. $^{*}P < 0.05$, $^{***}P < 0.001$. n = 4.

During the menopausal phase, there is a notable rise in bone turnover and an expedited decline in bone density, resulting in an average loss of 11% in spinal bone density over the course of 10 years following menopause (34). Accumulating evidence has proven that high level of bone turnover correlates with increased biochemical indicators of both bone formation and bone resorption (35, 36). BALP, a well-established and dynamic marker of osteoblasts, plays a crucial role in bone formation and serves as a reliable measure of osteocyte formation and activity levels (37). The concentrations of serum PINP serve as an indicator of the cumulative quantity of new bone production in the skeletal system (38). β-CTX is a metabolic marker for representing osteoblast activity (34). Our result showed that the L5 vertebrae of OVX mice experienced a significant reduction in spinal bone mass, amounting to a loss of nearly 40%. Additionally, we observed elevated levels of BALP and PINP as well as an increased concentration of β-CTX in serum. suggesting a state of heightened bone turnover in OVX mice. In line with the findings of bone metabolic markers, OVX mice was shown that the expression levels of Runx2 and Osterix, which are key transcription factors involved in the differentiation of osteoblasts, were found to be elevated and meanwhile an increase in osteoclasts

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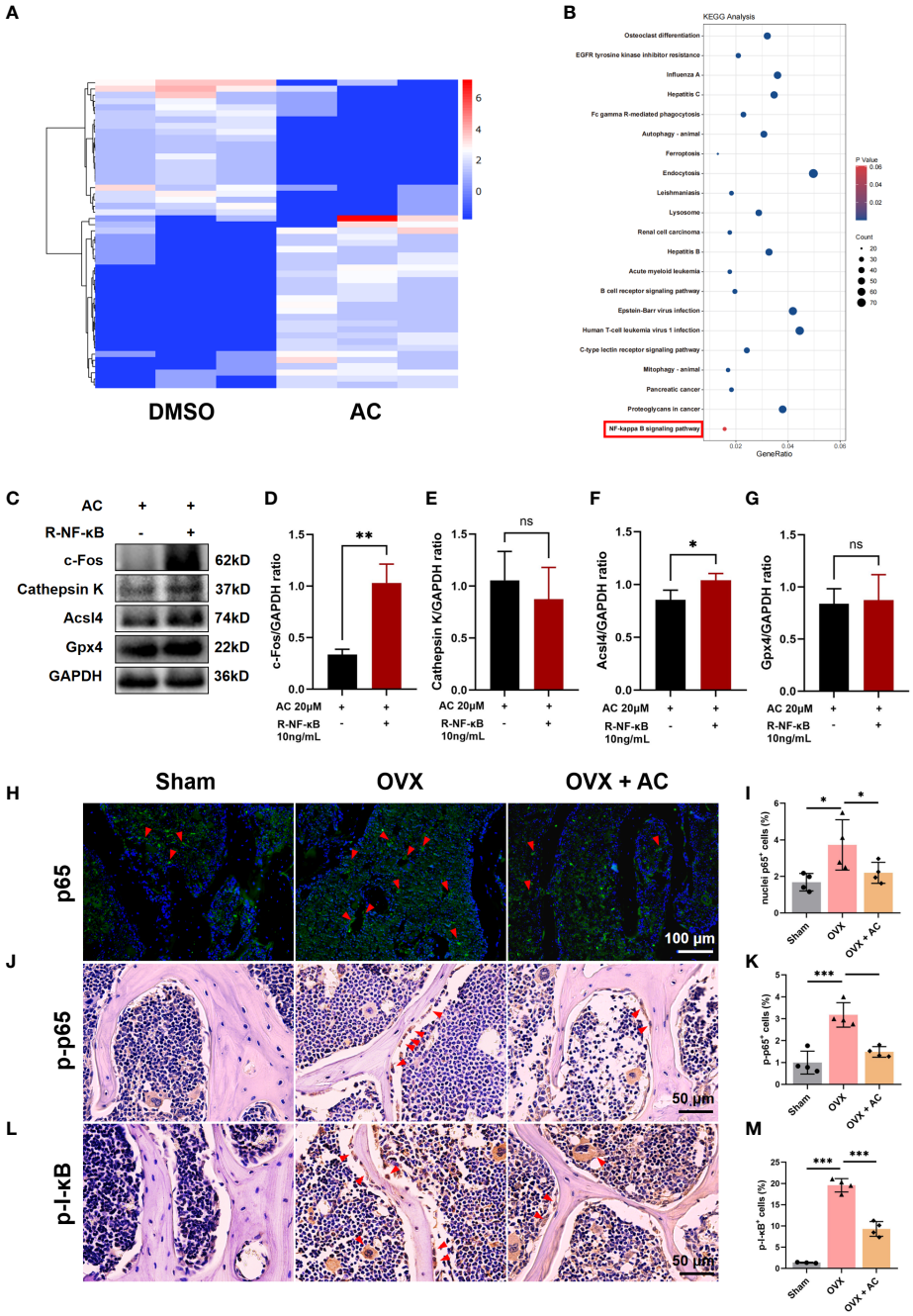

FIGURE 5
AC suppresses the NF-κB signaling pathway in osteoclasts. **(A)** The top 50 differentially expressed genes within up-and-down gene expressions were presented by heatmap analysis. **(B)** The KEGG analysis of differentially expressed genes between the AC and DMSO groups. **(C)** BMM cells were pretreated with 10 ng/mL recombinant NF-κB for 2 h followed by osteoclast-stimulating medium in the presence of 20 μM AC for another 24 h. Effect of recombinant NF-κB on expression of c-Fos, Cathepsin K, Acsl4 and Gpx4 proteins in AC-treated cells. GAPDH was used as an internal control. **(D–G)** Quantification of the expression levels of c-Fos, Cathepsin K, Acsl4 and Gpx4 proteins in **(C)**. **(H)** p65 nuclei translocation was presented by IF staining. Scale bar: 100 μm. **(I)** The relative percentage of p65 nuclei-positively stained cells to the total number of cells. **(J)** The expression of p-p65 in the lumbar vertebrae of each group. Scale bar: 50 μm. **(K)** Quantification of p-p65^{+} cells in each group. **(L)** The expression of p-I-κB in the lumbar vertebrae of each group. Scale bar: 50 μm. **(M)** Quantitative analysis of p-I-κB^{+} cells in each group. The red arrows represented the positive cells. Data presented as means ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001. n = 3 - 4.

number was detected through TRAP staining. However, AC effectively restored bone loss, reduced rapid bone turnover and reversed the upregulated state of both bone formation and bone resorption. These observations collectively suggest that AC could regulate bone homeostasis by remodeling the OVX-induced osteoporotic microenvironment.

It is hypothesised that the inhibitory effect of AC on osteoclast function may outweigh its impact on bone production, resulting in

protection against bone loss. Prior studies have revealed that OVX mice exhibit an abnormally high expression of osteoclasts-specific markers, such as NFATc1, c-Fos, Mmp9, and Cathepsin K (39, 40), Furthermore, the increased expression of genes linked with osteoclasts relies on the activation of NF-κB signaling (41–43). c-Fos is a critical factor for NFATc1 activation, and NFATc1 functions as the most transcription factor responsible for regulating the expression of osteoclast-specific genes, including Mmp9 and Cathepsin K (44). As expected, AC not only decreased NF-κB activity but attenuated the overexpression of NFATc1, c-Fos, Mmp9, and Cathepsin K in OVX mice. Additionally, the *in vitro* experiments showed a substantial anti-osteoclast effect in AC. These findings suggest that AC may effectively inhibit osteoclast activity by inactivating NF-κB signaling.

The NF-κB signaling pathway is involved in regulating several pathogenic processes (45, 46). Growing investigations have found that NF-κB signaling can modulate Gpx4-mediated ferroptosis in tumor cells (47, 48). Ferroptosis, a novel type of cell death mainly characterized by decreased activities of Gpx4 and cellular lipids composition by positive regulation of Acsl4 (49, 50), suggesting promoting Gpx4 or inhibition of Acsl4 may prevent ferroptosis (51). A current study used a RANKL-stimulated osteoclastogenesis assay to reveal that ferroptosis was involved in osteoclasts over the course of RANKL-induced differentiation (15). However, whether NF-κB signaling is associated with ferroptosis in osteoclast is largely elusive. Our results indicate that during the process of M-CSF/RANKL-induced osteoclastogenesis, the administration of AC treatment not only effectively suppressed the upregulation of c-Fos and Cathepsin K, which are crucial regulators of osteoclast formation, but also restored the abnormal expression of Gpx4 and Acsl4, two vital proteins involved in ferroptosis. These observed effects on inhibiting osteoclastogenesis and ferroptosis were found to be dependent on the activity of NF-κB signaling. These results suggest that NF-κB signaling pathway may play a critical role in regulating ferroptosis in osteoclasts and inhibition of NF-κB activity to modify osteoclast-mediated ferroptosis could be a valuable approach in the treatment of OP.

Besides, our study has certain limitations. *In vivo* experiments, we exclusively employed a singular dose concentration of AC, hence resulting in a dearth of comparative analysis including different concentrations of AC. Moreover, this study primarily identified the principal regulators of Gpx4 and Acsl4 in relation to ferroptosis, other the molecular alterations associated with AC targeting in the context of ferroptosis are needed for further investigation. Hence, in further study, we will set concentration gradients to treating OP models and perform *in vitro* osteoclastogenesis assay to further explore the ferroptosis phenotype in osteoclasts, this will enable us

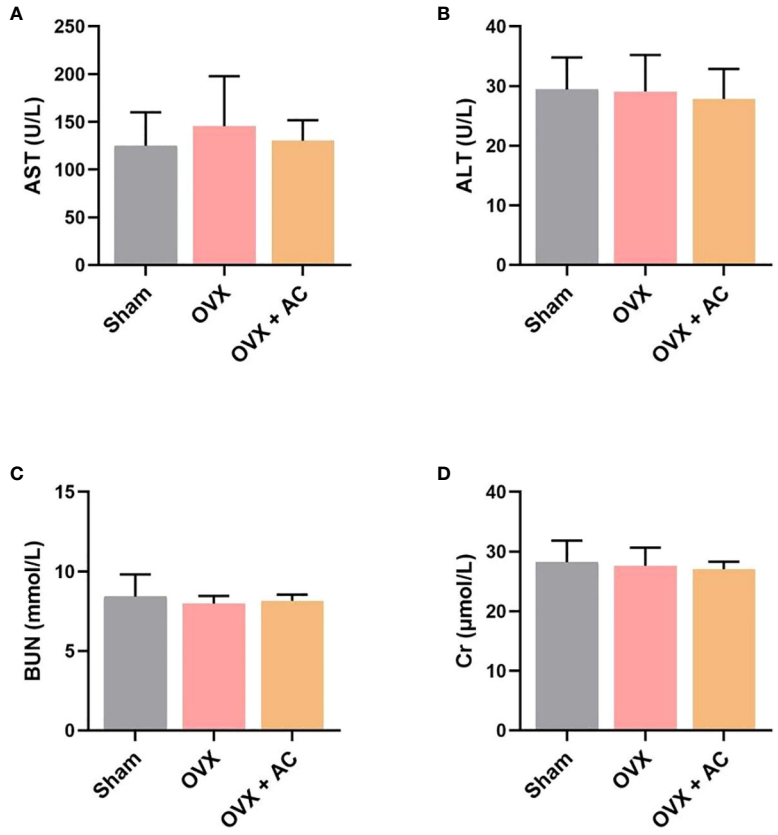

FIGURE 6
Effect of AC on chronic toxicity in OVX mice. Evaluation of the hepatotoxicity and nephrotoxicity of AC treatment by measuring the serum levels of **(A)** AST, **(B)** ALT, **(C)** BUN and **(D)** Cr. Data presented as means ± s.d., n = 4 - 6.

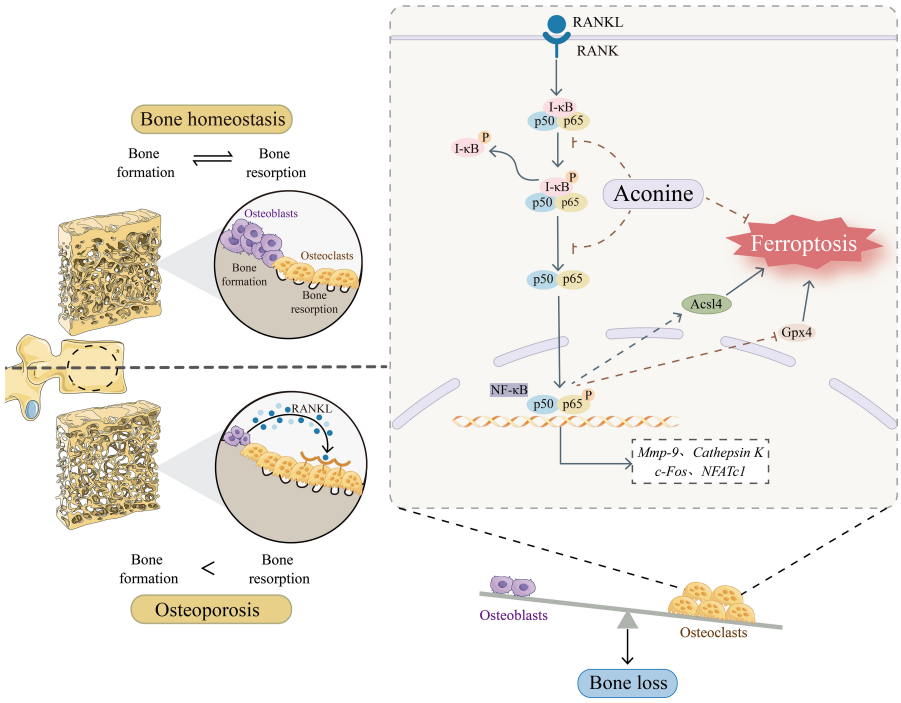

FIGURE 7
Schematic representation of the mechanism of AC treatment in OP.

to enhance the logical understanding of anti-osteoclast and anti-ferroptosis in osteoclasts mediated by AC in the treatment of OP.

Conclusion

In conclusion, our study demonstrated that AC, a natural product, could block the high concentration of bone turnover markers and remodel the OVX-induced osteoporotic microenvironment by inhibiting osteoclast formation and bone resorption function, thereby ameliorating OVX-induced OP phenotype in mice. Moreover, AC inhibited osteoclast ferroptosis by regulating the Gpx4 and Acsl4 expression via suppressing NF-κB signaling. These findings suggest that AC has the anti-resorptive and anti-ferroptosis properties, making it an excellent potential treatment option for postmenopausal OP.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

Ethics statement

The animal study was approved by Animal Experiments Ethical Committee of Shanghai Municipal Hospital of TCM. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

XL conceived and designed the experiments. CX and HL performed the experiments and drafted the manuscript. LW performed the *in vitro* experiments and analyzed the data. QD, WK, WWD, LC, SL, YX, JY. LL, and WLD contributed to data collection. WK drafted the scheme of this study. XL takes responsibility for the integrity of the data analysis. QS supervised the work. All authors approved the final manuscript. All authors contributed to the article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1234563/full#supplementary-material

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Targeting cellular senescence in senile osteoporosis: therapeutic potential of traditional Chinese medicine

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Senile osteoporosis (SOP) is a prevalent manifestation of age-related bone disorders, resulting from the dysregulation between osteoblast (OB)-mediated bone formation and osteoclast (OC)-mediated bone resorption, coupled with the escalating burden of cellular senescence. Traditional Chinese medicine (TCM) herbs, renowned for their remarkable attributes encompassing excellent tolerability, low toxicity, heightened efficacy, and minimal adverse reactions, have gained considerable traction in OP treatment. Emerging evidence substantiates the therapeutic benefits of various TCM formulations and their active constituents, including Zuogui wan, *Fructus Ligustri Lucidi*, and Resveratrol, in targeting cellular senescence to address SOP. However, a comprehensive review focusing on the therapeutic efficacy of TCM against SOP, with a particular emphasis on senescence, is currently lacking. In this review, we illuminate the pivotal involvement of cellular senescence in SOP and present a comprehensive exploration of TCM formulations and their active ingredients derived from TCM, delineating their potential in SOP treatment through their anti-senescence properties. Notably, we highlight their profound effects on distinct aging models that simulate SOP and various senescence characteristics. Finally, we provide a forward-looking discussion on utilizing TCM as a strategy for targeting cellular senescence and advancing SOP treatment. Our objective is to contribute to the unveiling of safer and more efficacious therapeutic agents for managing SOP.

KEYWORDS

senile osteoporosis, traditional Chinese medicine, cellular senescence, anti-senescence, therapeutic efficacy

Introduction

Osteoporosis (OP) is a prevalent metabolic bone disorder that affects individuals worldwide with a substantial incidence rate, primarily characterized by reduced bone mass, heightened bone fragility, and deterioration of microstructural bone tissues (1). Given its high prevalence among the elderly population, senile osteoporosis (SOP) has emerged as a significant global health concern (2). In China, the incidence of OP has risen dramatically, affecting approximately 110 million individuals in the past four decades (3). This prevalent condition poses a significant health risk to the elderly population, necessitating extensive resources for treatment, nursing, and management of SOP, osteoporotic fractures, and associated complications. Consequently, it places a substantial burden on families and society, both in terms of labor and financial resources. Beyond the imbalance between osteoblast (OB)-mediated bone formation and osteoclast (OC)-mediated bone resorption, research findings indicate that the aging process leads to a disproportionate differentiation of bone marrow mesenchymal stem cells (BMSCs) into adipocytes rather than OB. Furthermore, these cells undergo senescence, resulting in bone loss and contributing to the SOP development (4, 5). Hence, targeting fundamental aging mechanisms such as senescence represents a promising avenue for SOP treatment. Although several medications, such as teriparatide, risedronate, and romosozumab, are currently available for SOP treatment, their limited effectiveness is hampered by potential side effects (6–8).

Traditional Chinese medicine (TCM) has garnered wide utilization for the treatment of various diseases, including SOP, due to its minimal adverse effects (9–12). Recent basic research has discovered the anti-senescence effects of numerous TCM formulations (such as the Yiqi Huayu decoction, Bazi Bushen formulations) and their active ingredients (Ginsenoside Rb2) (13–16). Moreover, emerging evidence demonstrates the applicability of TCM and its derivative compounds in treating SOP through their anti-senescence effects (17–19). Nonetheless, a comprehensive review encompassing the treatment of OP, particularly SOP, using TCM with a focus on targeting cellular senescence is currently lacking. To comprehensively understand the anti-senescence mechanisms by which TCM exerts its therapeutic effects on SOP, it is essential to gather evidence from both *in vivo* and *in vitro* studies. Firstly, it is necessary to elucidate the effects of TCM on various animal models of SOP induced by aging to gain insights into its therapeutic potential. Furthermore, after demonstrating the therapeutic effects of TCM on SOP induced by aging *in vivo*, it is essential to delve deeper into the regulatory effects of TCM on senescence characteristics during the treatment of SOP *in vitro*.

Within this review, we delineate the significance of cellular senescence in the context of SOP and provide a comprehensive overview of recent advancements involving TCM formulations and active ingredients derived from TCM that can be harnessed to treat SOP by leveraging their anti-senescence property. Specifically, we highlight the profound effects of TCM on different aging models employed to simulate SOP. Additionally, we recapitulate the therapeutic effect of TCM on SOP by modulating various senescence characteristics, such as oxidative stress, p53, p21, p16, and the senescence of BMSCs. Finally, we present prospects for SOP treatment using TCM by targeting cellular senescence and developing potential therapeutic drugs.

Cellular senescence in SOP

Cellular senescence is a vital process of irreversible cell cycle arrest that is instrumental in tissue remodeling during development and post-injury (20). It occurs in response to various stresses, resulting in DNA damage and the secretion of chemokines, cytokines, and extracellular matrix proteins, which creates a detrimental microenvironment known as senescence-associated secretory phenotype (SASP) (21). Besides, activation of the p53/p21 and p16/pRB tumor suppressor pathways assumes a central role in cellular senescence (22). These pathways, recognized as core markers of senescence, have been observed not only in aged mice but also in aged human bones (23). Accumulation of senescent cells accompanies the aging process and has been linked to the promotion of various age-related diseases, including SOP (24–26). Notably, Farr et al. demonstrated that eliminating senescent cells can prevent age-related bone loss in mice (26). Thus, cellular senescence plays a critical role in the development of SOP. The mechanism through which cellular senescence induces SOP is shown in Figure 1.

Since the development of OP is thought to be caused by increased OC activity, decreased OB activity, compromised osteocyte viability, and reduced osteogenic potential of BMSCs (27–29), the senescence of dysfunctional OB, BMSCs, and osteocytes, significantly impact the pathological progression of SOP (30–33). These senescent cells display senescent-like characteristics such as impaired proliferation, reduced lifespan, shortened telomeres, and increased production of reactive oxygen species (ROS). Given the close relationship between cellular senescence and aging-related diseases, including SOP, various animal models of aging, such as D-galactose (D-gal)-induced mice and senescence-accelerated mice/prone (SAMP) mice, have been established to elucidate the mechanisms underlying cellular senescence in SOP (34, 35).

Recent evidence has shed light on the significant relevance of senescent OB, BMSCs, and components of the SASP to SOP. D-gal has been found to trigger OB senescence through excessive nonmetabolized D-galactitol accumulation, leading to bone senescence and SOP (36). In p21-expressing senescent OB, upregulated HIF-2α expression hinders OB differentiation and function. On the other hand, upregulated HIF-2α expression in OC promotes the transcription of p16 and p21 by directly binding to hypoxia-responsive elements. This interplay between HIF-2α and p16/p21 in senescent OB and OC contributes to age-related bone loss and SOP (37). Studies also have shown that estrogen deficiency could induce bone loss and promote OB senescence, while estrogen administration effectively alleviates the senescence of OB, preserves their function, and accelerates p53 degradation in osteoblastic MC3T3-E1 cells (38–40). Additionally, senescent OB can modulate the function of endothelial cells, induce cellular senescence and apoptosis, and hinder cell proliferation through the exosomal pathway, thus affecting the bone environment during aging through the alterations of angiogenesis (41). The acquisition of SASP in both osteogenic and myeloid lineage cells may contribute to the

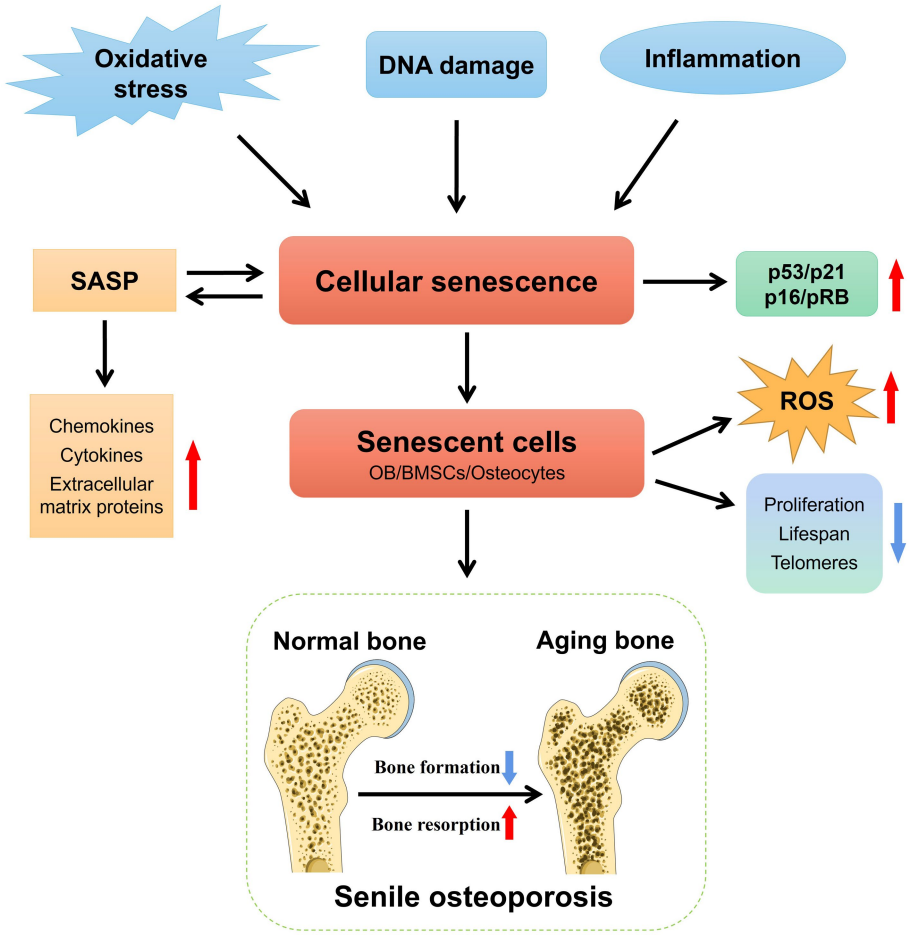

FIGURE 1
The mechanism underlying cellular senescence-induced SOP. The senescence of OB, BMSCs, and osteocytes plays a crucial role in the pathogenesis of SOP. Various interconnected factors, including heightened oxidative stress, DNA damage, chronic inflammation, and SASP, actively participated in the induction and progression of bone cell senescence in SOP. Red arrows indicate upregulated levels of these molecule entities, while blue arrows denote downregulated levels.

accelerated skeletal aging phenotype characterized by impaired osteogenesis (42).

Likewise, Geng et al. demonstrated that increased osteocyte senescence and SASP induced by estrogen deficiency accelerate bone loss (32), highlighting the potential role of senescence in estrogen deficiency-induced SOP. Furthermore, the removal of osteocytes leads to the accumulation of SASP in bone marrow osteoprogenitors and cells within the myeloid lineage, further boosting the OC formation while inhibiting osteogenesis, consequently resulting in an accelerated skeletal aging phenotype accompanied by OP (42).

According to a toxicology study, cadmium-induced cellular senescence of BMSCs through the activation of the NF-κB signaling pathway has been found to substantially impact the osteogenic differentiation and predispose BMSCs to adipogenesis, leading to an observable increase in the number of adipocytes and a decrease in mineralization within the bone marrow in Sprague–Dawley rats (43). Other studies have revealed that increased cellular senescence of BMSCs induced by diverse factors, including high-glucose concentrations, oxidative stress, and inflammation micro-environment, compromises the osteogenic differentiation ability of BMSCs and contributes to SOP (44–47). Furthermore, it has been shown that epigenetic modifications, include DNA methylation, histone modifications, chromatin remodeling and posttranscriptional processing through mRNA and noncoding RNAs, play a significant role in regulating BMSCs senescence (48). For instance, a decrease in the expression of lncRNA-*Bmncr* in senescent BMSCs has been associated with an increase in lipogenesis (49), while the suppression of H3K27me3 at the promoter of *p14* and *p16* leads to the activation of corresponding proteins, further exacerbating BMSCs senescence (50). These findings demonstrate the strong involvement of the epigenetic component in senescence-related OP.

Given the significant influence of senescent cells on bone homeostasis, the therapeutic targeting of cellular senescence in SOP has garnered considerable attention. Research into anti-senescence drugs for SOP has intensified, with substances such as Genistein, Lactoferrin, Resveratrol, and Angiotensin 1–7 being investigated (51–54).

TCM formulations and bioactive ingredients with anti-senescence properties in different aging models for treating SOP

TCM herbs have diverse properties and therapeutic qualities that have been utilized for the effective management of OP (55). Extensive research has highlighted the significant role of senescence in OP, particularly in the context of SOP, therefore, we compiled a summary of the anti-osteoporotic effects of TCM formulations and their active ingredients by using different aging models including naturally aging model, SAMP mice, and D-gal-induced aging model. These findings enhance our understanding of how TCM regulates senescence during SOP treatment, thereby facilitating further research and practical application in clinical settings.

Naturally aging model

SOP is known to become increasingly prevalent with aging, so animal models of natural aging serve as valuable tools to simulate the aging process and age-related diseases, including SOP, in the human body. Notably, increasing studies have demonstrated the anti-osteoporotic effects of TCM in animal models of natural aging.

Hu et al. found that administration of Xianzhen Gubao (XZGB) capsules to corticosteroid-induced aging rats for a period of 90 days resulted in a marked increase in trabecular area by suppressing bone resorption and a slight increase in bone formation, and higher doses of XZGB were found to yield greater effects (56). A study using metabolomics investigating Fufang Zhenzhu Tiaozhi (FTZ)'s protective effects against aging-induced OP showed that FTZ can reverse abnormal levels of metabolites associated with phospholipids and arachidonic acid metabolism, as well as energy metabolism in 21-month-old female mice when compared to the aging model group (57). Leptin (LEP), a peptide hormone synthesized and secreted by adipocytes, has been identified as playing a crucial role in the regulation of bone metabolism (58). Recent research has demonstrated that Qing'e Decoction (QED) treatment for 3, 6, and 9 months in naturally aging rats can reduce elevated levels of LEP in the serum, thus helping to regulate bone metabolism, maintain trabecular structure and bone quality, delay bone aging, and prevent OP (59).

Fructus Ligustri Lucidi (FLL), derived from the fruit of TCM *Ligustrum lucidum Ait*, is a component of numerous TCM formulations used in OP treatment. The combination administration of *Epimedium leaf* (EF) and FLL for 2 months has demonstrated beneficial effects by enhancing the expression of TGF-β1, BMP2, Wnt5a, and IGF-1 while reducing the increase of bone tissue adipocytes in 15-month-old male rats, thus ameliorating low bone mass associated with aging. These findings suggest that the combination of EF and FLL could serve as a potential alternative for treating SOP (60). In a study involving eight-month-old Kunming female mice, FLL did not show a therapeutic effect on aged mice with OP in terms of femur index, fecal Ca, and BMD value due to irreparable bone damage. Interestingly, it demonstrated a protective effect against OP and reduced damage in a model combining D-gal injection with a low calcium diet-induced SOP (61), highlighting the importance of early intervention in TCM-based SOP treatment.

Moreover, preclinical evidence of long-term Allicin treatment in aging male rats (ranging from 13 to 21 months) reveals it can provide protection against SOP and reverse deleterious bone biomechanical features associated with aging through the elevation of both bone formation and bone resorption, as well as an increase in bone mineral density (BMD), indicating Allicin may potentially delay the onset of OP (62). Oleanolic Acid (OA), a pentacyclic triterpenoid compound found in over 1,620 plants and medicinal herbs, has shown the ability to induce osteogenic differentiation of BMSCs (63), and effectively improve bone microarchitecture in aged rats (9-month-old) by improving calcium balance and modulating Vitamin D metabolism, making OA a potential drug candidate for the treatment of SOP (64).

SAMP mice

SAMP mouse model has been widely recognized as a valuable spontaneous experimental model to study age-related secondary OP (35), with numerous characteristics that resemble SOP in humans, including low peak bone mass and impaired OB formation (65), making them highly suitable for studying the therapeutic effects of various TCM interventions and their active components on SOP.

Zhao et al. found that *Eclipta prostrata* significantly improved bone microstructure in SAMP6 mice by regulating the dynamic balance of bone absorption and formation, while it also markedly increased the abundance of bacteria genera Lactobacillus and Lactococcus, suggesting that targeting gut microbiota might represent a novel treatment approach for SOP (66). Similarly, the combination of *Eucommia ulmoides* leaf water extract (EUL) and *Lactobacillus bulgaricus* (LB) has been proven to improve osteoporotic manifestations in SAMP6 mice, including reduced trabecular bone, increased intertrochanteric space, and decreased BMD. This combination treatment also regulates the diversity of gut microbiota (GM) to promote skeletal health (18). In parallel, a comparative study of SAMP6 mice explored the effects of 3 TCM formulations (Hachimi-jio-gan, Juzen-taiho-to, and Unkei-to) on bone loss in SOP disorder found that Hachimi-jio-gan and Juzen-taiho-to treatment could, respectively, decrease the number of mast cells in the bone marrow and serum parathyroid hormone levels while increasing the amount of bone-forming surface and the bone mass (67). Furthermore, Bu-Gu-Sheng-Sui decoction (BGSSD) accelerated the proliferation and differentiation of BMSCs and improved bone trabecular structure to protect bone mass in SAMP6 mice by stimulating the activity of Alkaline phosphatase (ALP) and increasing the expression of Runx2, suggesting BGSSD may constitute a potential drug for preventing and managing SOP by targeting cellular senescence (68). Another study conducted by Liu et al. evaluated the therapeutic potential of Antrodia camphorate alcohol extract (ACAE) for SOP recovery in SAMP8 mice and demonstrated ACAE could upregulate the level of osteogenic genes such as *RUNX2*, *OCN*, and *OPN in vitro* and inhibit bone loss as well as increase the percentage bone volume, trabecular bone number, and BMD *in vivo*, thereby promoting osteogenesis and preventing SOP (69). Another study on total glycosides and polysaccharides of *Cistanche deserticola* in SAMP6 mice exhibited improvements in decreased bone formation, damaged bone microstructure, and

regulation of RANKL, BMP-2, and OPG expression by activating the Wnt/β-catenin signaling pathway (19).

Moreover, Xu et al. revealed that OB from SAMP6 mice exhibited lower cellular development and differentiation activity compared to OB from normal aging mice (SAMR1). The expression of Connective tissue growth factor (CTGF) was also found to be lower in SAMP6 mice. Furthermore, it was demonstrated that Icariin, a flavonoid compound derived from *Epimedium brevicornum*, can activate the BMP signaling pathway and downregulate CTGF expression to enhance BMP-2-induced OB differentiation (70). Intragastrical administration of *Astragalus membranaceus* (AM, also known as Huangqi) to SAMP6 mice could improve the femoral BMD and bone microstructure, elevate the calcium and phosphorus contents, and increase the expression of Klotho, Vitamin D receptor (VDR), and CYP27B1 but decrease the expression of FGF23 and CYP24A1, suggesting the curative effect of AM on spontaneous SOP (3). Resveratrol, an edible polyphenolic phytoalexin from *Veratrum grandiflorum*, enhanced bone formation and counteracted accelerated bone loss in SAMP6 mice. It also improved the osteogenic differentiation of senescent BMSCs from SAMP6 mice through the amelioration of Mitofilin-mediated mitochondrial compromise (71). Orcinol glucoside (OG), derived from the extract of *Curculigo orchioides* Gaertn, could attenuate bone loss by inhibiting the formation and bone resorption activities of OC, thus aiding the prevention of SOP. Mechanistical analysis revealed that OG reduced the high levels of oxidative stress in the SAMP6 mice via the Nrf2/Keap1 and mTOR signaling pathways (72).

D-gal-induced aging model

D-gal, an aldohexose, facilitates the conversion of aldose and hydroperoxide, leading to the production of ROS (73). D-gal has the ability to induce osseous changes resembling senescence characteristics of natural aging, which may contribute to bone loss and SOP in the aging process (36, 74). Consistent with previous findings, TCM also plays a significant role in treating D-gal-induced aging in mice.

A study conducted by Xu et al. found that treatment with Bajitian Wan (BJTW) effectively mitigated D-gal-induced bone loss in aging mice. This effect was observed through the modulation of ALP, OCN, OPG, and RANKL levels (75), which suggests that BJTW exhibits promising anti-senescence properties and holds promise as a treatment option for SOP.

In the D-gal-induced aging rat model, Canthaxanthin treatment significantly increased the BMD, structural mechanics and biomechanics parameters, as well as bone calcium levels, thereby effectively preventing aging and SOP in the aging model rats injected with D-gal for 5 months (76). Orally administration of diosgenin to D-gal-induced aging rats has been found to significantly increase frame and femur volume while reducing porosity and frame density, suggesting diosgenin could potentially prevent bone loss during aging and provide beneficial effects in SOP (77). Similarly, Peptide-Calcium Chelate derived from Antler (*Cervus elaphus*) improved bone microstructure and alleviated age-related bone loss by enhancing calcium absorption in an aging mouse model (78). Cycloastragenol, an aglycone of astragaloside IV isolated from AM Bunge, could decrease serum bone resorption marker (TRACP), augment bone strength, reduce OC number, and improve bone formation in D-gal-treated rats. The potential mechanism behind these effects may be linked to the Cycloastragenol-induced increase in osteoactivin expression, providing preclinical evidence for its potential as a therapeutic agent in treating SOP (79).

Potential targets of TCM formulations and bioactive ingredients with anti-senescence properites in treating SOP

Cellular senescence, triggered by factors like oxidative stress and the activation of the p53/p21 and p16/pRB tumor suppressor pathways, plays a central role in SOP. In basic research, several TCM formulations and their active ingredients has demonstrated anti-senescence effects (13–16). To better understand the potential targets of TCM formulations and their bioactive ingredients in treating SOP, we also summarize the experiments focusing on reducing oxidative stress, senescence-associated markers and other characteristics such as BMSCs senescence.

Oxidative stress

Oxidative stress arises when cells generate an excessive amount of ROS, leading to a pathological process (80). Previous studies have demonstrated that senescent cells have higher ROS levels compared to normal cells, suggesting the involvement of ROS in cellular senescence (81). In addition, oxidative stress is closely associated with OP development (82, 83). Extensive research is now focused on the accumulation of ROS as well as ROS-mediated cellular senescence in SOP, with TCM playing a role in this domain.

As a major isoflavone glycoside extracted from the Chinese herb *Pueraria radix*, Puerarin effectively mitigates bone mass loss by inhibiting osteoclastogenesis and suppressing oxidative stress in bone tissues (84). Zhou et al. found that Resveratrol attenuates BMSCs senescence derived from aging rats and directs BMSCs differentiation towards OB lineage through the activation of AMPK and the down-regulation of ROS production. This research provides new insight into the application of TCM herbs and their active constituents for treating ROS/age-induced OP (85). Similarly, Ginkgolide B (GB), a small natural molecule from *Ginkgo biloba*, exhibits pharmacological activities in aging-related diseases, including SOP. It has been shown to significantly enhance bone mass in mice with aging-induced OP by increasing the OPG-to-RANKL ratio while promoting osteogenesis in aged BMSCs and inhibiting osteoclastogenesis in aged macrophages through ROS reduction. These findings suggest the promising potential of GB for further clinical investigation (86). Astragalus Polysaccharide (AP), derived from a commonly used anti-aging TCM herb AM, has a comparable effect in reducing ROS accumulation. It increases Nanog, Sox2, and Oct4 expression, and inhibits mitochondrial ROS accumulation to counteract the senescence of BMSCs induced by ferric ammonium citrate, ultimately promoting osteogenesis (87). Likewise, FLL reverses the decline in interleukin-2, tumor necrosis factor-alpha, and oxidative stress in the serum of D-gal-induced ICR mice, effectively preventing age-related OP by

maintaining bone microstructure and strength and inhibiting bone loss initiation (17). β-amyloid (Aβ) deposition and the resultant oxidative damage are significant contributors to aging diseases such as SOP, whereas *Humulus lupulus* L. Extract (HLE) treatment reduces Aβ deposition in bones of APP/PS1 mice, alleviating oxidative stress and modulating bone metabolism. It improves BMD and bone microstructure while substantially restoring the decreased expression of Nrf2, HO-1, NQO1, FoxO1, and SOD-2 in Aβ-damaged OB, indicating that HLE can alleviate Aβ deposition-induced oxidative stress through the activation of the Nrf2 and FoxO1 pathways, providing evidence for its clinical application in the prevention and treatment of SOP (88).

p53/p21/p16

Cellular senescence arises from replicative and stress-induced senescence, wherein the activation of p53 and p16 leads to the activation of p21, resulting in cell cycle arrest (89). OP mice and aged bones from humans have recognized p53, p21, and p16 as indicators of senescence (23, 90, 91). Research focused on these senescence markers in the context of OP holds significant potential. Indeed, numerous TCMs and their active constituents are currently under investigation in this area.

Zuogui Wan (ZGW), a classical TCM prescription for senile disorders and anti-aging, has been demonstrated to regulate Wnt signaling and suppress the expression of senescence-related factors such as p53, p21, and p16 to enhance cell proliferation, ameliorate DNA damage, and reduce SASP secretion, thereby maintaining osteogenic differentiation of rat BMSCs (92). Yuan et al. demonstrated that treatment with Si Jun Zi Tang (SJZT), another classical TCM prescription, improved OP in aging mice, as evident from micro-CT analysis results. The protein expression of p53, p21, and p16 were also significantly reduced in SJZT-treated mice (93). Network pharmacology, as an emerging discipline, provides a new network model of "multiple targets, multiple effects, and complex diseases" to explain the mechanism of TCM treatment. Several studies investigating the potential pharmacological process and specific mechanisms of TCM in treating OP (Liuwei Dihuang Pill, *Eucommia ulmoides* cortex, Ursolic acid, Jiawei Buguzhi Pill) have demonstrated that a potential association with p53 (94–98). Nevertheless, these predictions made by network pharmacology require further validation through future *in vivo* and *in vitro* experiments to strengthen the basic research evidence of TCM in the treatment of OP, particularly cellular senescence.

Moreover, treatment of human OB with Resveratrol and Anthocyanins led to a decrease in *p53* mRNA expression and an increase in cell proliferation, thus promoting OB differentiation and reducing RANKL-induced bone resorption. These compounds could also serve as a novel therapy for OP (99). Consistently, treatment with Resveratrol in human BMSCs resulted in a significant reduction in SASP secretion levels, as well as decreased expression of senescence-related genes (*p53*, *p16*, and *p21*) and intracellular ROS levels. Meanwhile, it inhibited adipogenic differentiation of human BMSCs, thereby achieving therapeutic effects for OP (53). Likewise, Echinacoside, a phenylethanoid glycoside isolated from a TCM herb *Herba Cistanches* (100), exerted a protective effect on OB by suppressing p53 expression, suggesting its potential for OP treatment (101).

Others

In addition to the aforementioned information, TCM formulations and their active constituents can address SOP using alternative approaches, such as improving the senescence of BMSCs and regulating multiple signaling pathways.

Du-Huo-Ji-Sheng-Tang (DHJST) and its active component Ligusticum augment *RUNX2* and *BMP-2* gene expression by activating SMAD1/5/8 and ERK signaling pathways, thereby promoting osteogenic activity in human mesenchymal cells (hMSCs). Meanwhile, they are capable of reducing senescence levels in hMSCs during the aging process (102).

Catalpol, the principal bioactive component of *Rehmannia glutinosa*, not only dose-dependently diminishes the proportion of senescent cells in BMSCs but also enhances their osteogenic differentiation and stimulates bone regeneration by partially activating the Wnt/β-catenin pathway (103). Tanshinone IIA (TSNA), a major active component found in *Salvia miltiorrhiza* Bunge (Danshen), can restore the cellular stemness of BMSCs and reverse aging in BMSCs. Mechanistically, TSNA primarily targets *PHGDH* mRNA, upregulating its levels and reducing the high methylation in the promoter region of PHGDH, thereby exerting anti-aging and anti-osteoporotic effects of TSNA on BMSCs (104). In parallel, AM exerts its effects by modulating the Vitamin D-FGF23-Klotho pathway, effectively reversing the reduced cellular viability and osteogenic capacity observed in senescent BMSCs after the *VDR* gene downregulation. This modulation enhances osteogenic ability and prevents age-related OP (105).

Conclusion and perspectives

SOP, a prevalent form of OP, represents a significant consequence of age-related bone disorders (106). Nevertheless, the pathogenesis of SOP remains intricate, and the precise mechanisms underlying its onset and progression require further elucidation. As discussed above, cellular senescence and the senescence-associated secretory phenotype (SASP) play a crucial role in various age-related conditions, including SOP. Hence, targeting senescence or eliminating senescent cells holds great promise.

TCM contains numerous bioactive compounds with diverse pharmacological activities. Moreover, when administered *in vivo*, TCM can generate additional bioactive or inactive metabolites. Importantly, TCM formulations, herbs, and their active ingredients, such as EF, FLL, Resveratrol, and ZGW, exhibit significant anti-senescence effects, whose efficacy against SOP has been verified through both *in vitro* and *in vivo* experiments involving naturally aging mice, SAMP mice, and D-gal-induced mice. These findings provide a theoretical basis for the clinical application of TCM in the treatment of SOP. However, current clinical research on TCM primarily focuses on postmenopausal osteoporosis (PMOP) (107, 108), and it does not adequately distinguish or assess the effects on PMOP and SOP, separately, which makes it difficult to gather specific clinical data for SOP treatment. It is important to have more clinical data in the future to establish the effectiveness of TCM in treating SOP. Nevertheless, clinical trials have been conducted on TCM formulations and their

active ingredients, such as QED, AM, and Resveratrol, for the treatment of other diseases (109–111). Moreover, preclinical studies have demonstrated the targeting of cellular senescence by these medicines in the context of SOP, as mentioned above, which provides confidence for the initiation of clinical trials on these TCM formulations for SOP treatment.

Apart from SOP, other aging-related diseases, including neurodegenerative diseases, cardiovascular diseases, and metabolic diseases, have been effectively treated with TCM (112–114). Since potential correlations may exist between senescence and the onset of different senescence-related diseases, exploring new therapeutic TCM drugs for treating SOP by targeting cellular senescence can draw inspiration from the treatments of other senescence-related diseases using TCM herbs, formulations, or their active ingredients. Current research has identified senescence as a modifiable through hormones, drugs, and inhibitors such as glucocorticoids, Dasatinib and Quercetin, metformin, SASP inhibitors (rapamycin and ruxolitinib), and senolytics (115–120). To gain further insights into the anti-senescence effects of TCMs and their active ingredients in SOP, combining TCM with proven anti-aging substances and drugs, alongside the aforementioned approaches, in basic research and preclinical trials is possible. Alternatively, conducting comparative studies to evaluate the effects of TCM and the aforementioned drugs (as positive control) separately is another avenue to explore. DNA damage is a crucial factor in maintaining genetic stability and is also implicated in triggering cellular senescence (121), contributing to the development of age-related diseases, including SOP (122). Notably, TCM formulations and their active compounds such as Bazi Bushen, Yifuning, Deoxyschisandrin, and Schisandrin B have shown therapeutic effects on age-related diseases by regulating DNA damage (123–125). Therefore, developing TCM formulations that target DNA damage repair for the treatment of SOP holds promising prospects. As mentioned earlier, epigenetic alterations play a crucial role in both normal bone formation and function, as well as in pathogenesis of SOP (48, 126). Natural TCM compounds like resveratrol, sulforaphane, specific phenolic acids and anthocyanins, have been shown to regulate bone remodeling by influencing the bone epigenome (127). Therefore, conducting further research on the mechanisms of TCM in treating SOP can be achieved through studying epigenetic modifications such as DNA methylation, histone modifications, and post-transcriptional regulation.

It is essential to note that the anti-senescence effects of TCM are not limited to a singular approach. For instance, Resveratrol not only enhances osteogenic differentiation of senescent BMSCs from SAMP6 but also reduces intracellular ROS levels and senescence-related genes (*p53*, *p16*, and *p21*). Consistently, FLL exhibits anti-senescence effects in both naturally aging mice and D-gal-induced aging mice. Furthermore, studies have demonstrated that D-gal can generate ROS (73, 128), which can contribute to the development of SOP. In this context, TCM formulations such as FLL have been shown to possess antioxidant properties when used to treat SOP induced by D-gal in mouse models. Thus, a comprehensive understanding of the anti-senescence effects of TCM in SOP necessitates consideration of different mechanisms of senescence. Noteworthy, when applying different aging models to simulate SOP, it is also necessary to consider their advantages and disadvantages. Naturally aging models can better replicate the gradual aging process of the human body, but they have the drawback of requiring prolonged modeling time. The D-gal-induced aging model is commonly utilized to investigate the pathological features associated with senescence, such as mitochondrial dysfunction, excessive formation of glycation products, and oxidative stress (74), however, additional research is necessary to explore these pathways in the D-gal-induced model and determine its similarity to the natural aging process. SAMP mice exhibit accelerated aging characteristics, allowing researchers to study the pathological manifestations of SOP in a relatively short period, however, they have a shorter lifespan compared to regular mice, which may limit long-term studies on SOP processes, and the accelerated aging phenotype observed in SAMP mice may not fully reflect the complexity and heterogeneity of human aging, potentially limiting the generalizability of findings.

Moreover, numerous studies have indicated that the bone microenvironment associated with senescence enhances the activity of OC progenitors and increases osteoclastogenesis. Interestingly, the elimination of senescent OC progenitors did not affect bone loss in age-related mice, instead, senescent osteocytes have emerged as a critical mechanism in bone aging, suggesting their involvement in age-related bone loss (129). Similarly, senescent immune cells, including macrophages and neutrophils, also contribute to the development of SOP (130). Based on the anti-senescence effects of TCM, it is speculated that TCM also has great potential in treating SOP by targeting senescent OC and immune cells. However, it is crucial to underscore that the clinical significance of TCM in age-related diseases, including SOP, remains incompletely understood. This is partly due to the early stages of research on TCM's anti-senescence effects and the scarcity of comprehensive and rigorous clinical trials documenting its efficacy. It is worth noting that age is a significant risk factor for another type of OP, PMOP. Research has found that targeting cellular senescence can be a potential approach for PMOP treatment (32, 46, 131). In contrast to SOP, which affects both men and women, PMOP predominantly occurs in women due to decreased ovarian function and a decline in estrogen levels in the body, so the mainstay of prevention of POMP was hormone therapy with estrogen and progestin (HT) or estrogen therapy (ET) (132), TCM has proven to be effective in reducing the risk of OP by its estrogen-like activity (133). Therefore, when using TCM to treat age-related PMOP, the combination of herbs or formulations with estrogenic effects and anti-senescence properties can be considered to enhance the therapeutic effects.

In conclusion, based on evidence from basic research, the utilization of TCM formulations and their active constituents for treating SOP by targeting cellular senescence exhibits promising prospects. However, there is still a lack of clinical data and validation regarding the use of TCM and its active constituents in the context of anti-senescence treatments for SOP. In addition, apart from senescence, the pathological mechanisms of OP also include other factors such as immune dysfunction and inflammation (134, 135). Therefore, further elucidation is warranted to determine whether a specific TCM exerts its anti-osteoporotic effects through a single pathway or multiple pathways, as well as the precise underlying mechanisms. Nevertheless, the mounting evidence strengthens our confidence in the potential of TCM for SOP treatment (Table 1).

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TABLE 1 The efficacy of TCM formulations and active constituents in treating SOP by targeting cellular senescence.

Compound	Medicinal materials	Animal model or Cells	Beneficial effects	Reference
N/A	XZGB	Naturally aging model	Increased the trabecular area	(56)
N/A	FTZ		Reversed the abnormal levels of metabolites	(57)
N/A	QED		Reduced the elevated levels of LEP and maintained trabecular structure and bone quality	(59)
N/A	SJZT		Increased the total average BMD	(93)
N/A	*Epimedium* leaf and *Fructus Ligustri Lucidi*		Improved low bone mass by enhancing the expression of TGF-β1, BMP2, Wnt5a, and IGF-1.	(60)
Allicin	*Allium sativum* L.		Reversed deleterious bone biomechanical features and increased BMD	(62)
Oleanolic Acid	N/A		Improved bone micro-architecture through improving calcium balance and modulating vitamin D metabolism	(63, 64)
N/A	BGSSD	SAMP mice	Protected the bone mass and improved bone trabecular structure	(68)
N/A	Hachimi-jio-gan and Juzen-taiho-to		Increased the amount of bone-forming surface and bone mass	(67)
N/A	*Astragalus membranaceus*		Improved the femoral BMD and bone microstructure, elevated the contents of calcium and phosphorus	(3)
N/A	*Eclipta prostrata*		Improved bone micro-structure by regulating the dynamic balance of bone absorption and formation	(66)
Eucommia ulmoides leaf water extract	*Eucommia ulmoides*		Increased trabecular bone, BMD, and decreased intertrochanteric space	(18)
Antrodia camphorata alcohol extract	*Antrodia camphorata*		Inhibited bone loss and increased the proportion of bone volume, trabecular bone number, and BMD	(69)
Resveratrol	*Veratrum grandiflorum*		Enhanced bone formation and counteracted accelerated bone loss	(71)
Orcinol glucoside	*Curculigo orchioides*		Reduced high levels of oxidative stress	(72)
Total glycosides and polysaccharides of *Cistanche deserticola*	*Cistanche deserticola*		Decreased bone formation and damaged bone microstructure as well as regulating the expression of RNAKL, BMP-2, OPG	(19)
Icariin	*Epimedium brevicornum*		Activated the BMP signaling pathway and downregulated CTGF expression	(70)
N/A	BJTW	D-gal-induced aging model	Alleviated bone loss by modulating the levels of ALP, OCN, OPG, and RANKL	(75)
N/A	*Fructus Ligustri Lucidi*		Reversed the decrease of interleukin-2, tumor necrosis factor-alpha, and oxidative stress in the serum	(17)
Cycloastragenol	*Astragalus membranaceus*		Decreased serum bone resorption marker (TRACP), increased bone strength, reduced OC number, and improved bone formation	(79)
Diosgenin	*Dioscorea rhizome*		Increased frame and femur volume and decreased porosity and frame density	(77)
Peptide–Calcium Chelate	Antler (*Cervus elaphus*) Bone		Ameliorated the bone microstructure and alleviated age-related bone loss by increasing calcium absorption	(78)
Canthaxanthin	N/A		Increased the BMD, parameters of structural mechanics and biomechanics, bone calcium	(76)

(Continued)

TABLE 1 (Continued)

Compound	Medicinal materials	Animal model or Cells	Beneficial effects	Reference
N/A	ZWG	BMSCs	Regulated Wnt signaling and suppressed the expression p53, p21, and p16 to enhance cell proliferation	(92)
N/A	*Astragalus membranaceus*		Regulated the VD-FGF23-Klotho pathway, reversed the decreased cell viability and osteogenic ability	(105)
Astragalus Polysaccharide	*Astragalus membranaceus*		Increased Nanog, Sox2, and Oct4 expression, and impeded mitochondrial ROS accumulation	(87)
Ginkgolide B	*Ginkgo biloba*		Promoted osteogenesis and inhibited osteoclastogenesis by reducing ROS	(86)
Resveratrol	*Veratrum grandiflorum*		Activated AMPK and down-regulated ROS production	(85)
Catalpol	*Rehmannia glutinosa*		Reduced the percentage of senescent cells and enhanced the osteogenic differentiation	(103)
Tanshinone IIA	*Salvia miltiorrhiza*		Restored the cellular stemness and improved the aging state	(104)
Resveratrol and Anthocyanins	N/A	OB	Reduced *p53* mRNA expression and reduced RANKL-induced bone resorption	(99)
Echinacoside	*Herba Cistanches*		Inhibited p53 expression	(101)
N/A	DHJST	hBMSCs	Increased *RUNX2* and *BMP-2* gene expression, decreased the level of senescence	(102)
Resveratrol	*Veratrum grandiflorum*		Decreased the secretion levels of SASP and the expression levels of p53, p16, p21	(53)
Humulus lupulus Extract	*Humulus lupulus*	MC3T3-E1 cells	Alleviated oxidative stress and regulated bone metabolism	(88)

Author contributions

YZ: Writing – original draft. XY: Writing – original draft. CZ: Writing – original draft. KF: Data curation, Formal analysis, Writing – review & editing. HL: Conceptualization, Data curation, Formal analysis, Supervision, Writing – review & editing. CW: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

OP	Osteoporosis
SOP	Senile osteoporosis
PMOP	Postmenopausal osteoporosis
OB	Osteoblast
OC	Osteoclast
BMSCs	Bone marrow mesenchymal stem cells
TCM	Traditional Chinese medicine
SASP	Senescence-associated secretory phenotype
ROS	Reactive oxygen species
D-gal	D-galactose
SAMP	Senescence-accelerated mouse/pron
EF	*Epimedium leaf*
FLL	*Fructus Ligustri Lucidi*
XZGB	Xianzhengubao
FTZ	Fufang Zhenzhu Tiaozhi
LEP	Leptin
QED	Qing'e Decoction
OA	Oleanolic Acid
EUL	*Eucommia ulmoides* leaf water extract
LB	*Lactobacillus bulgaricus*
GM	Gut microbiota
AM	*Astragalus membranaceus*
BGSSD	Bu-Gu-Sheng-Sui decoction
ACAE	Antrodia camphorata alcohol extract
OG	Orcinol glucoside
CTGF	Connective tissue growth factor
BJTW	Bajitianwan
GB	Ginkgolide B
AP	Astragalus Polysaccharide
Aβ	β-amyloid
HLE	*Humulus lupulus* L. Extract
SJZT	Si Jun Zi Tang
DHJST	Du-Huo-Ji-Sheng-Tang
hMSCs	Human mesenchymal cells
TSNA	Tanshinone IIA

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Cornus officinalis: a potential herb for treatment of osteoporosis

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Osteoporosis (OP) is a systemic metabolic skeletal disorder characterized by a decline in bone mass, bone mineral density, and deterioration of bone microstructure. It is prevalent among the elderly, particularly postmenopausal women, and poses a substantial burden to patients and society due to the high incidence of fragility fractures. Kidney-tonifying Traditional Chinese medicine (TCM) has long been utilized for OP prevention and treatment. In contrast to conventional approaches such as hormone replacement therapy, TCM offers distinct advantages such as minimal side effects, low toxicity, excellent tolerability, and suitability for long-term administration. Extensive experimental evidence supports the efficacy of kidney-tonifying TCM, exemplified by formulations based on the renowned herb *Cornus officinalis* and its bioactive constituents, including morroniside, sweroside, flavonol kaempferol, Cornuside I, in OP treatment. In this review, we provide a comprehensive elucidation of the underlying pathological principles governing OP, with particular emphasis on bone marrow mesenchymal stem cells, the homeostasis of osteogenic and osteoclastic, and the regulation of vascular and immune systems, all of which critically influence bone homeostasis. Furthermore, the therapeutic mechanisms of *Cornus officinalis*-based TCM formulations and *Cornus officinalis*-derived active constituents are discussed. In conclusion, this review aims to enhance understanding of the pharmacological mechanisms responsible for the anti-OP effects of kidney-tonifying TCM, specifically focusing on *Cornus officinalis*, and seeks to explore more efficacious and safer treatment strategies for OP.

KEYWORDS

osteoporosis, *Cornus officinalis*, kidney-tonifying herbs, bone homeostasis, effective ingredients, pharmacological mechanisms

Introduction

Osteoporosis (OP), a global skeletal disorder often referred to as the "silent disease," is characterized by bone mass loss and microstructure degeneration, leading to an increase in the risk of fractures and imposing a substantial socioeconomic burden (1, 2). It is estimated that the number of fractures causing by OP will reach 2.6 million in 2025, double the total number in 1990, and will reach 4.5 million by 2050 in the world (3). Moreover, increasing evidence indicates that women and older individuals are particularly vulnerable to OP and its consequences, with 1/3 of women and 1/5 of men aged 50 and above experiencing osteoporotic fractures globally (1). However, prolonged use of anti-OP medication such as denosumab, teriparatide, bisphosphonates, calcitonin, and estrogen, can result in undesirable side effects, including an

increased risk of malignancy, atypical femur fractures, osteonecrosis of the jaw, and cardiovascular issues (4). The concern over these side effects and the uncertain long-term efficacy of pharmacological treatments has prompted the search for alternative medications with fewer adverse events, low toxicity, high efficacy, and good tolerability.

An increasing number of researchers are exploring Traditional Chinese Medicine (TCM) as an alternative treatment for OP due to its fewer adverse events and long-term safety profile (5). *Cornus officinalis* (known as Shanzhuyu in Chinese), an ingredient commonly found in TCM formulas for bone-related diseases such as Zuo Gui Pill (ZGP) (6, 7), You Gui Pill (YGP) (7), and Liuwei Dihuang Pill (LWDHP) (8), all of which are known for its kidney-nourishing properties, exerts beneficial effects in the prevention and treatment of OP by alleviating common symptoms experienced by individuals with OP such as lumbar and knee discomfort (9, 10). Furthermore, recent research has highlighted the therapeutic potential of certain monomeric components derived from *Cornus officinalis*, independent of its inclusion in TCM formulations including flavonoids, tannins, iridoids, organic acids, polysaccharides, and lignans (11). Among them, gallic acid, morroniside, loganin, sweroside, quercetin, notoginsenoside R1, cornuside I, kaempferol, and 5-HMF, extracted from *Cornus officinalis*, may play a crucial role in OP treatment (12).

In this review, we comprehensively summarize the research on *Cornus officinalis* in the context of OP, focusing primarily on its mechanisms of action involving bone homeostasis, immunomodulation, vascularity, and bone microarchitecture, thus providing a better understanding of the therapeutic role played by *Cornus officinalis* in the pathological process of OP and its potential clinical application.

Pathomechanism of OP

OP is a common metabolic bone disease associated with a variety of factors such as bone homeostasis, immune mechanisms, vascular changes, estrogen deficiency, mechanical stress, and the nervous system. In this section, we will discuss the pathological of OP related to these factors.

Bone homeostasis

Several key cells in bone tissue, such as bone mesenchymal stem cells (BMSC), osteoblast (OB), and osteoclast (OC), play critical roles in bone remodeling, including bone formation and bone resorption (13). Particularly, BMSC can mainly differentiate into adipocytes and OB in bone, to play an important role in the regulation of normal bone homeostasis (14). The capacity of BMSC from OP patients to differentiate into OB is lower than that in healthy individuals (14). The shift in preferential differentiation of MSCs from OB to adipocytes accompanied by reduced bone mineral density (BMD) can contribute to OP progression (15). OB secrete various components of osteoid, such as collagen I, alkaline phosphatase (ALP), osteopontin (OPN), and osteocalcin (OCN), which then mineralize to form mature bone (16, 17). Additionally, precursor OC are enlisted and attached to the bone matrix, subsequently undergoing further differentiation into mature OC, which can release acids and lytic enzymes that facilitate the degradation of the bone matrix and absorption of aging and damaged bone tissue (18, 19). As evidenced by disturbed bone homeostasis, altered bone microstructure, and reduced bone strength, OP arises from the imbalance of bone formation and bone resorption, resulting from excessive absorption by OC or impaired generation of OB (17, 20).

Moreover, various regulatory factors and signaling pathways impact the activity of BMSC, OB, and OC, thus governing the process of bone resorption and formation processes. Significant roles are played by signaling pathways such as Wnt/β-catenin, bone morphogenetic proteins (BMP)-Smad, Hedgehog, receptor activator of nuclear factor-B ligand (RANKL)/receptor activator of nuclear factor-B (RANK)/osteoprotegerin (OPG), along with several regulatory factors. Notably, the canonical Wnt/β-catenin signaling pathway has emerged as a crucial regulator of bone formation, promoting the osteogenic process, preventing apoptosis of OB precursors, facilitating OB differentiation and inhibiting BMSC differentiation into adipocytes (21, 22). Conversely, inhibiting Wnt pathway impedes bone formation, rendering individuals more susceptible to early-onset OP and osteogenesis imperfecta (13). Similarly, activation of Hedgehog signaling pathway promotes the differentiation of BMSC into OB rather than adipocytes by upregulating Runx-2 expression, thereby enhancing bone formation (23, 24). Moreover, specific BMP and canonical TGF-β positively regulate osteogenic activity by phosphorylating downstream Smad proteins, thereby influencing the balance between OB-mediated bone formation and OC-mediated bone resorption (23, 25, 26).

Furthermore, the RANKL/RANK/OPG signaling pathway represents the most extensively studied pathway concerning OC differentiation and activity. OB release RANKL, which binds to RANK, a specific receptor on the surface of OC, triggering the transcription of downstream factors, such as c-FOS, NFATc1, tartrate-resistant acid phosphatase (TRAP), and cathepsin K (CTSK), ultimately leading to the differentiation and activation of OC (27). Meanwhile, OPG, which is also secreted by OB, competitively binds to RANK, suppressing OC activity and safeguarding bones against excessive resorption (28, 29).

The role of Notch signaling pathway in bone remodeling relies on the type of Notch receptor involved: Notch 1 fosters increased OPG production and decreased sclerostin, exerting osteoprotective effects by inhibiting OC formation and bone resorption, while Notch2 promotes osteoclastogenesis and enhances bone resorption by stimulating RANKL expression (30). In summary, OP arises from an imbalance between bone formation and bone resorption within bone homeostasis, stemming from the dysregulation of multiple signaling pathways.

In summary, BMSC and OB play roles in bone formation, while OC affect bone resorption. Together, they mediate OP through different factors (Figure 1).

Vasculature

The vascular system in bones contributes to the supply of oxygen, nutrients, hormones, growth factors, and neurotransmitters necessary for the normal growth, development, regeneration, and remodeling of bone. Emerging evidence reveals that the metabolic imbalance in the bone microenvironment caused by blood vessel supply impairment,

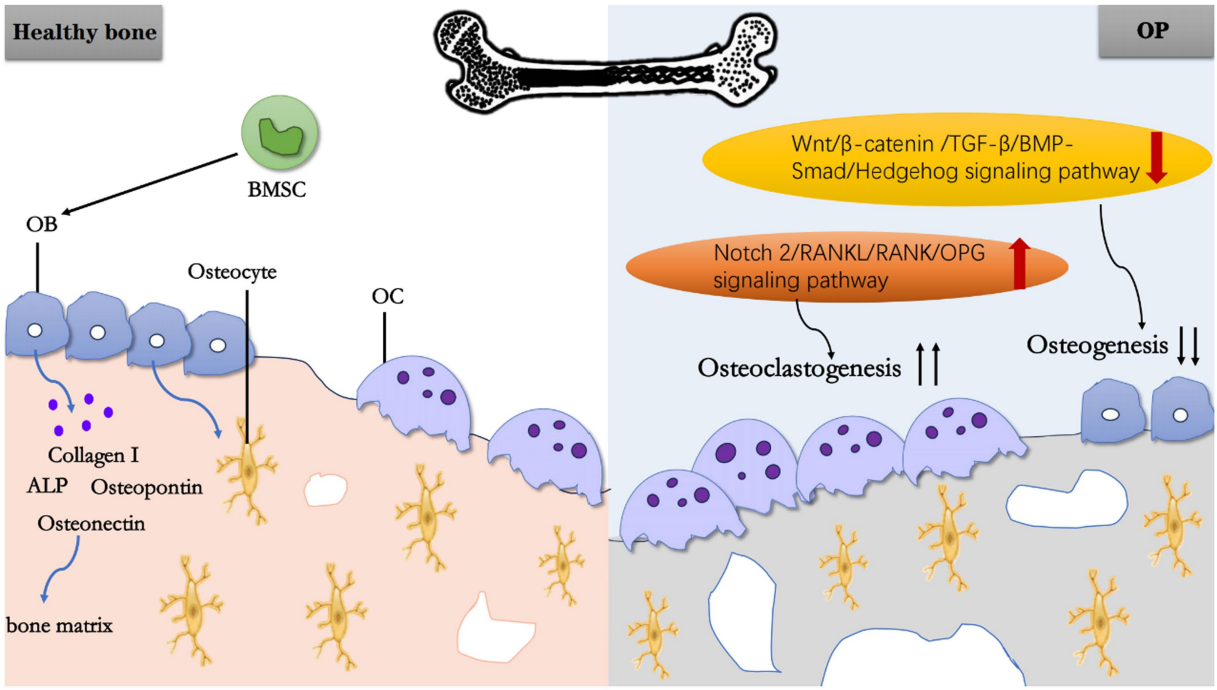

FIGURE 1
Schematic working model of OP. In a normal physiological state of healthy bone, BMSC can differentiate into OB, and OB further mature into osteocytes. Meanwhile, OB secretes Collagen I, ALP, OPN, Osteonectin, and other substances to constitute bone matrix. Under pathological state, the upregulation of Notch 2/RANKU/RANK/OPG signaling pathway promoting OC generation and the downregulation of Wnt/β-catenin/TGF-B/BMP-Smad/Hedgehog signaling pathway inhibiting osteogenesis, ultimately lead to OP.

along with the imbalance of vascular calcium, phosphorus and glucose metabolism, is intricately associated with the pathological mechanisms of OP (31). For instance, adjustment of the number and size of blood vessels along the flow has confirmed that the skeletal system occupies 10 and 15% of the total cardiac output, which is crucial for skeletal system health. And insufficient blood flow can lead to delayed bone repair and other low bone mass diseases due to impaired osteogenesis (32, 33). Moreover, a female patient with a rare Hajdu-Cheney syndrome causing periarticular OP showed reduced the height and density of blood vessels in all affected fingers (34). Triggering the activation of the HIF signaling pathway in OB could prevent the reduction of blood vessels in the bone marrow of postmenopausal OP patients to prevent bone loss (35). Another study showed that supplementation of ribonuclease-rich lactoferrin promotes new vessels formation, achieving a significant reduction in bone resorption and an increase in bone formation to restore bone homeostasis (36).

Blood vessels inside bones are generally identified two types: The type H vessels with high expression of CD31 and endomucin mainly localized in the vicinity of the chondro–osseous junction, and the type L vessels with low expression of CD31 and endomucin mainly localized in the diaphysis (37). The number of Type L vessels, which does not change over time, are basically not involved in bone metabolism (37). Contrary to type L vessels, type H vessels maintain OB around blood vessels, which has been proved to be an important carrier of to induce angiogenesis and bone formation (38–40). Researches indicate a corresponding reduction in the number of type H blood vessels in aged OP mice, ovariectomized (OVX) induced OP mice, as well as in elderly and OP patients, accompanied by loss of bone precursor cells (37–40). In turn, promoting the maturation of Type H vessels by activating Notch signaling can regulate the differentiation of perivascular osteoprogenitor cells and accelerate osteogenesis (41).

The levels of calcium, phosphorus, and glucose in blood vessels, as well as their metabolic balance, are also closely related to OP. Studies have observed significant decreases in BMD, osteoclastic strength, serum ALP, and calcium and phosphorus in the OP model after ovariectomy (42–46). In addition, abnormal calcium loss of bone tissue, accompanied by calcium deposits within blood vessels causing vascular calcification, contributes to the pathogenesis of OP, known as the "calcium paradox" (47, 48). The aggravation of vascular calcium deposition obstructs nutrient supply to bone tissue and further promotes the pathogenesis of OP. On the other hand, output venous vessels in Haversian and Volkmann ducts allow immune cells to migrate from the basement membrane to the deeper layers of dense bone, becoming a site for mineral reabsorption during OP (49). Furthermore, abnormal blood glucose levels downregulate PI3K-AKT signaling pathway to inhibit OB activity and promote OC activity in the trabecular bone region with reduced serum level of OCN and ALP (50, 51). All these findings underscore the close relationship between blood vessels and the pathological process of OP, particularly the number of type H vessels and capillaries, bone blood flow and vascular calcium deposition besides vascular calcium, phosphorus and glucose metabolism (31, 34–36, 48, 49).

In conclusion, activating neovascularization, promoting bone blood flow, and balancing calcium level between blood vessels and bone tissue are therapeutic strategies for OP management.

Immune system

Osteoimmunology is an interdisciplinary field arising from mounting evidence of the close relationship between the immune system and bone metabolism (52–54). Relevant studies have shown that many immune cells in the bone system, including T lymphocytes, B lymphocytes and macrophages, affect bone cells in the bone system directly or indirectly through the secretion of mediators by immune cells such as OPG/RANKL, COX-2, interleukins, and tumor necrosis factor (TNF) (52, 54–64).

Th2 lymphocytes, through the release of IL-4 and IL-13, act to prevent the formation of OC by downregulating prostaglandin dependent on COX-2, thereby suppressing bone resorption (54, 56). Conversely, Th17 lymphocytes, as the main source of IL-17, promote osteoclastic differentiation *in vitro* and the generation of RANKL, resulting in bone loss in mice with primary hyperparathyroidism (57–59). Meanwhile, IL-17 also promotes the early differentiation of OB by increasing the expression of ALP, RUNX2, OCN, and OPG (61–63). Besides, the upregulation of TNF-α expression in T-lymphocytes, under the regulation of RANKL, promotes the apoptosis of OB (60, 64).

The B lymphocytes, on the other hand, reduce OB differentiation by acting through CCL3 and TNF, which target ERK and NF-κB signaling pathways (65). In addition, B lymphocytes secrete various cytokines that play a dual role in OC: On one hand, they produce IL-7 (66), RANK (67), and approximately half of the total OPG (68) in the bone marrow, suppressing OC activation; On the other hand, B lymphocytes secrete G-CSF and RANKL under inflammatory conditions, promoting the differentiation and proliferation of OC, thus leading to bone resorption (69). Surprisingly, IL-18, initially considered an up-regulator of OPG that inhibits osteoclastogenesis, was subsequently found to increase the expression of RANKL on T lymphocytes, ultimately promoting bone mass loss (70).

Macrophages presented in bones are known as various populations: bone marrow macrophages (BMMs), OC, and osteal macrophages (71), all of which can categorized into two phenotypes—M1 (inflammatory phenotype) and M2 (reparative phenotype)—playing different roles in bone homeostasis. M1 macrophages, considered as precursors of OC (72), polarize after stimulation by pro-inflammatory cytokines IL-6, TNF-α, and IFN-γ (73), triggering osteoclastogenesis and subsequent bone destruction (74). Interestingly, RANKL-induced M1 macrophages contribute to the expression of *OPN* and *RUNX2* in BMSC, inducing osteogenesis as a contrary effect (75). Conversely, M2 macrophages polarize under stimulation by anti-inflammatory cytokines such as IL-4 and IL-13, and stimulate MSCs or pre-osteoblastic cells to differentiate into OB, promoting bone formation. Moreover, increased transition from M1 to M2 macrophages enhances this trend (53, 73, 76). Hence, regulating the ratio of M1/M2 macrophages holds the potential therapeutic effect of anti-OP.

Other factors

Many other factors, including nervous system, mechanical stress, estrogen, and oxidative stress, contributing to OP based on available data. To maintain proper bone balance, the nervous system enters mature bones, regulates blood flow and metabolism, and secretes neurotransmitters (77). Neuropeptide-Y (NPY), a classic neuronal regulator of energy homeostasis, directly inhibits BMSC proliferation and OB differentiation through the Y1 receptor on the surface of BMSC or OB and the Y2 receptor in hypothalamus, thereby suppressing bone formation and leading to OP (78–81). Moreover, mechanical stress also impacts OP, preventing osteoporotic bone loss through the Pl3k/Akt signaling and erythropoiesis (82). Estrogen, in order to protect the bones, inhibits OB apoptosis and OC formation by reducing the expression of RANKL, which also promotes the apoptosis of OC (83), which contributes to an increase in the incidence of OP among postmenopausal women. Furthermore, increased oxidative stress raises TNF-α levels in serum while reducing Sirtuin 6 (Sirt6) expression in long bones, promoting NF-κB acetylation as well as CTSK over-expression and activation (84), consequently leading to bone destruction (85, 86).

Anti-OP effects of *Cornus officinalis* and effective ingredients or Chinese formulations

BMSC and OB are the therapeutic targets of *Cornus officinalis* and its active ingredients or compounds to exert an anti-OP role

Cornus officinalis has been traditionally employed in East Asia for the treatment of OP. This botanical resource boasts abundant active ingredients that exert diverse effects on OP by modulating the proliferation and differentiation capacity of BMSC, promoting osteogenic differentiation, and ameliorating the OP phenotype.

The aforementioned findings, presented in Table 1, support the notion that promoting BMSC proliferation and osteogenic differentiation, inhibiting lipogenic differentiation, enhancing osteogenesis related protein such as BMP2, Osx, RUNX2, ALP, OPN, OCN by regulating Wnt/β-catenin, BMP, PI3K/AKT/mTOR, AMPK, JNK, ERK, NF-κB signaling pathways represent promising therapeutic strategies for the treatment of OP mediated by the efficacious components of *Cornus officinalis*.

OC is another therapeutic targets of *Cornus officinalis* and its active ingredients or compounds to exert an anti-OP role

OC is bone-resorbing cell that degrades bone through acid secretion and the release of proteolytic enzymes (107). *Cornus officinalis* processes the ability to restrict the differentiation of bone marrow-derived macrophages (BMMs) into OC, and suppress the translation and genetic transcription of OC-associated markers. These anti-OP effects are achieved via the active ingredients of *Cornus officinalis* (Table 2) (119).

In conclusion, *Cornus officinalis* and its active constituents modulate the function, activity, and quantity of OC by inhibiting MAPK, AKT, ERK, JNK signaling pathways to reduce the expression of OC-related proteins RANKL, c-Fos, NFATc1 and CTSK, thus inhibit bone resorption. These novel findings designate *Cornus*

TABLE 1 Active ingredients of *Cornus officinalis* for BMSC and OB.

Sorts of compounds	Active constituents	Results	References
Flavonoids	Quercetin	Enhances osteoblastogenesis while inhibiting adipogenic differentiation through Wnt/β-catenin, BMP, AMPK, JNK, and ERK signaling pathways	(87–91)
		Restores impaired BMSC function and activity induced by TNF-α by inhibiting NF-κB activation and β-catenin degradation	(92)
	Kaempferol	Reduces apoptosis induced by LPS, stimulates MSCs proliferation, and regulates non-coding RNAs, including miR-124-3p and miR-10a-3p, to control MSCs differentiation toward osteogenic lineages, promoting bone formation	(93, 94)
		Promotes OB autophagy while decreasing OB apoptosis, elevates the expression of osteogenic markers, including ALP, OSX, COL-1, OCN, and OPN, and facilitates osteoid mineralization and calcium deposition, resulting in an increase in calcium nodules	(95)
Iridoids	Sweroside	Enhances osteogenic differentiation by activating the mTOR1/PS6 signaling pathway, resulting in upregulation of OCN, Runx2, and Osx expression in osteoporotic mice BMSC and increased mineralized nodules formulation	(96, 97)
		Contributes to considerably higher levels of BMP2, RUNX2, ALP, OPN, and bone sialoprotein-1 (BSPH1) in OVX mice, along with increased bone matrix production	(98)
	Morroniside	Boosts BMSC proliferation *in vitro*, counteracts BMSC dysfunction and impaired osteogenic differentiation and bone loss induced by high glucose via downregulating the AGE-RAGE pathway	(99, 100)
		Interacts with sodium-glucose cotransporter 2 and adenosine A2AR to improve precursor cell viability (MC3T3) and promote proliferation	(101, 102)
		Activates PI3K/AKt/mTOR pathway, leading to Beclin1-and Atg13-dependent autophagy, facilitating the transformation of MC3T3-E1 cells into mature OB	(103, 104)
	Cornuside I	Increases ALP expression and calcium deposition, while stimulating MSCs proliferation through the activation of the PI3K/AKT signaling pathway to enhance osteogenic differentiation	(105)
	Notoginsenoside R1	Enhances migration and differentiation of human adipose-derived MSCs into OB, upregulating osteogenesis marker expression, such as ALP and OCN	(106)

TABLE 2 Active ingredients of *Cornus officinalis* for OC.

Sorts of compounds	Active constituents	Results	References
Flavonoids	Quercetin	Reverses increased OC in OVX rats by promoting OC apoptosis and autophagy, involving the MAPK pathway activation	(46, 108)
		Inhibits osteoclastic progenitor cell differentiation, disrupts the actin ring of mature OC, and exerts anti-OP activity while counteracting OC function	(109, 110)
		Prevents the increase of OC-promoting factors RANKL, TNF-α, IL-6, and IL-8 in RA-fibroblasts-like synoviocytes (RA-FLS) induced by proinflammatory factor IL-17	(109)
	Kaempferol	Downregulates elevated bone turnover in OVX rats, reduces the number of TRAP-positive multinucleate cells, and suppresses the transcriptional expression of OC-related markers such as c-Fos, NFATc1 in RANKL-induced RAW264.7 cells (precursor cells of OC), thereby inhibiting osteoclastogenesis	(111–113)
iridoids	Loganin	Restrains OC precursor cell differentiation in the OB–OC co-culture system, decreases TRAP activity (a molecular marker of OC number and bone resorption)	(114, 115)
	Morroniside	Suppresses TRACP enzyme activity and the expression of OC-related genes, exhibiting therapeutic potential in the treatment of OVX-induced OP in rats by inhibiting osteoclastic differentiation.	(116)
	Notoginsenoside R1	Inhibits RANKL-induced mitogen-activated protein kinases (MAPK) signaling pathway activation and subsequent OC production *in vitro*	(117)
phenolic acid	Gallic acid	Inhibits AKT, ERK, and JNK signaling pathways to reduce the expression of NFATc1 and CTSK, thus suppressing OC differentiation, reducing bone loss in the OVX-induced OP model, demonstrating prophylactic and therapeutic effects on OP	(118)

TABLE 3 Active ingredients of *Cornus officinalis* targeting vasculature system.

Sorts of compounds	Active constituents	Results	References
Flavonoids	Quercetin	Increases blood calcium and phosphorus contents, regulates autophagy and apoptosis of bone cells, thus preventing OP	(44, 46)
		Improves serum calcium, phosphorus, and other biochemical indexes, as well as the thickness, length, and density of the femur, and the tensile strength of the osteoporotic femur	(43)
		Increases bone turnover markers (serum ALP, OCN and urinary calcium, phosphorus, creatinine), HIF-1α gene expression, and NF-κB levels, while decreasing vascular endothelial growth factor (VEGF) and β-catenin expression	(42)
		Elevates the levels of OCN expression and ALP activity in serum, as well as urinary deoxypyridine base in diabetic rats	(50)
Iridoids	Morroniside	Upregulates the expression of CD31 and VEGFA in mice with myocardial infarction	(120)
		Upregulates the expression of Ang-1 and Tie-2 in rats with cerebral ischemia/reperfusion	(121)
Phenolic acid	Gallic acid	Regulates estrogen and improves calcium and phosphorus levels in the blood	(45)

officinalis and its active constituents as potential therapeutic anti-OC targets for the treatment of OP.

The vascular system is one of the therapeutic targets of *Cornus officinalis* and its active ingredients or compounds exert an anti-OP role

Angiogenesis, nutritional support function, and the metabolism of blood calcium, phosphorus, and glucose are directly related to bone development and regeneration. In the context of bone diseases, vascular function is often impaired, accompanied by metabolic imbalances (42–46, 50, 51). An increasing body of evidence demonstrates the significant role of *Cornus officinalis*, its compounds, and active ingredients in addressing this issue. The following Table 3 elaborates a detailed account of their respective targets in the prevention of OP.

These findings suggest that *Cornus officinalis* and its active component and compounds hold potential as a therapeutic option for OP prevention by promoting angiogenesis, enhancing bone blood flow through regulation of HIF-1α, CD31, VEGF and its receptors, and balancing calcium, phosphorus and glucose metabolism between blood vessels and bone tissues (122). Based on the aforementioned experimental data, *Cornus officinalis*, in conjunction with its formula and monomer compounds, offers potential advantages in improving blood vessels in bones and thus playing a role in the management of OP.

The immune system is one of the therapeutic target of *Cornus officinalis* and its active ingredients or compounds exert an anti-OP role

It has been found that several kinds of immune cells can interact with OB and OC to combat OP (52). As mentioned above (the immune system part), immune cells in bone system, including T lymphocytes, B lymphocytes and macrophage, can participate in the regulation of differentiation into OB or OC by the secretion of inflammatory factors such as interleukins and TNF. Therefore, improving the inflammatory microenvironment may have the potential to regulate the function of immune cells, promote BMSC differentiation into OB and inhibit the mature of OC precursor. Previous research has indicated that active ingredients in *Cornus officinalis*, such as 5-HMF, Cornuside, loganin, and sweroside, possess anti-inflammatory properties, however, it remains uncertain whether these substances also serve as preventatives for OP (123–126). In this context, a recent study has discovered that kaempferol may suppress the upregulation of proinflammatory cytokines induced by LPS in BMSC, promote the production of anti-inflammatory factors, and inhibit the process of osteoclastogenesis and bone resorption induced by proinflammatory factor IL-1β (94). Other studies also showed that quercetin, loganin or morroniside could enhanced the M2 macrophage polarization by targeting NF-κB and Nrf2 signaling pathways indicating potential ability to promote osteoblastic differentiation and inhibit osteoclastic differentiation (127–129). Consequently, we conclude that *Cornus officinalis* and its active ingredients represent a potential therapeutic class with anti-inflammatory properties that can inhibit the progression of OP.

Other factors are the other therapeutic targets of *Cornus officinalis* and its active ingredients or compounds to exert an anti-OP role

Oxidative stress and estrogen play important roles in maintaining bone balance, and abnormal expression of these factors leads to OP (83–86). Numerous reports have confirmed the significant effects of the active ingredients and compounds in *Cornus officinalis* for the treatment of OP. Table 4 elaborates on their respective targets in treating OP.

These results suggest that *Cornus officinalis*, along with its active components, can inhibit OB apoptosis and OC formation by suppressing oxidative stress response and binding estrogen receptors, which aids in promoting OB differentiation. Based on the above findings, both *Cornus officinalis* and its active

TABLE 4 The active ingredients of *Cornus officinalis* for other factors.

Sorts of compounds	Active constituents	Results	References
Flavonoids	Quercetin	Blocks the oxidative stress of chondrocytes, reduces apoptosis, NLRP3-mediated pyroptosis and ECM degradation	(130)
	Kaempferol	Phosphorylates ER, activates downstream ALP, Runx-2, OSX, COL1, OCN, and osteonectin, thus inhibiting OC differentiation but inducing OC apoptosis, and preventing OB apoptosis	(131)
	Notoginsenoside R1	Suppresses oxidative stress of MC3T3-E1 cells by blocking JNK pathway, thereby restoring the ability of osteogenic differentiation	(132)
		Binds to estrogen receptor as a phytoestrogen, promoting the transcription of COL1, osteonectin, OCN, Runx2, and osterix, thereby facilitating osteogenic differentiation and mineralization	(133)

TABLE 5 The *Cornus officinalis*-containing formulations for anti-OP effect.

TCM formula	Model	Dosage and duration	Results	References
LWDHP	OVX-treated SD rats	100 g/day for 12 weeks	Stimulates osteogenetic process by activating the Wnt/β-catenin signaling pathway	(134)
	Citrate buffer-induced diabetic mice	1.8 or 3.6, or 5.4 g/kg/day for 12 weeks	Improves BMD, BV, bone microstructure, maximum load, and bending resistance in the femurs of osteoporotic rats	(135)
ZGP	OVX-treated rats	32 g/kg/day for 12 weeks	Reverses the Th17/Treg ratio, leading to increased BMD and inhibition of bone loss in OVX mice	(136)
	OVX-treated SD rats	2.3 or 4.6 g/kg/day for 12 weeks	By combining with anti-OP medicines, ZGP treatment significantly reduces bone resorption markers such as TRACP, TRACP-5b, urine oxidative deamino acid/creatinine ratio D-Pyr/Cr, and β-cross-linked C-terminal type 1 collagen	(137)
	glucocorticoid-induced SD rats	62.3 g/kg/day for 1 month	Modifies the orderly arrangement of bone trabecular compositions and bone microarchitecture, intensifies bone mechanics, and substantially increases BMD in the lumbar spine	(138)
		3.8 g/kg/day for 6 weeks	In combination with ED-71, it reduces blood glucose levels in diabetic mice and promotes osteogenic differentiation through the PI3K-AKT signaling pathway	(51)
BSTLD	OVX-treated rats	6 or 12 g/kg/day for 12 weeks	Increases blood perfusion in the bone marrow cavity	(33)

components offer potential advantages in maintaining bone balance and thus taking an important part in the treatment and prevention of OP.

An anti-OP effect exerted by *Cornus officinalis*-containing formulations

Cornus officinalis is a constituent of many TCM formulations such as ZGP, YGP, LWDHP, Bu-Shen-Tong-Luo decoction (BSTLD), all of which are known for their kidney-nourishing properties, and extensive evidence supports the favorable impact of these formulations in the prevention and treatment of OP (Table 5) (33, 51, 134–138).

These results suggest that *Cornus officinalis*-containing formulations could mainly improve BMD and bone microstructure, stimulate osteogenetic process, increases blood perfusion in bone marrow by reversing the Th17/Treg ratio or targeting PI3K-AKT and Wnt/β-catenin signaling pathway. Based on the above findings, *Cornus officinalis*-containing formulations offer potential advantage in promoting osteogenesis in the treatment and prevention of OP.

Conclusion and perspectives

OP is a skeletal condition characterized by reduced BMD and compromised trabecular bone structure, which significantly increases the likelihood of fractures and imposes substantial physical and financial burdens. Within TCM application, *Cornus officinalis* is widely employed for OP treatment. Both preclinical and clinical investigations have demonstrated the effectiveness of the chemical constituents and associated formulations of *Cornus officinalis* in preventing OP, which efficacy is attributed to various mechanisms, including the modulation of bone homeostasis, promotion of angiogenesis, anti-inflammatory effects, and regulation of the immune system, etc (Figure 2). Therefore, phytochemicals from *Cornus officinalis* possess significant potential for the development of novel anti-osteoporotic medications. Herein, we provide a comprehensive review of the role of *Cornus officinalis* in multiple anti-OP mechanisms, which aligns with the multifactorial nature of OP's etiology and surpasses the traditional model of single drug targeting single aspects of medicine. By thoroughly investigating the therapeutic properties of *Cornus officinalis* in the context of OP treatment, our aim is to enhance our understanding of TCM's underlying mechanisms in addressing

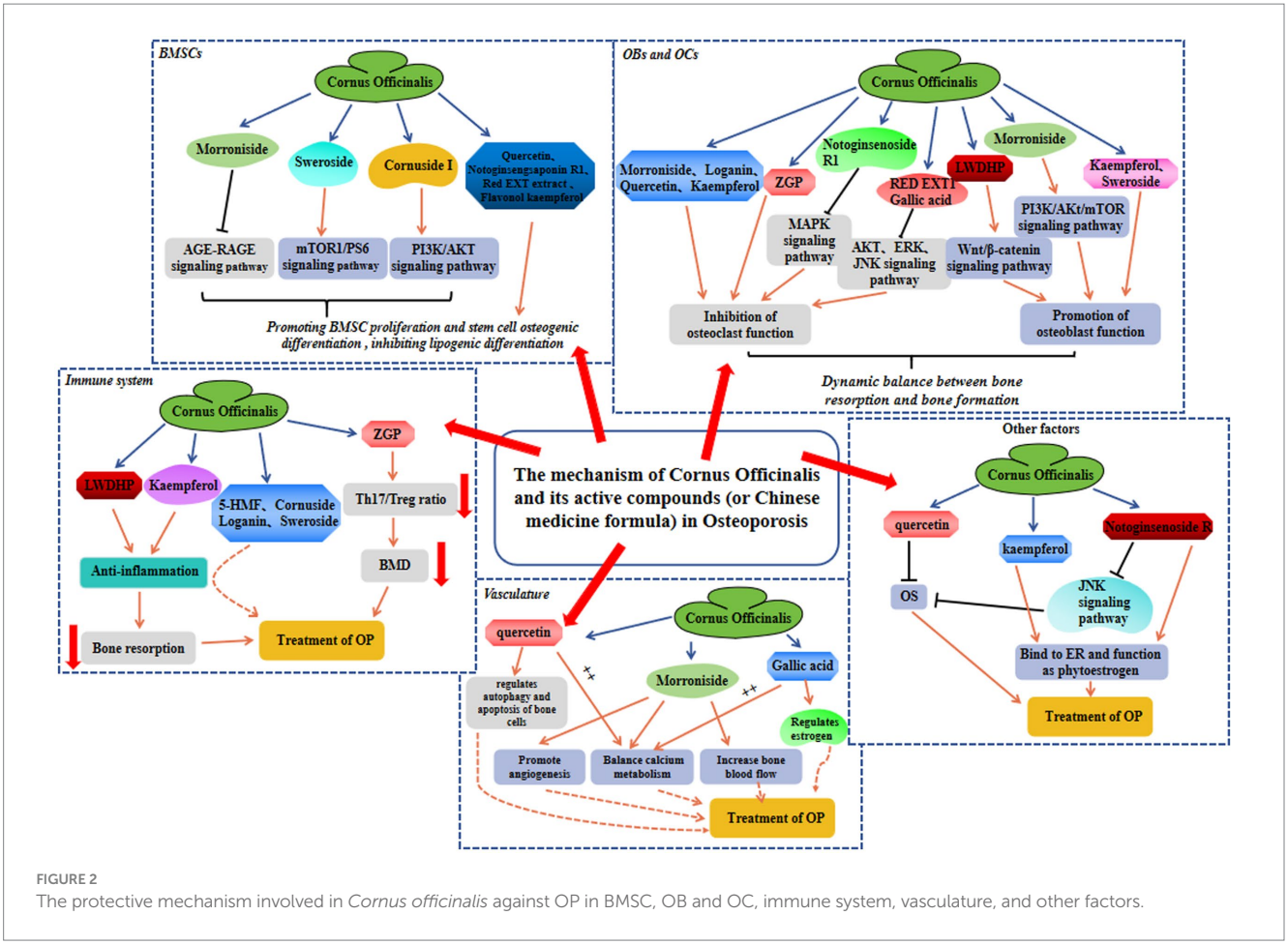

FIGURE 2
The protective mechanism involved in *Cornus officinalis* against OP in BMSC, OB and OC, immune system, vasculature, and other factors.

this condition. This endeavor is expected to greatly contribute to the advancement of more efficacious pharmaceutical interventions for OP. Thus, systematic data mining of the existing *Cornus officinalis* database can undoubtedly aid in the drug discovery process by identifying safe candidates.

While several compounds, such as quercetin and kaempferol, extracted from *Cornus officinalis*, have been extensively studied in relation to OP, further investigation is necessary to explore the potential effects of sweroside, notoginsenoside R1, cornuside I, morroniside, and loganin. Additionally, it is essential to identify the active components of *Cornus officinalis* through comprehensive investigations. Moreover, limitations exist in the current use of animal models for OP research. The majority of *in vivo* studies employ rodent models, which possess dissimilar cortical-to-cancellous bone ratios compared to humans, and inter-species cellular differences result in deficiencies in both *in vivo* and *in vitro* experiments, which must be further corroborated using in mammalian or primate models (139, 140). Furthermore, the existing research primarily focuses on the efficacy of *Cornus officinalis* in preclinical experiments, necessitating the need for clinical trials to substantiate its effectiveness and safety.

Author contributions

XTa: Writing – original draft. YH: Writing – original draft. XF: Writing – original draft. XTo: Writing – original draft. QY: Writing – original draft. WZ: Writing – review & editing. FF: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Efficacy of the Chinese herbal medicine Jintiange capsules in the postoperative treatment of osteoporotic vertebral compression fractures: a systematic review and meta-analysis

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Background: In traditional Chinese medicine, Jintiange capsules are frequently used to treat metabolic bone diseases and strengthen bones and tendons. The main component of Jintiange capsules is bionic tiger bone powder. However, the active ingredients and proteins are derived from other animal bones, with chemical profiles similar to that of natural tiger bone. This study aimed to explore the efficacy of Jintiange capsules, a Chinese herbal medicine, in the postoperative treatment of osteoporotic vertebral compression fractures (OVCFs).

Methods: In this systematic review, literature was retrieved using PubMed, the Cochrane Library, the Chinese National Knowledge Infrastructure, the Web of Science, the Wanfang Database, the Chinese Biomedical Literature Database, and the Chinese VIP Database from inception to July 2023. The primary outcome measures were the bone mineral density (BMD) and effective rate. The secondary outcome measures were the visual analog pain score (VAS), Oswestry disability index (ODI), Cobb's angle, serum osteocalcin, serum alkaline phosphatase, and adverse events. RevMan 5.4 and STATA 17.0 software were used for data analysis.

Results: We enrolled randomized controlled trials (RCTs) focusing on 1,642 patients in the meta-analysis. The meta-analysis illustrated that Jintiange capsules significantly increased the BMD of the lumbar spine ($p < 0.00001$), femoral neck ($p = 0.0005$), and whole body ($p = 0.01$). The subgroup analysis of Jintiange capsules combination therapy showed that the BMD of the lumbar spine and whole body was significantly improved with Jintiange capsules ($p < 0.00001$). The test for the overall effect showed that Jintiange capsules had a significantly higher effective rate than the control groups ($p = 0.003$). Additionally, the overall effect test showed that Jintiange capsules decreased the VAS and ODI ($p < 0.00001$) and Cobb's angle ($p = 0.02$), and improved serum OC and ALP ($p < 0.00001$) compared with the controls. Furthermore, the pooled analysis of adverse reactions showed no serious impacts on the treatment of OVCFs.

Conclusion: Jintiange capsules demonstrate high safety and efficacy in the treatment of OVCFs, including increasing BMD, the lift effect rate, serum OC levels, and pain relief, decreasing the ODI, serum ALP levels, and adverse events, and improving Cobb's angle. Additional research is required to validate the efficacy of Jintiange capsules for the postoperative treatment of OVCFs.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO.

KEYWORDS

Chinese herbal medicine, Jintiange capsules, osteoporosis, osteoporotic vertebral compression fractures, systematic review and meta-analysis

1 Introduction

Osteoporosis (OP) is a systemic disease characterized by low bone mass, decreased bone strength, increased skeletal fragility, and bone tissue destruction (1). Osteoporosis affects approximately 10 million people aged over 50 years in the United States and 34.65% of those over 50 years of age in China (2). Among older adults, osteoporotic vertebral compression fractures (OVCFs) are one of the most severe manifestations of osteoporosis (3). The clinical symptoms of OVCFs, including severe kyphosis, lower back pain, and frequent relapse, impact the physical and mental health, quality of life, and life expectancy of patients with OVCFs (4). The most common non-surgical treatment of OVCFs is bed rest, analgesics, and estrogen replacement therapy (5). Although beneficial, these have several side effects that prevent pain relief and progression over the long term (6). Although percutaneous vertebroplasty and percutaneous kyphoplasty (PVP/PKP) are frequently used to treat OVCFs, they do not relieve osteoporosis-related pain or prevent adjacent vertebral fractures (7). Therefore, developing alternative medications with fewer adverse effects and novel therapeutic techniques for postoperative OVCF treatment is essential and feasible.

Currently, bisphosphonates, calcium agents, and estrogens are widely used to prevent and treat OVCFs. Although numerous anti-OVCF medications with diverse pharmacological properties are available, the targeted therapeutic effect is not attained in significant numbers of individuals with OVCFs (8). Some studies have shown that bisphosphonates can selectively adhere to and remain within the bone and promote the apoptosis of osteoclasts, but the long-term use of the medication can lead to gastrointestinal side effects and induce bone microdamage accumulation and atypical insufficiency fractures in the skeletal system (9). Calcium has been reported to increase the risk of myocardial infarction in the long term because OVCFs require long-term treatment, in which the harm and benefits of medications are unavoidable and need to be well balanced (10).

Traditional Chinese medicine has long been a treatment for OP, tonifying the kidneys and strengthening the bones in accordance with Chinese medicine treatment principles (11). For the treatment of OP, natural Chinese herbal medicine possesses distinctive advantages that improve bone quality and biomechanical properties and influence the fate of bone cells during bone remodeling (12). Jintiange capsules are a common Chinese medicine, the primary ingredient of which is artificial tiger bone powder (13). Modern pharmacological studies have demonstrated that tiger bone is abundant in collagen, amino acids, and minerals and can play important anti-inflammatory and analgesic roles as well as strengthening tendons and bones (14). Existing evidence shows that natural tiger bone can reduce fracture risk and relieve pain in OVCFs (15). However, the use of natural tiger bone is prohibited because the tiger is a protected animal in China. To meet the medical demand, artificial tiger bone powder has been developed to substitute natural tiger bone, and its structure closely resembles that of natural tiger bone (16, 17). Several clinical studies have indicated that multiple organic constituents of Jintiange capsules, such as amino acids, calcium, magnesium, phosphorus, iron, copper, manganese, and zinc, can improve bone formation, inhibit bone absorption, and accelerate the metabolism to absorb calcium for the postoperative treatment of OVCFs (18, 19). Although Jintiange capsules have been regarded as an effective Chinese patent medicine for patients with OVCFs in China and are recommended in Chinese OP treatment guidelines, there is currently a lack of high-quality evidence regarding the efficacy of Jintiange capsules in the treatment of OVCFs (20). Therefore, it is imperative to conduct this Systematic Review and meta-analysis to evaluate the efficacy of Jintiange capsules in the postoperative management of OVCFs.

2 Materials and methods

This study strictly followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (21). The ethical review was unnecessary because the data from the articles included in this meta-analysis were publicly available. The study was registered on PROSPERO (CRD42023456068).

2.1 Search strategy

We conducted an electronic search of the following seven authoritative literature databases: PubMed, the Cochrane Library, the Chinese National Knowledge Infrastructure (CNKI), the Web of Science, the Wanfang Database, the Chinese Biomedical Literature Database, and the Chinese VIP Database from their inception until July 2023. Furthermore, each database employed a combination of MeSH terms and free words for comprehensive study searches, and the language of the published literature was unrestricted. The relevant keywords for Jintiange were "Jintiange capsule" OR "artificial tiger bone" OR "Jintiange" OR "bionic tiger bone." We retrieved keywords of OP, including "osteoporosis" OR "postmenopausal osteoporosis" OR "bone mass" OR "bone loss" OR "bone mineral density." The keywords for OVCFs were "compression fractures" OR "lumbar compression fracture" OR "thoracic vertebral compression fractures" OR "osteoporosis vertebral compression fractures" OR "OVCFs."

Abbreviations: OP, Osteoporosis; OVCFs, Osteoporotic vertebral compression fractures; PVP, Percutaneous vertebroplasty; PKP, Percutaneous kyphoplasty; CNKI, China National Knowledge Infrastructure; BMD, Bone mineral density; VAS, Visual analog scale; ODI, Oswestry disability index; OC, Osteocalcin; MD, Mean difference; CI, Confidence interval; RR, Risk ratio.

2.2 Eligibility criteria

We only included RCTs that compared Jintiange capsules with conventional western therapies and placebos for the postoperative treatment of OVCFs. The intervention group used Jintiange alone or with other conventional western therapies. The control group received only conventional western therapies or a placebo. Participants were diagnosed with OVCFs regardless of age, gender, dosage, duration, or disease course. Primary outcomes included bone mineral density (BMD) and effective rate; secondary outcomes included visual scale (VAS), the Oswestry Disability Index (ODI), Cobb's angle, serum osteocalcin (OC), and adverse events. Additionally, all outcome indicators had to specify the results of the pooled data.

2.3 Exclusion criteria

The exclusion criteria included the following: (1) non-randomized controlled trials; (2) participants that were not diagnosed with OVCFs; (3) RCTs with participants who did not receive postoperative treatment; (4) studies that did not use Jintiange capsules or any other Chinese herbals or Traditional Chinese Medicine therapies; and (5) meeting abstracts, reviews, duplicate publications, animal experiments, and insufficient clinical data.

2.4 Data extraction

Two researchers extracted and collated the basic information of the final studies based on inclusion and exclusion criteria. Additionally, a third researcher examined the results independently. For this meta-analysis, author, publication year, study type, sample size, age of participants, operation method, number of study centers, follow-up time, intervening measures, and course of treatment were required as general information about the study. Disagreements between the two researchers about the pooled data were determined and resolved by consulting with a third researcher.

2.5 Quality assessment of the included studies

Two independent authors evaluated the risk of bias for each trial study using the Cochrane systematic reviews. This evaluation tool has seven items: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias. Each domain can be judged as low bias risk, high bias risk, and unclear bias risk in the literature (22, 23).

2.6 Statistical analysis

All the enrolled studies were conducted on the Review Manager Software (RevMan5.4), and the STATA software (STATA Software Version 17.0) was used to evaluate publication bias and execute sensitivity analysis. The continuous outcome variables were presented as mean difference (MD) with a 95% confidence interval (95% CI), and we measured the same outcomes using standardized mean differences (SMD) of 95% CIs. We extracted the number of positive events based on binary outcome variables and calculated the risk ratio (RR) with 95% confidence intervals for both categories. The heterogeneity of the trial was assessed using I^2 statistics, among which $p < 0.05$ or $I^2 > 50\%$ was evaluated to have high heterogeneity, and we used a random-effects model. Each article that contributed to heterogeneity was excluded from the sensitivity analysis; otherwise, the fixed-effects model was used when the heterogeneity between the enrolled studies was small ($p \geq 0.05$ or $I^2 \leq 50\%$). Furthermore, subgroup analysis was applied to the trial based on Jintiange capsules, separated into Jintiange combined intervention subgroup and Jintiange alone intervention subgroup. A funnel plot was constructed to evaluate potential publication bias, which was analyzed using Egger's test.

3 Results

3.1 Screening results of the studies

According to the screening strategy, 148 articles were initially identified after removing duplicate literature. Next, we eliminated 102 articles by scanning the titles and abstracts. After applying the inclusion and exclusion criteria, the full text of the 42 studies met the requirement with the Jintiange Capsule, of which 16 studies on the postoperative treatment of patients with OVCFs ultimately met the inclusion criterias of this meta-analysis (18, 24–38). Figure 1 shows the search process and selection details.

3.2 Characteristics of the included studies

Through searching and screening, this meta-analysis included 16 clinical studies, all of which were RCTs on the postoperative treatment of patients with OVCFs with Jintiange capsules. These enrolled studies were all conducted in China. Overall, 16 RCTs included 1,642 participants, of which 822 were in the intervention group and 820 were in the control group. The year of publication of the included trials ranged from 2014 to 2023. All the reported groups in the studies were matched in terms of age, gender, operation, course of disease, and outcome. The operation methods for OVCFs were PVP, PKP, and DHS. Among all studies of the intervention group, 10 studies involved combined therapies and six were single treatment studies with only Jintiange capsules. The daily dose of Jintiange capsules is 1.2 g. The course of interventions was between 1 month and 12 months. Table 1 shows the specific details and characteristics of all the included studies.

3.3 Literature quality assessment

Each RCT employed the Cochrane Collaboration's tool to evaluate the risk of bias. Most of the 16 articles enrolled in this meta-analysis were randomized controlled trials, among which three studies did not document the random allocation approach (21, 26, 29). Although only one study mentioned the concealment allocation and others did not clearly describe the method (12), it

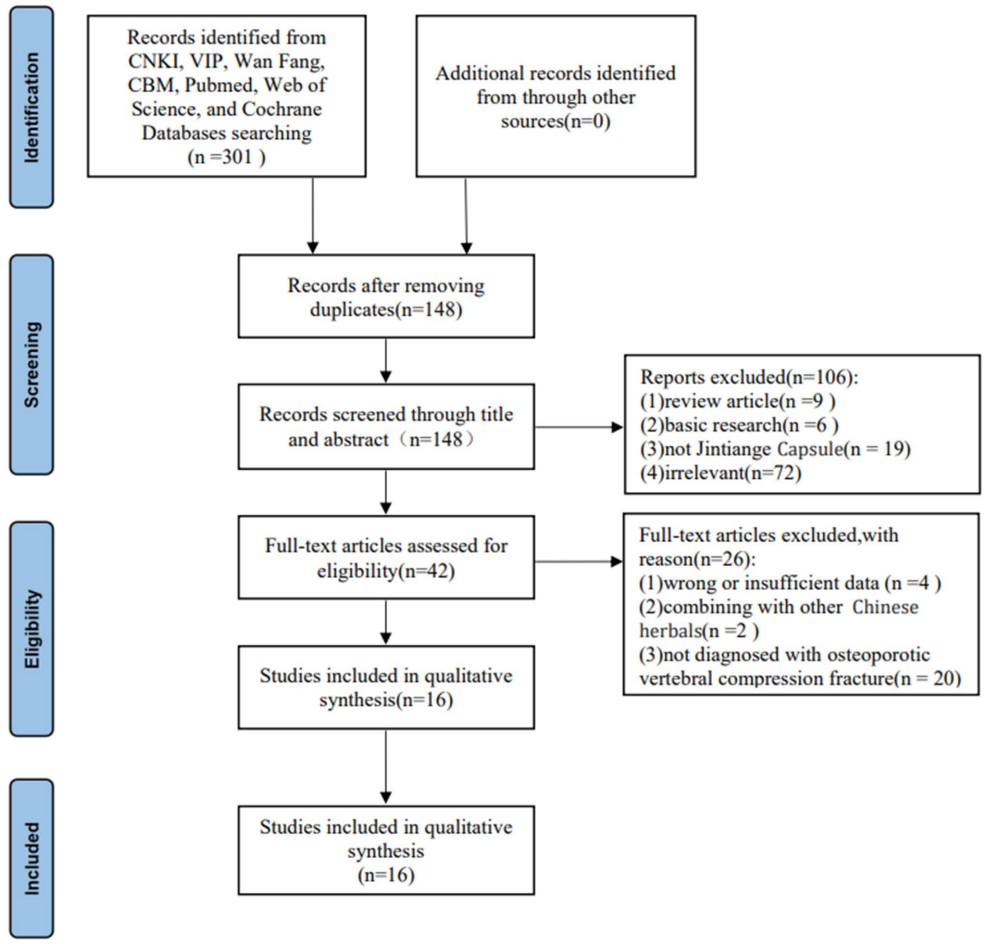

FIGURE 1
Flowchart of the literature search process.

was unlikely to affect the data evaluation. With one of the 16 studies considered to have a low risk of performance bias for the blinding of participants and blinding of outcome assessment (12), the residual studies included remained unclear. Fourteen RCTs did not meet the incomplete outcome data criterion or had no drop-out patient data (12, 18–22, 24, 25, 27–32). One study did not provide a precise explanation of the selective reporting of data (19), which may lead to potential outcomes risk bias. Baseline comparisons were in accordance with the published prespecified analysis plan. No information mentioned that the baseline comparisons were imbalanced, and other biases were not present in the enrolled studies. The details about the risk of bias evaluation for each study are shown in Figure 2.

3.4 Results of the meta-analysis

3.4.1 Primary outcomes

3.4.1.1 BMD of the lumbar spine

Seven studies (28–30, 32, 34, 36, 38) reported BMD changes at the lumbar spine. These enrolled studies showed that the test for the overall effect of the Jintiange capsules significantly increased the BMD of the lumbar spine compared with the controls (SMD = 1.12, 95% CI: 0.96 to 1.28, $p < 0.00001$). Subgroup analyses of the Jintiange capsules combined intervention reported that the Z value of the test for overall effect was larger than the Jintiange capsules alone intervention group ($Z = 14.42$), which further suggests that the Jintiange capsules combined intervention appears to be superior at improving the BMD of the lumbar spine (Figure 3).

3.4.1.2 BMD of the femoral neck

Three studies reported the effects of the Jintiange capsules on the BMD in the femoral neck (24, 34, 36). The pooled data demonstrated a significant difference in lifting BMD at the femoral neck between the Jintiange capsules and control groups (MD = 0.08, 95% CI: 0.04 to 0.13, $p = 0.0005$) (Figure 4).

3.4.1.3 BMD of the whole body

Eight studies (18, 25–27, 30, 33, 35, 37) reported percentage changes in whole-body BMD. The available data illustrated that compared with the control groups, the overall effect test of the Jintiange capsules showed a significant difference in the BMD of the whole body (SMD = 1.67, 95% CI: 0.40 to 2.93, $p = 0.01$). Additionally, the subgroup analysis showed that the Jintiange capsules combined intervention had a superior effect on

TABLE 1 Characteristics of the 16 included studies.

Study	Study design	Sample size (EG/CG)	Operation method	Mean age		Interventions		Course of treatment
				EG	CG	EG	CG	
Xu (2023)	RCT	116 (58/58)	PVP	61.51 ± 5.23	61.54 ± 5.24	Jintiange capsules (1.2 g, tid) + CG	Alendronate (70 mg, qw) + calcium carbonate D3 (600 mg, bid) + salmon calcitonin (20 μg, qd)	3 months
Sun et al. (2023)	RCT	58 (28/30)	PKP	77.64 ± 5.96	76.76 ± 6.47	Jintiange capsules (1.2 g, tid)	Calcium carbonate D3 (600 mg, bid)	3 months
Shu and Zhang (2022)	RCT	106 (53/53)	PKP	67.5 ± 2.7	67.8 ± 2.8	Jintiange capsule (1.2 g, tid)	Conventional western medication	3 months
Qiu et al. (2022)	RCT	150 (75/75)	PVP	65.71 ± 6.85	65.42 ± 7.29	Jintiange capsules (1.2 g, tid) + CG	Calcium carbonate D3 (600 mg, qd) + calcitriol capsule (0.25 μg, qd)	6 months
Yang (2021)	RCT	66 (33/33)	NA	69.32 ± 1.52	69.31 ± 1.55	Jintiange capsules (1.2 g, tid) + CG	Calcium carbonate D3 (750 mg, tid) + alendronate (60 mg, qw)	6 months
Wu et al. (2021)	RCT	78 (39/39)	PKP	66.15 ± 8.12	65.37 ± 8.28	Jintiange capsules (1.2 g, tid) + CG	Calcium carbonate D3 (600 mg, qd) + alendronate (70 mg, qw) + salmon calcitonin (20 μg, qd)	6 months
He et al. (2021)	RCT	86 (43/43)	IFS	66.42 ± 8.82	65.22 ± 6.78	Jintiange capsules (1.2 g, tid)	Calcium carbonate D3 (1,200 mg, bid)	1 month
Han et al. (2021)	RCT	80 (40/40)	PKP	68.8 ± 5.70	70.2 ± 6.2	Jintiange capsules (1.2 g, tid)	Calcium carbonate D3 (600 mg, bid)	6 months
Huang (2020)	RCT	58 (29/29)	NA	70.21 ± 6.58	70.85 ± 6.49	Jintiange capsules (1.2 g, tid)	Calcium carbonate D3 (100 mg, tid) + alendronate (70 mg, qw)	1 month
Huang and Pang (2020)	RCT	128 (64/64)	NA	68.83 ± 2.91	69.02 ± 2.74	Jintiange capsules (1.2 g, tid) + CG	Calcium carbonate D3 (600 mg, bid)	6 months
Wang (2019)	RCT	96 (48/48)	IFS	62.5 ± 8.0	62.30 ± 6.7	Jintiange capsules (1.2 g, tid)	Alfacalcidol (0.5 μg, qd)	6 months
Tan (2019)	RCT	104 (52/52)	PVP	NA	NA	Jintiange capsules (1.2 g, tid)	Calcium (600 mg, qd) + vitamin D3 (125 U, qd)	6 months
Li (2019)	RCT	60 (30/30)	NA	72.5 ± 5.2	73.0 ± 5.3	Jintiange capsules (1.2 g, tid)	Calcium carbonate (750 mg, tid) + alendronate (70 mg, qw)	1 month
Li et al. (2018)	RCT	136 (68/68)	PVP	76.6 ± 8.4	75.9 ± 8.9	Jintiange capsule (1.2 g, tid)	Calcium carbonate D3 + zoledronic acid + calcitriol capsules	3 months
Yan (2015)	RCT	120 (60/60)	PVP	72.5	72.5	Jintiange capsules (1.2 g, tid)	Calcium (200 mg, qd)	3 months
Chen and Bi (2014)	RCT	200 (100/100)	PVP	65.4	65.4	Jintiange capsule (1.2 g, tid) + CG	Calcium carbonate D3 (600 mg, bid) + salmon calcitonin (50 IU, qd)	3 months

RCT, randomized controlled trial; EG, experimental group; CG, control group; PVP, percutaneous vertebroplasty; PKP, percutaneous kyphoplasty; IFS, internal fixation surgery; qd, once a day; bid, twice a day; tid, three times a day; qw, once a week; NA, not available.

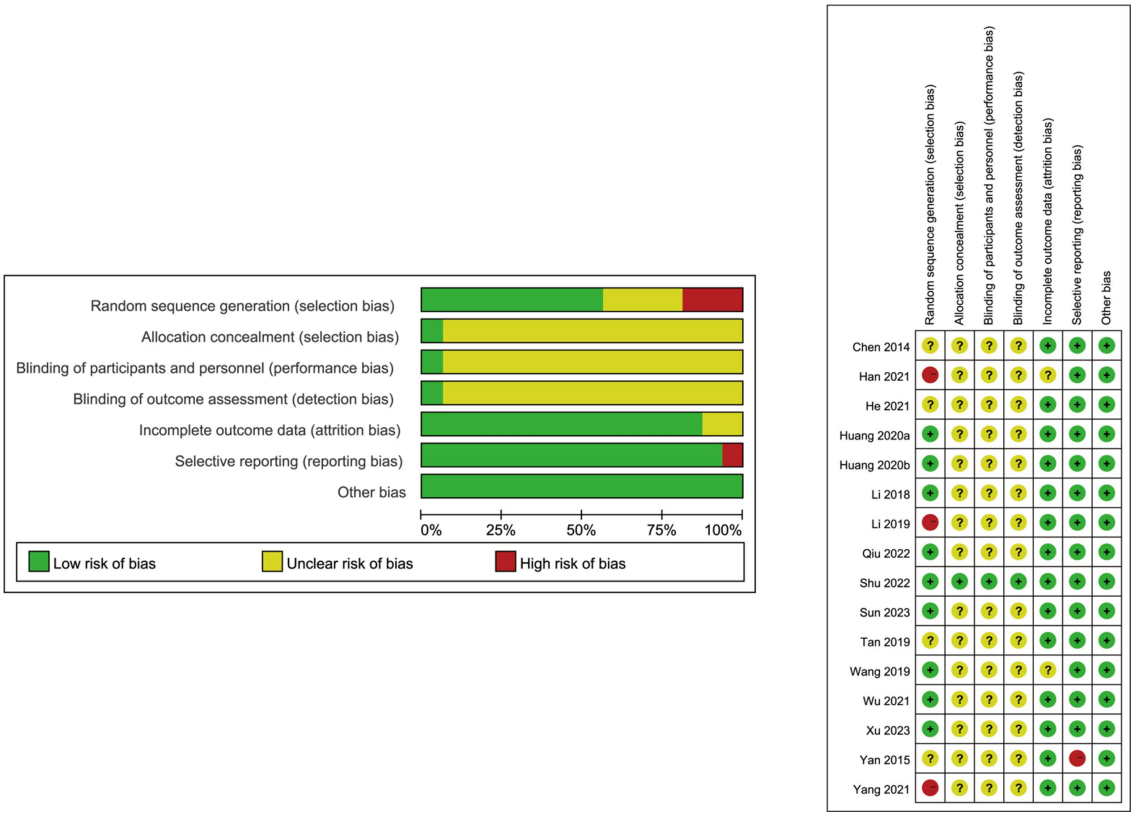

FIGURE 2
Risk of bias summary of the 16 included studies.

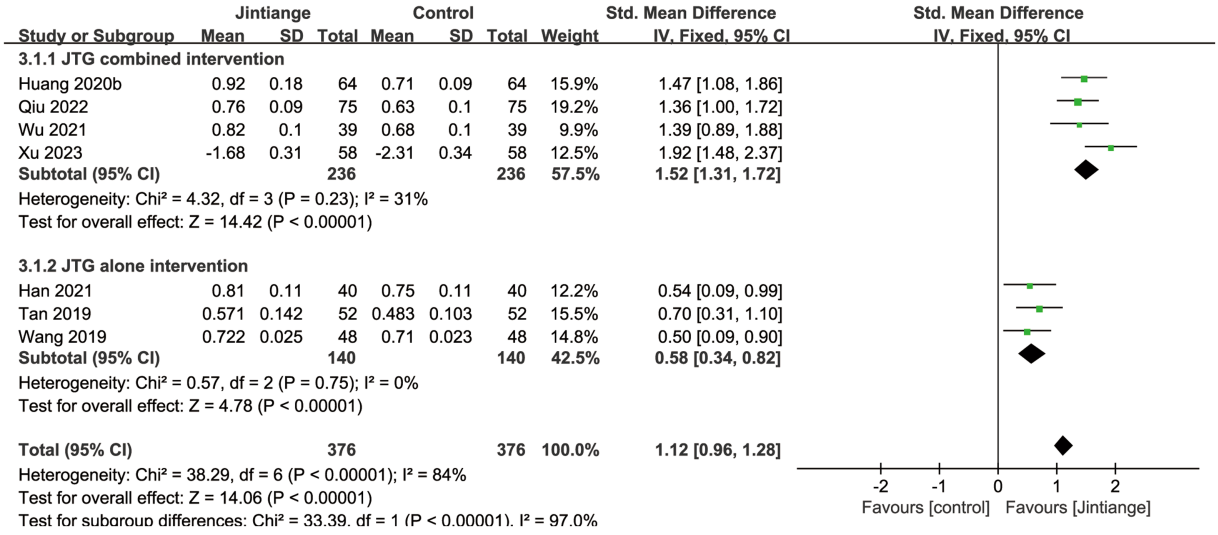

FIGURE 3
Forest plot of the change in lumbar spine BMD.

whole-body BMD relative to the Jintiange capsules alone (SMD =10.0, 95% CI: 8.18 to 11.82, $p < 0.00001$). However, the pooled results showed no significant difference in whole-body BMD changes between the Jintiange capsules and the control groups ($p = 0.22$) (Figure 5).

3.4.1.4 Effective rate

Nine studies (18, 24, 25, 27, 31, 33–35, 38) showed percentage changes in the effective rate. The test for the overall effect of these nine studies showed that Jintiange capsules significantly improved the effective rate in contrast with control groups (RR = 1.14, 95% CI:

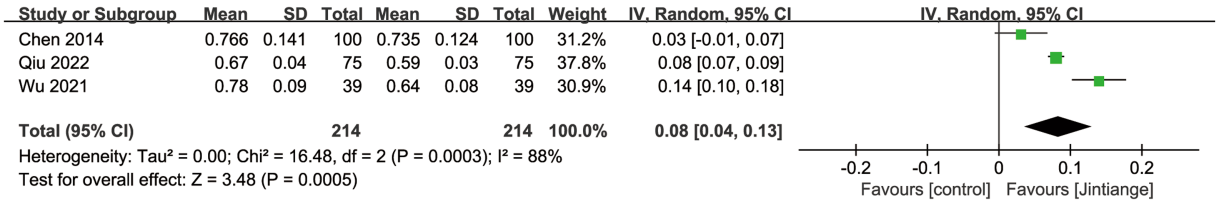

FIGURE 4
Forest plot of the change in femoral neck BMD.

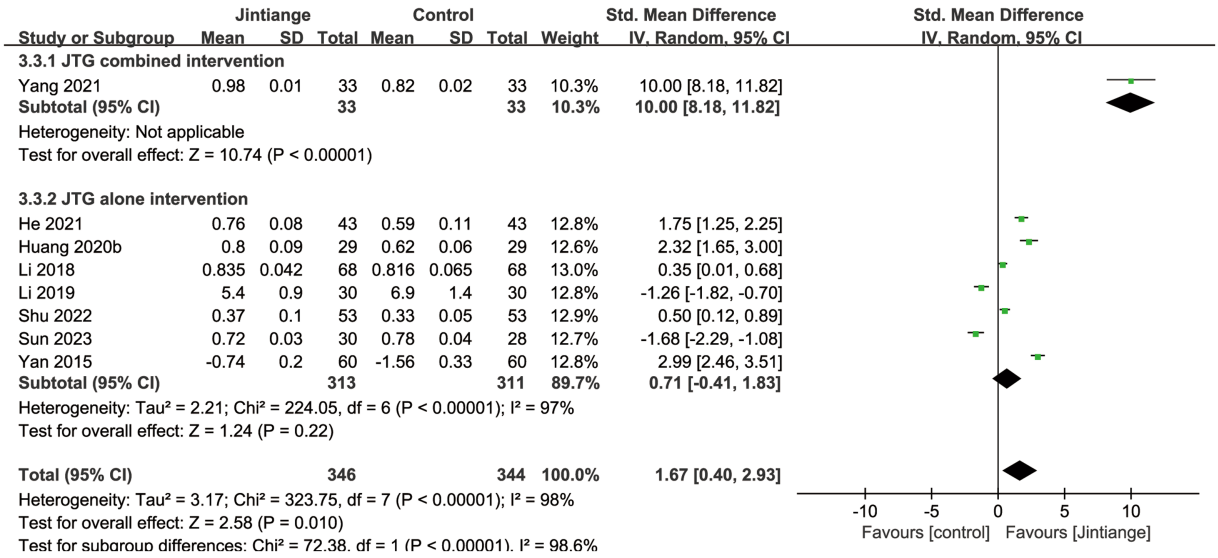

FIGURE 5
Forest plot of the changes in whole-body BMD.

1.05 to 1.25, $p = 0.003$). Subgroup analysis of the effective rate showed that the Jintiange capsules alone intervention was also superior to that of the control groups ($p = 0.003$). However, the pooled results showed that the Jintiange capsules combined intervention did not significantly impact the effective changes between the Jintiange capsules group and the control group ($p = 0.12$). Therefore, the heterogeneity of the Jintiange capsules combined intervention was eliminated after removing Wu et al. The subgroup analysis demonstrated that the Z value of the Jintiange capsules combined intervention increased ($Z = 4.54$), the p-value decreased ($p < 0.00001$), and the 95% confidence interval was narrowed down (1.09 to 1.24). These changes further suggest that the Jintiange capsules combined intervention appears to be better at increasing the effective rate (Figure 6).

3.4.2 Secondary outcomes

3.4.2.1 VAS

Ten studies showed the assessment of the VAS. Meta-analysis results showed that the overall effect test reported a significant difference between the Jintiange capsules and the control groups (MD = −1.24, 95% CI: −1.78 to −0.70, $p < 0.00001$) (18, 24–27, 31, 32, 34, 36, 37). Similarly, the subgroup analyses illustrated that both the Jintiange capsules combined and alone interventions remarkably reduced the VAS score relative to controls ($p = 0.02$, $p = 0.0001$) (Figure 7).

3.4.2.2 ODI

Five studies (18, 29, 31, 32, 36) reported the percentage changes in the ODI. This meta-analysis illustrated that the overall effect test showed a statistically significant difference in the comparison of ODI levels with the control group (MD = −7.39, 95% CI: −9.36 to −5.42, $p < 0.00001$). The subgroup analysis of the Jintiange capsules combined and alone interventions showed that the Jintiange capsules treatment resulted in a significant decrease in the ODI compared with the control group ($p < 0.00001$) (Figure 8).

3.4.2.3 Cobb's angle

This meta-analysis analyzed three studies related to Cobb's angle (18, 36, 37). The available data of both overall effect test and the subgroup analysis of the Jintiange capsules combined intervention showed that the Jintiange capsules had a superior effect in improving Cobb's angle than the control group ($p < 0.00001$, $p = 0.02$). However, there was no statistically significant difference between the subgroup of the Jintiange capsules alone and the control condition ($p = 0.11$) (Figure 9).

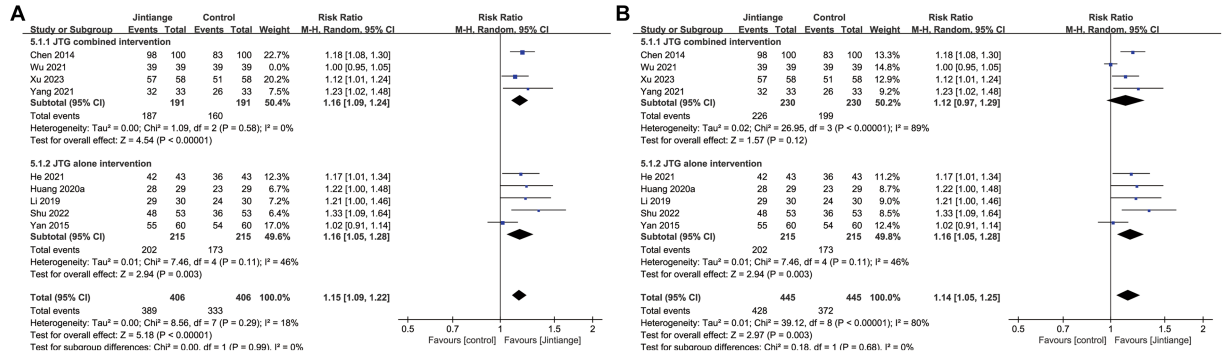

FIGURE 6
(A) Forest plot of the effective rate. **(B)** Forest plot of the effective rate after eliminating the study by Wu.

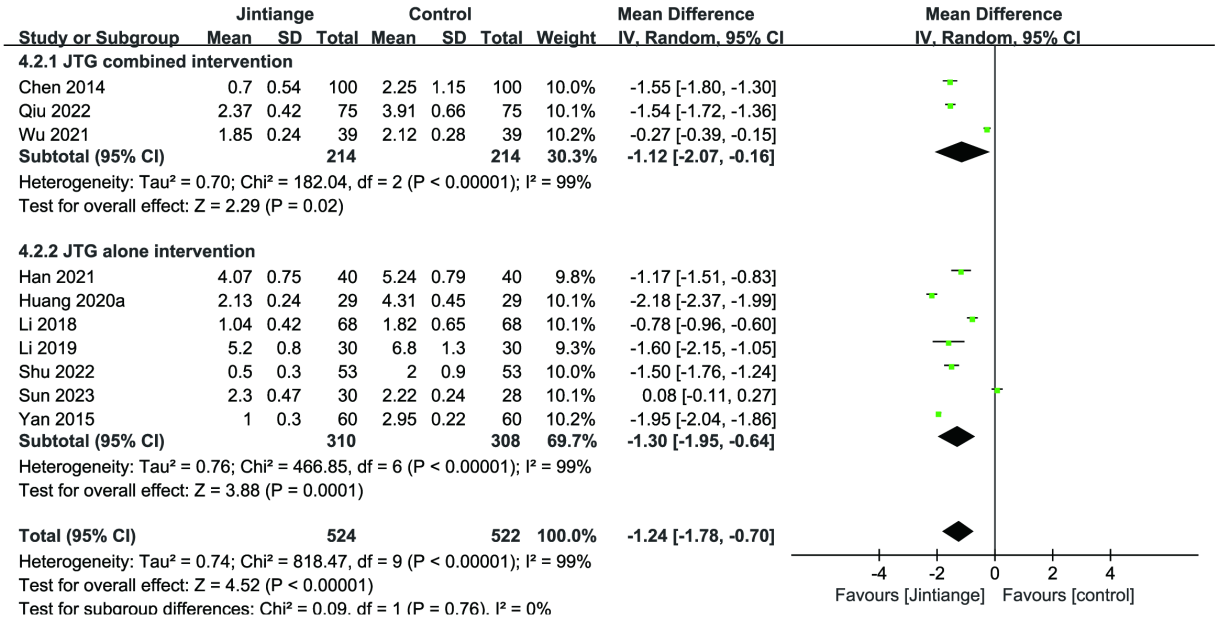

FIGURE 7
Forest plot of the changes in the VAS.

3.4.2.4 Serum OC

Four studies (30, 32, 34, 35) reported the changes in the serum OC. As illustrated in Figure 5, the pooled data depicted that the overall effect test showed a significant difference between the Jintiange capsules and control groups (MD =2.14, 95% CI: 0.86 to 3.42, $p = 0.001$). The results of the subgroup analysis indicated that the Jintiange capsules combined and alone interventions also significantly increased serum OC in contrast with the control group ($p = 0.01$, $p < 0.00001$) (Figure 10).

3.4.2.5 Serum ALP

Two studies (30, 35) analyzed the changes in serum ALP. The overall effect test of the pooled data demonstrated that the Jintiange capsules significantly reduced serum ALP in contrast with the control group (SMD = −11.35, 95% CI: −16.19 to −6.50, $p < 0.00001$). This result further suggested that the Jintiange capsules combined intervention was remarkable in improving the changes in serum ALP (Figure 11).

3.4.3 Adverse events

Six trials reported information on adverse events and 10 did not (21, 24, 25, 27, 29, 32). The incidence of adverse events was 10/238 in the Jintiange capsules group and 45/238 in the control group. The reporting of adverse events included dry mouth, recurrent fractures, constipation, belching, abdominal distension, diarrhea, nausea, and vomiting. Four studies showed that five patients in the Jintiange capsules group and 28 patients from the control group developed mild gastrointestinal symptoms. In the other two studies, 17 subjects developed recurrent fractures in the controls, among which, one study revealed that five subjects developed recurrent fractures in the

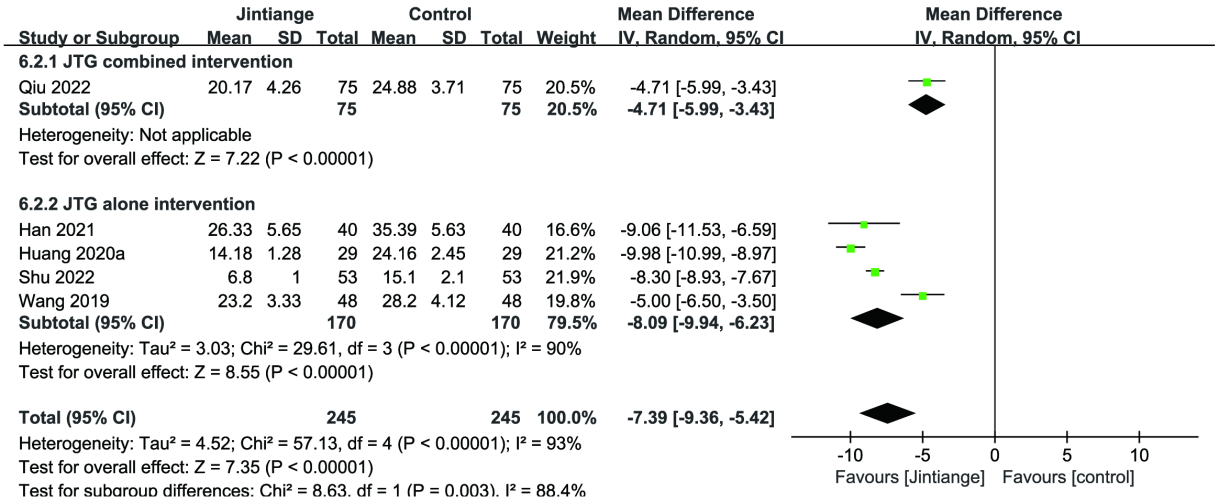

FIGURE 8
Forest plot of the changes in the ODI.

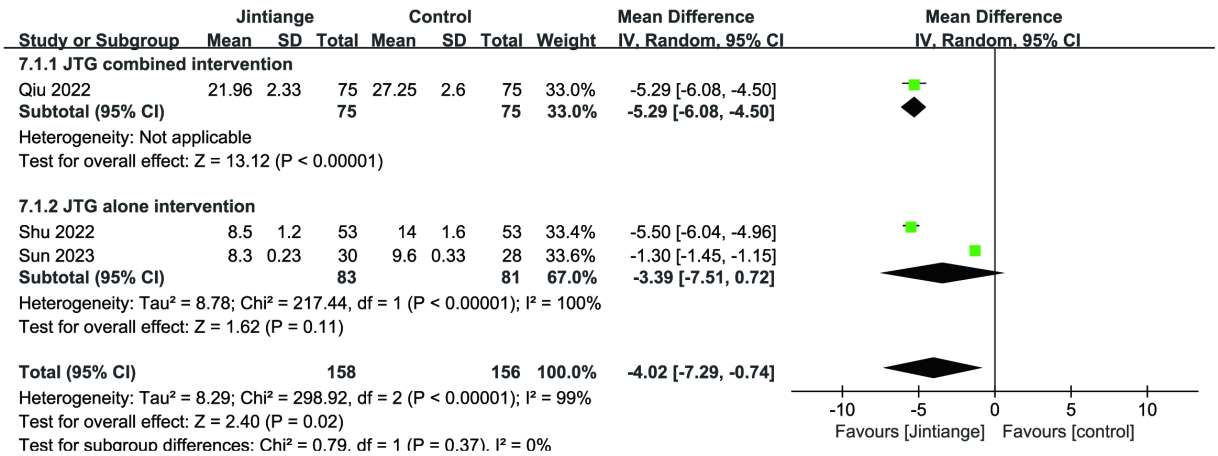

FIGURE 9
Forest plot of the changes in Cobb's angle.

Jintiange capsules group. These studies evidenced that the frequency of adverse events was mild, and the treatment was less limited than in the control group, with no severe adverse impacts.

3.5 Publication bias and sensitivity analysis

We plotted the funnel plots along with Egger's test to evaluate the publication bias of the VAS score in STATA 17 software. The funnel plot showed that these studies were approximately symmetrically distributed on both sides of the regression line. Additionally, the results from Egger's test showed no significant publication bias for the VAS score ($p = 0.838$) (Figure 12).

We also performed sensitivity analyses for these 10 studies by excluding each article to validate the stability of this meta-analysis, which revealed that there was no statistical effect on the pooled data when any of the articles were eliminated except Sun 2023. However, this study had to be cautiously interpreted when explaining the prognostic results of the VAS due to Sun 2023 appearing to have a potential impact on the subgroup analysis of the Jintiange capsules alone (Figure 13).

4 Discussion

OP is a metabolic bone disease characterized by bone density and susceptibility to fracture (39). OVCF is a common secondary disease of OP, with an incidence of 0.307% among people over 50 years old, and is closely associated with low-energy trauma and advancing age. OVCFs may cause severe back pain and kyphosis and increase patient mortality (40). Surgery, such as PVP and PKP, are common alternative therapies for patients who fail to restore the height of the vertebral body through conservative treatments. However, there are inevitable adverse events of cement leakage,

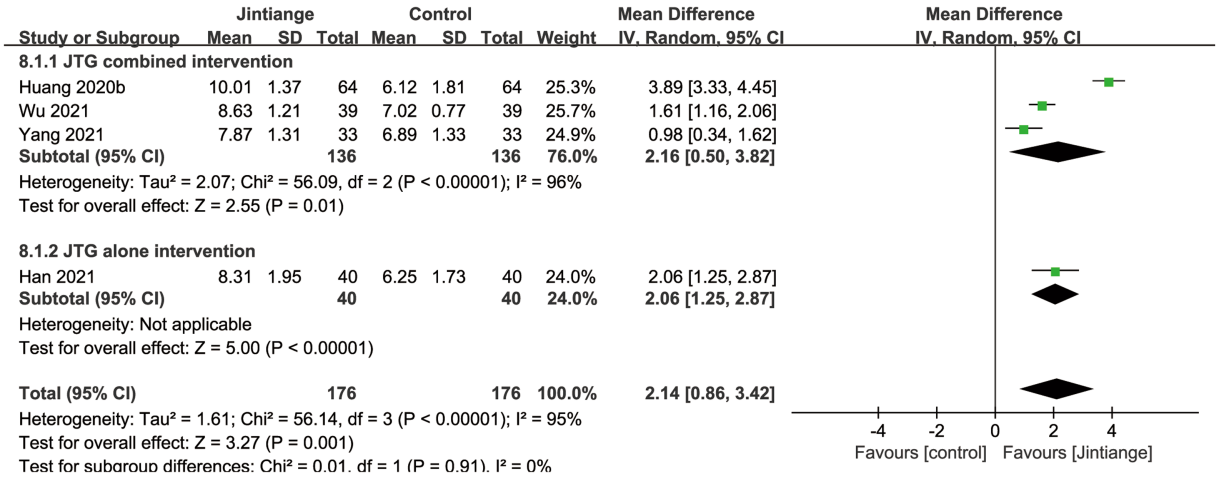

FIGURE 10
Forest plot of the changes in serum OC.

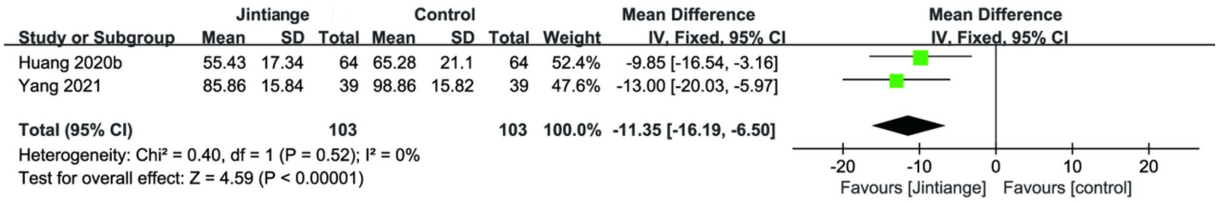

FIGURE 11
Forest plot of the changes in serum ALP.

pulmonary cement embolism, and new vertebral compression fractures caused by PVP or PKP (41, 42). Similarly, current guidelines suggest that calcium and bisphosphonates can be used as routine oral medications for OP, but these medications may cause adverse events with long-term use, such as renal dysfunction and an increased risk of breast cancer (43). Therefore, effective and safe medications for OVCFs are required to improve this situation. The therapeutic effect of Jintiange capsules on OVCFs has received considerable attention, but research on its anti-OVCF mechanism is limited due to the lack of evidence regarding the effectiveness and safety of Jintiange capsule administration.

Our current study demonstrated that Jintiange capsules exert a prominent anti-OVCF effect. The potent anti-OVCF effect of Jintiange capsules can possibly be attributed to its primary ingredient, artificial tiger bone. Tiger bone has been reported to contain abundant calcium, phosphorus, peptides, and proteins (44). Calcium and phosphorus are fundamental bone minerals involved in many biological processes and can promote the development of bone, maintain the stability of the cytoskeleton, and inhibit osteoclast activity (45). The peptides and proteins can degrade into amino acids *in vivo*, which can potently stimulate increases in intracellular calcium and osteoblast differentiation (46). This meta-analysis indicates that Jintiange capsules maintained and improved the BMD of the lumbar spine, femoral neck, and whole body, and the subgroup analysis of the Jintiange capsules combined intervention showed the same conclusion regarding the lumbar spine and whole-body BMD compared with the control group. After selecting studies with substantial heterogeneity, the overall effect test of subgroup analysis revealed that Jintiange capsules improved the overall effective rate (RR = 1.15, 95% CI: 1.09 to 1.22, $p < 0.00001$, $I^2 = 0\%$). Chronic pain is the major symptom of postoperative treatment for OVCFs (47). In this meta-analysis, the pooled data of the subgroup analysis showed that the Jintiange capsules combined intervention reduced the VAS compared with controls. In addition, Egger's test revealed no significant publication bias for the VAS. The subgroup analyses of Jintiange capsules also indicated that the Jintiange capsules combined intervention significantly improved ODI and Cobb's angle compared with the control group. Osteocalcin (OC) is a protein derived from osteoblasts that is vitamin K-dependent and has biological effects on bone metabolism (48). A previous study has shown that Jintiange capsules can prevent bone loss and promote the osteogenic differentiation of BMSCs by regulating the BMP and Wnt-β-catenin pathways in ovariectomized rats, and BMP-2 can increase the expression of osteoblastic markers in cultures of pluripotent cells (49). The result showed that Jintiange capsules significantly raised serum OC levels, and subgroup analyses of the Jintiange capsules alone and combined interventions suggested similar conclusions. Serum ALP is regarded as a diagnostic marker of bone loss in osteoporotic patients and can play an important role in the activation of osteoblasts (50). Empirical evidence in ovariectomized rats has shown that Chinese medicine can significantly increase bone mass and suppress the formation of osteoclasts by inhibiting serum ALP levels (51, 52). The result of this meta-analysis also showed that the Jintiange capsule decreased ALP levels.

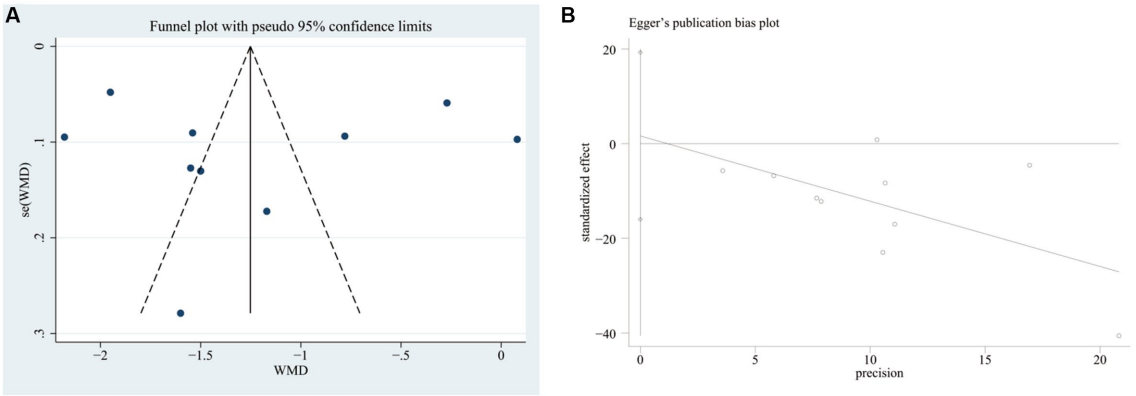

FIGURE 12
(A) Funnel plot of the VAS change. **(B)** Publication bias test for the VAS change.

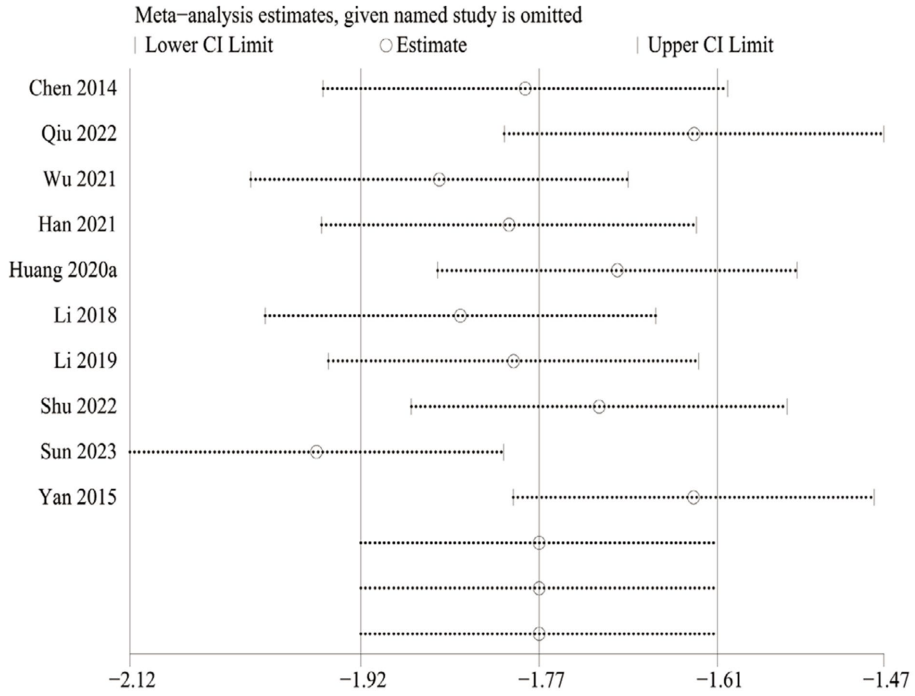

FIGURE 13
Sensitivity analysis of the VAS change.

Toxicity is the most severe adverse effect of Jintiange capsules. Four studies reported that the adverse effects are relatively mild gastrointestinal symptoms experienced by patients with OVCFs receiving Jintiange capsules, which can be alleviated by stopping treatment. Five participants of the Jintiange capsules group developed recurrent fractures compared with the control group after treatment with Jintiange capsules, which showed a significant reduction in the rate of new vertebral fractures in patients treated with Jintiange capsules. This also showed that Jintiange capsules had a positive effect on reducing severe bone loss in older populations suffering from OVCFs. According to our analysis, the data suggested that Jintiange capsules are safe for the postoperative treatment of OVCFs in the present situation.

Jintiange capsules, the artificial bone tiger powder prepared from several farmed animal skeletons, have similar chemical constituents and pharmacological properties to natural tiger bone, which significantly exhibit anti-inflammatory, analgesic, fracture-healing, and bone metabolism-improving effects that can treat OVCFs (19, 53). An experimental study found that Jintiange capsules can alter the proliferation, differentiation, and mineralization of MC3T3-E1 osteoblasts to increase osteogenesis, which also can inhibit their apoptosis and enhance autophagy by reversing the regulatory effects of the PI3K-AKT and ER stress pathways (54). Another study showed that the main ingredient of tiger bone is calcium phosphate, which can interact with the targets of CALR and CALM1 to promote the synthesis of extracellular phosphate. Therefore, Jintiange capsules can increase bone and cartilage mineralization and regulate multiple signaling pathways (55). Therefore, this meta-analysis showed that Jintiange capsules increased clinical efficacy in improving biochemical markers. Simultaneously, the pooled

data showed that Jintiange capsules maintained a relatively high level of serum OC compared with the control treatment, which could be due to the calcium and phosphate in the capsules increasing osteogenesis and the mineralization of osteoblasts, thereby increasing serum OC. Based on the stabilizing regulatory role of Jintiange capsules in bone metabolism, its clinical application deserves further attention in the postoperative treatment of OVCFs.

5 Limitations

The 16 included studies had several limitations that should be considered when interpreting the data. First, the sample sizes of the RCTs were too small, and relevant studies may have been overlooked, although we retrieved seven databases without any restriction on language. Second, there were various methodological quality biases because the report on the assessment methods was uneven for the included studies, such as selection bias, performance bias, and reporting bias. Third, the surgical treatment follow-up period ranged from 1 to 12 months, and thorough groupings with regard to more uniform treatment lengths were not put together. Lastly, there is high heterogeneity of the subgroup analysis in the included articles, indicating that diverse conservative Western medication may be utilized in the Jintiange capsules combined intervention group. Therefore, given the limitations of the designs of the studies, future clinical studies targeting the postoperative treatment of OVCFs with Jintiange capsules should avoid the above situations, as the reliability and evidence-based data on the positive effect of Jintiange capsules on the postoperative treatment of OVCFs remains to be improved.

6 Conclusion

The data from the studies showed that Jintiange capsules are effective in the postoperative treatment of OVCFs when combined with other therapeutic measures or alone. Their mechanism of action can increase BMD, increase lift effect rates, relieve pain, decrease the ODI, improve Cobb's angle, raise serum OC levels, inhibit serum ALP levels, and lower the incidence of adverse events, making it a potential medication for treating OVCFs with high safety and effectiveness. Given the limitations of this study, the efficacy of Jintiange capsules for the postoperative treatment of OVCFs will need to be validated in future clinical studies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

YF: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. WW: Writing – review & editing, Conceptualization, Funding acquisition, Project administration, Supervision, Visualization. MZ: Writing – review & editing, Formal analysis, Validation. JZ: Software, Validation, Writing – review & editing. MT: Validation, Writing – review & editing, Formal analysis.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1289818/full#supplementary-material

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Research progress of plant medicine and Chinese herbal compounds in the treatment of rheumatoid arthritis combined with osteoporosis

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Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease. The clinical manifestations of various joint pain and bone destruction are common. RA has a high disability rate and is closely related to local and systemic osteoporosis (OP). RA can occur at any age, however, its incidence increases with age. Most patients are 40 to 50 years old with an incidence among women approximately 3 to 5 times more than among men. Osteoporosis is a kind of metabolic bone disease characterized by bone mass and bone microstructure damage and is one of the common complications of RA. Currently, in the clinic, more patients develop RA with OP symptoms. Therefore, both OP and RA-related factors should be considered in the OP treatment of RA. Currently, there is more and more research on RA combined with OP drugs, including basic drugs, bone resorption inhibitors, bone formation promoters, and anti-rheumatic drugs to improve the condition. The high cost or limited efficacy of certain Western drugs, coupled with their potential for adverse reactions during treatment highlight the pressing need for novel pharmaceuticals in clinical practice. In recent years, traditional Chinese medicine (TCM) can improve the bone formation and bone resorption indexes of patients with RA, regulate the balance of osteoclasts and osteoblasts, and regulate the immune inflammatory response, so as to treat RA combined with OP. This article discusses the advancements in single Chinese medicine and Chinese medicine combination treatments for RA complicated with OP, focusing on the mechanism of action and syndrome differentiation and classification, to offer new ideas for future clinical prevention and treatment.

KEYWORDS

rheumatoid arthritis, osteoporosis, bone metabolism, traditional Chinese medicine, geriatric medicine, plant-based natural products

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that leads to synovial cell proliferation and pannus formation in newborns. The occurrence, development, and production of RA are closely correlated with inflammatory cell infiltration and capillary involvement in the joints. The main clinical manifestations include pain, swelling, deformity, and functional decline. In addition to joint involvement, trabecular bone destruction, reduction in bone mass, and increased bone fragility are secondary effects. Studies have shown that patients with rheumatoid experience increased bone resorption and a higher incidence of osteoporosis (OP) (1). A Korean epidemiological study revealed that nearly 50% of postmenopausal women with RA had osteoporosis. In contrast, the prevalence of osteoporosis among Korean adults over 50 years old is 35.5% for women and 7.5% for men (2, 3). Currently, the pathogenesis of RA-induced OP remains poorly studied. However, it primarily involves factors such as receptor activator of nuclear factor κB ligand (RANKL), receptor activator of nuclear factor κB (RANK), the osteoprotegerin signal transduction pathway system, inflammatory mediators, and pharmacological agents such as glucocorticoids—all of which can contribute to the pathogenesis of OP (4). OP, the most common complication of RA, significantly impacts the treatment effectiveness, prognosis, and quality of life for patients (5). Currently, there are various drugs available with their own advantages and disadvantages, particularly concerning adverse reactions in the gastrointestinal tract that hinder patient acceptance and lead to poor compliance. Traditional Chinese medicine (TCM) monomers and their compound medicines have gradually proved to be significantly effective in treating RA combined with OP. The advantage lies in treating patients' symptoms and improving their constitution based on clinical symptoms and TCM constitutional defects. The research on the pathogenesis of RA leading to OP has increased, but there is insufficient research in the field of TCM to explain how TCM treats RA combined with OP (6). Therefore, it is necessary to comprehensively study recent research in traditional Chinese medicine prevention and treatment of RA combined with OP. This article aims to accumulate clinical experience and provide new treatment ideas.

Understanding of RA combined with OP in TCM

Modern medical theory posits that the kidneys primarily assume responsibility for metabolite excretion and body fluid balance regulation. This concept is more specific and limited, diverging significantly from the TCM interpretation of the "kidney." TCM scholars perceive the kidney as a functional complex encompassing aspects of renal function, endocrine regulation, nervous system involvement, and other systems in modern medicine. Notably relevant to rheumatoid arthritis combined with osteoporosis is the belief in TCM that the kidney harbors essence while governing bone health and marrow vitality. Chinese medicine scholars believe that kidney deficiency is the key to the occurrence of rheumatoid arthritis combined with osteoporosis. According to the pathogenesis and clinical symptoms of RA combined with OP, Chinese medicine classifies it into the category of "bone Bi" and "Bi syndrome." "Medical Jing Yi" explained that when the kidney qi is abundant, it nourishes the kidney essence, promotes active marrow generation, strengthens and densifies the bones, and allows for free movement of limb joints. On the contrary, when there is a deficiency in kidney essence, the bone marrow lacks a source of biochemicals and insufficient marrow essence cannot support bone health. As a result, weak bone tendons and bone destruction may occur. Qing Dynasty physician Wang Qingren put forward the theory of "stasis causing Bi," and Lin Peiqin that "stagnation for a long time must be blood stasis." Therefore, the invasion of external evil causes the meridians to be blocked and a deficiency of Qi and blood, and blood stasis is formed for a long time. Now medical science has found that RA patients have abnormal hemorheology and blood circulation disorders, which can cause blood stasis and venous stasis, aggravate microcirculation disorders around RA joints, and lead to abnormal calcium absorption, which damages the joint synovium and bone tissue. This can eventually lead to secondary osteoporosis (7). As mentioned previously, there are many mechanisms of RA causing OP, and the clear causes need to be studied. Studies have found that rheumatoid arthritis patients exhibit varying degrees of bone mineral density loss, which is associated with disease activity, duration, inflammatory markers, and other factors. Dual-energy X-ray absorptiometry results indicate decreased bone mineral content in the femur and lumbar spine of rheumatoid arthritis patients (8). Assessing fracture risk in patients with rheumatoid arthritis and osteoporosis is crucial. Studies have demonstrated that a comprehensive assessment method combining clinical indicators, bone mineral density detection, and TCM syndrome characteristics improves the accuracy of predicting fractures. Early diagnosis and treatment of underlying conditions along with lifestyle changes can help increase bone mineral density to prevent fractures (9). Studies have found that TCM syndromes in RA patients with osteoporosis mainly include kidney deficiency, liver depression, and spleen deficiency. In terms of TCM treatment, tonifying the kidneys, soothing the liver, and invigorating the spleen can effectively improve symptoms (10).

Treatment of RA with OP by TCM monomers

When herbal medicine is used to treat RA and OP, there is an overlap in the traditional Chinese medicines commonly employed for both diseases. By conducting an analysis of the TCM prescription patterns for RA and OP, Zhong Liyan discovered that in the treatment of RA, there is a strong correlation with the administration of white mustard seed, honeysuckle, Lonicerae japonicum, Tripterygium wilfordii, ground beetle, turmeric, Kadsura pepper stem, Rhizoma Dioscoreae Nipponicae, and Paniculate Swallowwort (11). Similarly, in the treatment of OP, Moutan PI, Amphorae fructus, Dipsacus, Pinnellia pinellia, Morinda officinalis, and Ligustri lucidum were found to have significant correlations. The advantage of herbal medicine in the treatment of RA combined with OP is that the mechanism of action of traditional Chinese medicine in the treatment of osteoporosis is achieved through systemic, multi-link, and multi-pathway regulation. The Chinese medicine monomers for the treatment of RA and OP with their interactions are shown in Figure 1 and Table 1.

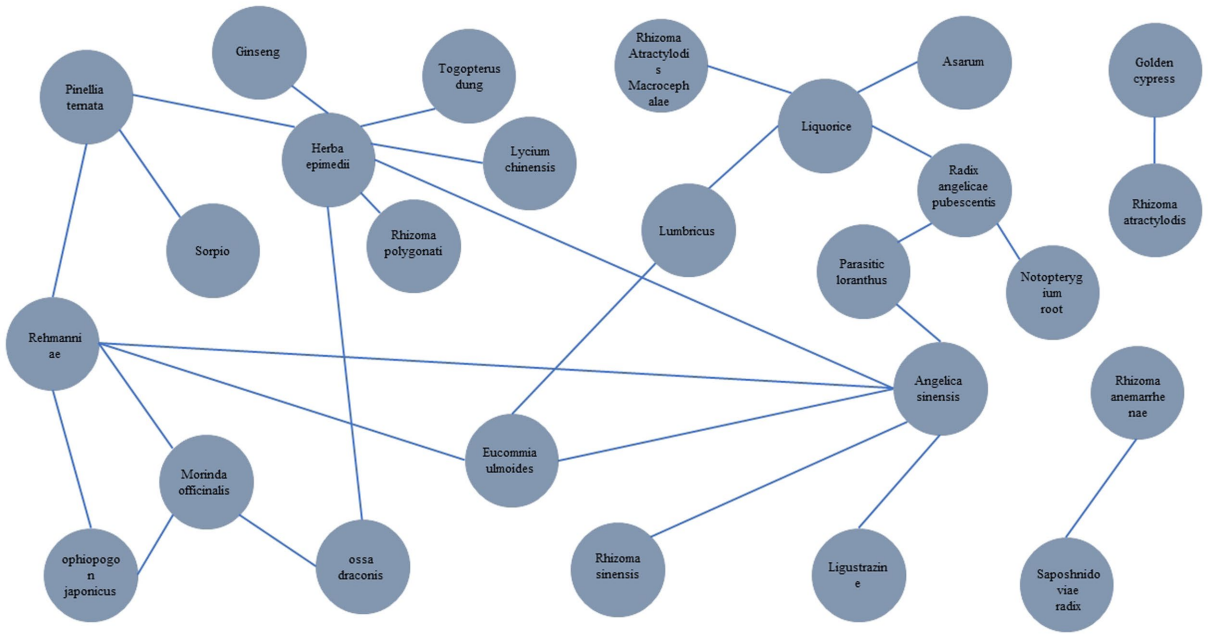

FIGURE 1
Visualization of the interaction network of drugs for the treatment of RA and OP.

Tripterygium hypoglaucum (Levl.) Hutch

The monomeric extract of Tripterygium hypoglaucum (Levl.) Hutch, known in traditional Chinese medicine, is derived from the root of this plant, which is a unique variety belonging to the genus Tripterygium in the Epimonaceae family. It has an effect on the three channels of the liver, spleen, and kidney. It has a bitter, punic, and mild taste, and has the functions of dispelling wind and removing dampness, promoting blood circulation and stopping bleeding, soothing tendons and bone healing, etc. Its main active ingredients include diterpenoids such as triptolide, triptolide triol, triptolide triterpenoid quinone A, and triptolide triterpenoids (21). According to the introduction, the basic pathological change of RA is the chronic inflammation of the synovial membrane of the joint, which leads to pannus formation, invasion of articular cartilage and subchondral bone, bone resorption, bone destruction and osteofibrosis, and finally joint malformation and loss of function. On the one hand, Tripterygium hypoglaucum Hutch can be used for therapeutic purposes for RA combined with OP by regulating bone metabolism. Mo Danya et al. (22) found by observing joint X-ray films of experimental mice that tripterygium, the active ingredient in Malus Kunmingshan, can inhibit synovium hyperplasia, antagonize cartilage destruction, improve bone density, and prevent osteoporosis. In addition, triptolide can significantly reduce the levels of vascular endothelial growth factor (VEGF) in synovial tissue and IL-6 in synovial fluid of joints, as well as the formation of new vessels in synovial tissue of rats, inhibit the proliferation of synovial cells, and show protective effects on synovial tissue and cartilage tissue (23). Liu et al. (24) found that triptolide could inhibit osteoclast formation by reducing the expression of nuclear transcription factors and osteopG in the joint cavity at the mRNA and protein levels, confirming that triptolide could inhibit osteoclast formation. On the other hand, in the prognosis of RA combined with OP, due to long-term and heavy use of anti-rheumatic drugs, adverse reactions may cause kidney damage. Studies (25) have pointed out that renal dysfunction with no obvious clinical manifestations is more common in RA patients, kidney involvement has become an important factor affecting the prognosis of RA, and renal failure is also a common cause of death for RA. In addition, traditional Chinese medicine states that "the kidney governs the bone," so in the prognosis and treatment of RA combined with OP, taking into account the protection of the kidney is also the key to preventing and treating the symptoms of osteoporosis. Zeng Hongbing et al. (26) found that Tripterygium hypoglaucum Hutch may reduce proteinuria and delay kidney injury by reducing the expression of transforming growth factor, inhibiting the proliferation of mesangial mesangium. Wu Xiabo et al. (27) found that the therapeutic effect of Malus Kunmingshan on nephrotoxic nephritis in rats was achieved by reducing urinary protein content, serum urea nitrogen, triglyceride, and total cholesterol, increasing serum albumin and total protein, improving renal function and pathological changes of glomerulus.

Radix angelicae pubescentis and other monomer TCMs with a similar mechanism

Radix Angelicae Pubescentis has the effect of dispelling wind dehumidification, relieve pain and dredging channel blockage. Radix Angelicae Pubescentis was first published in the "Shen Nong Bencaojing," which said that "it [is] mainly used in the treatment of wind-cold damp pathogen and incised wound knife injurious to health. And treat all kinds of orthopedic diseases caused by wind evil entering the channels." Methoxyparsley and dihydroparsley are two of the most active ingredients in Lonosol. Studies have shown (28) that methoxyparsley is the main component of the antitumor,

TABLE 1 Chinese herbs used to treat RA and OP.

Herbs	Main active ingredient	Clinical effect	Reference
Tripterygium Hypoglaucum (Levl.) Hutch	Triptolide, triptolide triol, triptolide triterpenoid quinone A, and triptolide triterpenoids	Immunosuppressive, anti-inflammatory, osteoclastogenesis-inhibiting	(12)
Radix angelicae pubescentis	RA: Methoxyparsley and dihydroparsley OP: Osthole Cnidium lactone	RA: Anti-inflammation, anti-oxidation, and eliminate wind and dampness OP: Inhibition of osteoclast bone absorption and bone metabolism	(5) (6)
Rhizoma Drynariae	Naringin and Drynaria total flavonoids	RA: Inhibition of inflammatory reaction OP: Anti-osteoporosis and renal protection	(13) (14)
Radix Cyathulae (achyranthes bidentata)	Ecdysterone (ECR)	Anti-apoptotic and anti-inflammatory	(15)
Salvia miltiorrhiza Bunge (Tanshinone)	Salvianolic acid B and Tanshinone VI	RA: Anti-inflammatory and anti-oxidation OP: Increases bone mineral density and promotes bone formation	(16) (17)
Angelica sinensis (Oliv.) Diels	Angelica including volatile oil, organic acids, amino acids, and coumarin.	Analgesic, anti-inflammatory, and anti-osteoporosis	(18)
Radix paeoniae alba	Albiflorin std., triterpenoid, and flavonoid	Analgesic, anti-inflammatory, antidepressant, and regulates immune system	(19)
Red peony root	Albiflorin std., triterpenoid, and flavonoid	Anti-inflammatory, anti-oxidation, nerve protection, and improves microcirculation	(20)

anti-angiogenesis, and anti-proliferation properties of doxine. The anti-inflammatory activity of dihydroparsley has been confirmed, but its mechanism is still unclear. In recent years, it has been confirmed that inflammatory factors, as inflammatory mediators, on the one hand, led to the occurrence of the primary disease of RA, and on the other hand, destroy the normal bone metabolic balance of the body and affect bone loss (29). Chao et al. (30) studied dihydroparvyl angelic acid and found that it inhibits the LPS-mediated inflammatory response mainly by inhibiting the activation of nucleotide-binding oligomerization domain 1 (NOD1) /NF-κB. Naringin, the active ingredient of the osteocalcin supplement, can promote the differentiation and proliferation of bone marrow stromal cells, increase the expression of osteocalcin, and effectively reverse the process of osteoporosis. Ang et al. (31) suggested that naringin inhibited the activation of NF-κB by inhibiting the degradation of IκB-α mediated by RANKL, and inhibited osteoclast generation and bone resorption by interfering with the RANKL-mediated NF-κB and extracellular signal-regulated kinase ERK signaling pathways. Achyranthes root extract can improve the biomechanical quality of bone and trabecular structure (32). Ecdysterone (ECR) is the main component of achyranthes and has been used for the prevention and treatment of osteoarthritis. Zhang et al. (15) showed that ECR plays an anti-apoptotic and anti-inflammatory role in rat chondrocytes induced by interleukin-1β, which may be related to the NF-κB signaling pathway. It has also been demonstrated that salvianolic acid B prevents bone loss in glucocorticoid-treated rats by stimulating osteogenesis and bone marrow angiogenesis, and inhibiting fat formation (33). Nicolin et al. (34) demonstrated that tanshinone VI inhibits osteoclast differentiation by inhibiting the expression of RANKL and NF-κB. In summary, dysregulation of the NF-κB signaling pathway may be the basis of the pathogenesis of RA complicated with OP. The aforementioned herbs can exert a favorable therapeutic impact on RA complicated with OP by modulating the NF-κB signaling pathway. In conclusion, the pathogenesis of RA complicated with OP is related to the interaction between signal transduction pathways and inflammatory factors and is closely related to vitamin D level and physical exercise. The effect of RA treatment drugs on OP should not be underestimated.

Angelica sinensis

Angelica sinensis, which is medicinal sweet, pungent, humoral, and returns to the liver, heart, and spleen channel, has the effect of tonifying blood and promoting blood circulation, regulating the menstrual flow, and relieving pain, moistening the bowels. The main chemical components of Angelica include volatile oil, organic acids, amino acids, and coumarin, which have pharmacological effects such as analgesic, anti-inflammatory, anti-osteoporosis, anti-platelet aggregation, anti-anemia, and protection against cardiovascular and cerebrovascular diseases (18). The inflammation in RA is one of the causes of OP. Studies by Ma et al. (35) have shown that Artemisonolactone, an extract of Angelica sinensis, has obvious anti-inflammatory effects on ovariectomized rats with osteoporosis. Ferulic acid, an effective component of angelica, can reduce the expression of IL-1β, TNF-α, matrix metalloproteinase-1, and matrix metalloproteinase-13 in chondrocytes induced by hydrogen peroxide, thus playing a protective role in articular cartilage (36).

Radix paeoniae alba and red peony root

Paeoniae alba, which is medicinal bitter, acidic, slightly cold, and returns to the liver and spleen meridian, has the effect of nourishing blood and regulating the meridian, collecting Yin and stopping perspiration, soothing the liver, and relieving liver Yang. Modern

pharmacological studies have shown that Paeony has anti-inflammatory, analgesic, immune regulation, liver protection, anti-depression, and other pharmacological effects, and has been widely used in the clinical treatment of RA, chronic hepatitis B, cancer pain, and other kinds of pain (19). Studies have shown (37) that Paeony can reduce inflammation in RA and delay the bone destruction and progression of RA by reducing the levels of IL-1, IL-1β, IL-17, and TNF-α, inhibiting the activation of NF-κB and the proliferation of synovium and regulating the levels of IL-2 in both ways and through other mechanisms.

Red peony root, which is bitter, slightly cold, and affects the liver channel, has the effect of clearing heat and cooling blood, dissipating blood stasis, and relieving pain. Modern pharmacological studies have shown that peony has various pharmacological effects such as protecting nerve cells and cardiomyocytes, anti-atherosclerosis, lowering pulmonary artery pressure, anti-thrombus, stabilizing microcirculation, hypoglycemic, anti-gastric ulcer, protecting the liver, anti-depression, antiviral, anti-inflammatory, anti-tumor, and anti-radiation. It has been widely used in the treatment of circulatory, nervous, blood, digestive, and other multi-system diseases (38). Qin Yanqin et al. (39) studied the effects of the effective components of Paeonia lactis on inflammatory factors in an A549/THP-1 cell co-culture inflammatory model, and found that it could reduce the levels of IL-1β, IL-6, IL-23, and MMP-9, thereby inhibiting the inflammatory response.

In conclusion, although there is insufficient evidence to strictly infer the use of Chinese medicine monomers in treating RA-OP, the above studies have demonstrated their potential efficacy in reducing inflammation and oxidative stress. Although many studies have shown the anti-inflammatory and anti-oxidative effects of TCM monomers, as well as their therapeutic potential for RA-OP in laboratory and animal experiments, further verification is needed for clinical application. To more fully evaluate the efficacy of TCM monomers in treating RA-OP, attention should be given to the following studies: (1) Large randomized double-blind controlled trials are the most reliable way to assess drug efficacy and safety. More such trials on using TCM monomers for treating RA-OP will help confirm their clinical efficacy. (2) Clinical case reports and retrospective analysis provide initial clinical data on using TCM monomers for treating RA-OP, although their reliability is relatively low. Paying attention to this literature helps one understand the application value of TCM monomers in actual clinical practice. (3) Research on the mechanism of drug action helps optimize treatment plans and predict possible side effects by gaining an in-depth understanding of how Chinese medicine monomers affect RA-OP.

TCM prescription to treat RA combined with OP

In 2002, the Guiding Principles of Clinical Research on New Chinese Medicine (Trial Implementation) divided RA into five syndrome types: damp-heat obstruction, cold-dampness obstruction, kidney qi deficiency and cold, liver and kidney Yin deficiency, and blood stasis obstruction (40). Zeng Zhaoyang et al. (41) classified osteoporosis into four syndrome types: kidney Yang deficiency syndrome, liver and kidney Yin deficiency syndrome, spleen and kidney Yang deficiency syndrome, and blood stasis and qi stagnation syndrome. Zhang Hongyue (42) clinical study on traditional Chinese medicine syndromes found that among 161 RA patients with OP, wind-cold-dampness syndrome accounted for 4.96% (8 cases), cold-damp obstruction syndrome accounted for 14.90% (24 cases), damp-heat obstruction syndrome accounted for 16.77% (27 cases), phlegm and blood stasis obstruction syndrome accounted for 17.39% (28 cases), qi and blood deficiency syndrome accounted for 9.93% (16 cases), and liver and kidney deficiency was diagnosed in 36.02% of the cases (58 cases), as shown in Figure 2.

TCM compound of tonifying kidney and dredging collaterals

Kidney deficiency is the key to the occurrence of rheumatoid arthritis combined with osteoporosis. Medical Jing Yi states that the kidney qi is sufficient; the kidney essence is abundant; the medullary generation is active; the bone is dense, firm, and powerful; and the limbs and joints move freely. On the contrary, the kidney essence is deficient, the bone marrow is biochemically deficient, and the medullary essence is insufficient, so the bones and tendons are weak and the bone is destroyed. Therefore, the treatment of arthralgia should take the specimen into account, dispelling the evil. Wang Fang (43) found that a Pei-Bushenyang Decoction supplemented with flavor could significantly relieve clinical symptoms in RA combined with OP patients; reduce the levels of ESR, CRP, and BALP; increase the indexes of 25-OH-D, osteocalcin, and blood calcium; and improve bone mineral density, with significant differences. On the basis of Western medicine treatment, Tian Jiexiang (44) added a representative prescription of a Bushentongluo Gutabaikang pill (antler cream, ejiao, bone crushing supplement, cinnamon, turtle plate, turtle shell, etc.) to treat the disease, and the patients' clinical symptoms were alleviated and bone mineral density was significantly improved. According to many years of clinical experience, Professor Pang Xuefeng (45) summarized the empirical prescription for the treatment of RA in the active stage: a Hanbikang Decoction, which is composed of kidney toning drugs such as epimedium and Guji and anti-rheumatic drugs, can significantly improve the clinical symptoms and signs of patients with RA combined with OP, and the improvement of bone mineral density is better than that of Western medicine alone. In addition, the animal experiments with the Hanbikang Decoction have also proved that it can reduce the expression of RANKL and RANK in bone tissue and increase the expression of OPG by affecting the RANKL/RANK/OPG signaling pathway, so as to achieve the effect of anti-RA bone erosion and can be used to treat RA combined with OP. To sum up, traditional Chinese medicine kidney prescriptions for patients with RA and OP, alongside kidney anti-rheumatism medicine, can effectively improve the clinical curative effect, low bone mineral density values, morning stiffness, joint pain, swelling, and wait for a symptom, and is worthwhile for clinical use.

TCM compound for liver and kidney deficiency

Professor Fan Yongsheng believed that Yin deficiency in the liver and kidneys can be seen in the early, middle, and late stages of RA (46), and advocated that nourishing the liver and kidneys should run

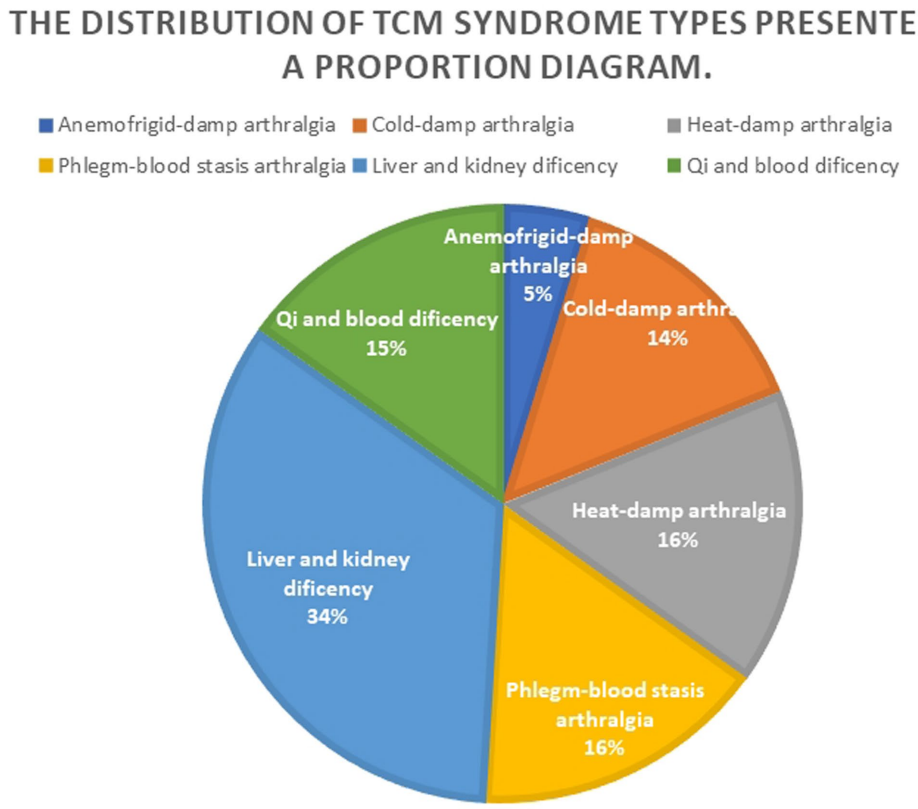

FIGURE 2
Proportion diagram of TCM syndrome types distribution.

through the whole process of RA. Clinical treatment of RA combined with OP syndrome of liver and kidney deficiency has achieved satisfactory clinical efficacy. According to Wang Xiaoqing (47), Zhiyin Yigu prescription (Rehmannia rehmannii, Mulberry parasitic, Achyrano rhizoma, yellow juniper, Euperia ulmoides, stucca, etc.) has the functions of nourishing liver and kidney, dispelling wind and dampness, clearing heat deficiency, and inhibiting osteoclast activity by increasing the content of OPG and RANKL in serum, thereby reducing bone loss and controlling the development of disease. It has a definite effect on RA combined with OP patients with liver and kidney deficiency syndrome. In addition, the core prescription for the treatment of RA combined with OP is Duhuo parasitic decoction, which has been proved by modern pharmacology to inhibit platelet aggregation, inhibit thrombus, and facilitate the blood circulation of joints. The ethanol extract in mulberry parasitic can dilate blood vessels and has the effect of reducing blood pressure. Rhizoma Drynariae can promote the absorption and utilization of calcium in bones, increase the levels of calcium and phosphorus in blood, improve chondrocytes, delay cell degeneration, reduce the incidence of bone and joint lesions, and play a sedative and analgesic role (48). A Liuwei Dihuang pill with cooked Rehmannia nourishes the liver and kidneys, benefits the essence, and fills the marrow. According to Compendium of Materia Medica, it can "fill bone marrow, grow muscle and produce essence blood." Modern pharmacological studies (49) show that a Liuwei Dihuang pill can inhibit Th2-mediated immune inflammation, inhibit overexpression of Th2, regulate the balance of Th1 and Th2, and enable patients to achieve a balanced immune function. Zhou Jinsheng (50) observed the treatment of 88 patients with rheumatoid disease and found that the combined treatment with Liuwei Rehmannia could reduce the blood sedimentation rate index and reduce the inflammatory response of patients, which was helpful in improving clinical symptoms. Long Kuanbin et al. (51) used a Liuwei Dihuang pill to treat 60 patients with rheumatoid disease complicated with osteoporosis and the patients' clinical symptoms were improved.

TCM prescriptions for resolving phlegm, promoting blood circulation and pathways

Professor He Dongyi (52) argues that the pathological basis of RA combined with OP is based on the deficiency of kidney essence, the blockage of blood stasis as the standard, the congenital deficiency of kidney deficiency leads to the loss of the essence of the muscles, bones, and joints to replenish the blood, the warmth of the kidney Yang, and the evil of wind and cold dampness invading the human body, blocking the Qi and blood, making the operation of Qi and blood not smooth, the long-term phlegm and blood stasis interlock, the muscle and bone dystrophy, causing joint pain and deformity and eventually leading to osteoporosis. Li Xiaodan et al. (53) treated 76 patients with rheumatoid arthritis with a Dianteng Yimu decoction, and found that bone metabolism indexes were improved compared

with before treatment, confirming that a Dianteng Yimu decoction can inhibit bone absorption in patients with rheumatoid arthritis after treatment, especially in promoting bone formation. Jiang Yi et al. (54) treated RA combined with OP patients with Bixie Qufengyin combined with methotrexate. After treatment, the joint function index, bone pain index, joint swelling index, joint tenderness index, and morning stiffness time of patients in the observation group were decreased compared with those before treatment and the control group, and the difference was statistically significant, indicating that Bixie Qufengyin combined with methotrexate achieved the clinical therapeutic purpose. Zhong Weijing et al. (55) discussed the mechanism of action of Shexiang Oolong pills (artificial musk, Radix aconitum, Radix aconitum, Radix scorpion, Radix black beans) from the perspective of cytokines. The Chinese medicine uses musk as its principal component, which can move the stagnation in the blood, promote blood circulation, and diffuse the nodules. Musk is also the most fragrant fragrance, which can penetrate the meridians and collars, so it can relieve pain. Studies have shown that Musk Oolong pills can increase the serum OPG level and reduce the level of RANKL in patients with RA, inhibit bone resorption, regulate bone metabolism, and then delay bone destruction and promote bone repair.

To conclude, TCM prescriptions can well improve RA combined with OP clinical symptoms. Chinese herbal formulas have the potential to significantly improve the treatment of RA combined with OP therapy, offering a wide range of opportunities for development in this field. The micro-therapeutic effects mentioned above are primarily mechanical, but some studies have demonstrated the efficacy of TCM in treating RA combined with OP. For instance, a randomized controlled trial involving 40 RA patients with OP revealed significant improvements in pain levels, joint inflammation symptoms, and quality of life after 24 weeks of TCM treatment (56). In another clinical study with 60 RA patients suffering from OP, those treated with traditional Chinese medicine experienced notable alleviation in symptoms such as joint pain, swelling, and stiffness after 24 weeks of treatment while also reducing the risk of fractures (57). Furthermore, a separate study involving 120 RA patients with OP found that traditional Chinese medicine led to a significant reduction in serum inflammatory factor levels (such as tumor necrosis factor-α and interleukin-6) after 12 weeks of treatment along with improved bone mineral density (58). Although not all of the studies mentioned above were randomized controlled trials (RCTs), they demonstrated TCM treatment's efficacy in improving clinical symptoms and bone metabolism in RA patients with OP.

Discussion

In recent years, with the deepening of research on rheumatoid arthritis, more and more attention has been paid to the situation of bone destruction and systemic bone loss caused by RA combined with OP. OP is a difficult condition and a key point in the clinical treatment of RA. TCM has accumulated a lot of practical experience in the treatment of RA combined with OP. It has also achieved ideal results, and has its unique advantages and characteristics in syndrome differentiation and multi-target multi-approach holistic treatment. At present, there is no expert consensus on TCM syndrome differentiation and treatment of RA combined with OP. Researchers have mostly carried out relevant studies based on the traditional Chinese medicine syndrome types of RA. Although the classification criteria of RA are not the same and there are few relevant studies, the existing results mostly show that patients with RA syndrome of liver and kidney insufficiency and phlegm and blood stasis are more serious in bone destruction and more likely to be complicated with OP. In terms of treatment, many methods such as supplementing the liver, spleen, and kidneys; eliminating phlegm; promoting blood circulation; and clearing collars are adopted. Western medicine also has certain advantages and limitations in the treatment of RA combined with OP. In terms of advantages, Western medicine has clear targets and drugs for the treatment of RA and OP, such as biological agents, anti-resorption drugs, and drugs that stimulate bone formation, and the curative effect is clear. In terms of limitations, the method of Western medicine in the treatment of RA and OP is relatively myopic, focusing on drug therapy, and some drugs have side effects, which may affect the quality of life of patients (59). In view of the above-mentioned, there are still many mechanisms that are not exact. For example, how botanic drugs interact with inflammatory factors IL-1, IL-17, IL-6, and IL-4 in the RANKL/RANK/OPG system, and what role each inflammatory factor plays in regulating bone metabolism. In addition, the specific mechanism of action of Chinese medicine prescriptions is still not completely clear. Furthermore, for RA combined with OP, the theoretical documentation of Chinese medicine is not complete, and there are few studies on syndrome differentiation and classification. Compared with Western medicine intervention methods, the use of TCM internal and external treatment intervention in clinical treatment still has limitations. Finally, at present, there are more and more animal experimental studies on osteoporosis by traditional Chinese medicine researchers, but most of them only focus on a single disease of osteoporosis, mainly in the field of postmenopausal osteoporosis (PMOP), and there are few studies on RA combined with OP. In addition, the use of plant medicine and TCM prescription for treatment is the "internal treatment" in the concept of TCM. In the aspect of "external treatment," there is still a lack of existing research, but relevant clinical studies have shown that the "external treatment" of traditional Chinese medicine has a certain therapeutic effect on RA combined with OP. Wu Mingxia et al. (60) treated 18 female patients with OP with acupuncture, and the main acupoints were Dazhu, Dazhui, and Mingmen. The bone mineral density of the lumbar spine and femoral neck was increased after treatment at Xuanzhong (GB 3), Geshu (BL 23), and Zusanli (ST 36). Xiong Fangli et al. (61) collected the auricular points of the uterus, kidneys, endocrine, ovaries, and spleen. The needles were retained for 2 days, and the two ears were treated alternately. Thirty days of solid embedding was taken as a course of treatment. After 3 courses of treatment, the symptoms, signs, X-ray films, bone mineral density, and urine calcium of 60 middle-aged and elderly women with OP combined with fracture were improved by 95%. Therefore, we believe that the improvement of the existing treatment scheme combined with the external treatment of TCM may achieve twice the result with half the effort, with traditional exercises such as Tai chi, Wu qinxi, Ba duanjin, possibly being a measure to improve the quality of life in the late stage of RA combined with OP. Acupuncture and moxibustion can promote the relief of symptoms. Auricular intradermal needling is one of the non-drug treatment methods, which can stimulate acupoints such as the liver,

kidneys, and spleen, to achieve the effect of invigorating the spleen and nourishing the liver and kidneys.

Conclusion and Prospect

In summary, epidemiological survey data has confirmed the presence of osteoporosis secondary to RA, which has gained increasing attention from rheumatologists. Western medicine primarily employs combination therapy, administering traditional anti-osteoporosis drugs alongside RA treatment. With the rapid development of traditional Chinese medicine, research on RA combined with OP is progressing and showing remarkable clinical efficacy. Clinical practice has demonstrated that utilizing TCM syndrome differentiation and treatment, focusing on holistic therapy, effectively alleviates the disease with minimal side effects for improved patient quality of life. Extracting monomers from single TCM ingredients to produce medicines offers clear quantification and accurate testing in laboratories while enjoying high recognition and acceptance abroad. This enhances the competitiveness of TCM in international markets and represents a direction for successful internationalization. Simultaneously, there will be increased and in-depth research on the causes, development, differentiation of symptoms, and treatment of rheumatoid arthritis with osteoporosis (OP) in the future. Efforts will be made to reach an academic consensus as soon as possible to fully utilize the unique advantages of TCM in treating rheumatic diseases. Therefore, it is necessary to deepen research on its mechanism of action through improving scientific and rigorous experimental design, conducting large-scale observations, and utilizing big data for research purposes, as well as engaging in effective interdisciplinary collaboration that provides a solid theoretical foundation and detailed data support for clinical practice. Research on TCM treatment for RA combined with OP has broad application prospects; however, further discussion is needed regarding its pathogenesis which plays a key role in medical disease treatment. Additionally, we suggest focusing future efforts on multidirectional and multitargeted research into plant medicine mechanisms and treatment of osteoporosis and rheumatoid arthritis with TCM compounds at metabolomic, proteomic, and cellular molecular gene levels while addressing the aforementioned research limitations. In terms of clinical treatment approaches, we believe that strengthening health education about OP among RA patients should be prioritized. We should promote a healthy lifestyle and control all factors related to osteoporosis. Additionally, we should monitor bone metabolism indexes, BMD, and fracture risk assessment in RA patients with OP during diagnosis and treatment. It is important to advocate for RA patients to receive the lowest possible dose of glucocorticoids to reduce the risk of osteoporosis. In clinical practice, we need to consider drug efficacy, adverse reactions, and treatment costs based on each individual's situation in order to promptly prevent and treat osteoporosis while relieving symptoms of RA, diminishing fracture risks, and bringing greater benefits to patients.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Potential mechanism of tea for treating osteoporosis, osteoarthritis, and rheumatoid arthritis

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Osteoporosis (OP), osteoarthritis (OA), and rheumatoid arthritis (RA) are common bone and joint diseases with a high incidence and long duration. Thus, these conditions can affect the lives of middle-aged and elderly people. Tea drinking is a traditional lifestyle in China, and the long-term intake of tea and its active ingredients is beneficial to human health. However, the mechanisms of action of tea and its active ingredients against OP, OA, and RA are not completely elucidated. This study aimed to assess the therapeutic role and related mechanisms of tea and its active ingredients in OP, OA, and RA. Moreover, it expanded the potential mechanisms of tea efficacy based on network pharmacology and molecular docking. Results showed that tea has potential anti-COX properties and hormone-like effects. Compared with a single component, different tea components synergize or antagonize each other, thereby resulting in a more evident dual effect. In conclusion, tea has great potential in the medical and healthcare fields. Nevertheless, further research on the composition, proportion, and synergistic mechanism of several tea components should be performed.

KEYWORDS

***Camellia sinensis*, osteoporosis, osteoarthritis, rheumatoid arthritis, cyberpharmacology, molecular docking**

1 Introduction

Musculoskeletal disorders, such as osteoporosis (OP), osteoarthritis (OA), and rheumatoid arthritis (RA), are prevalent health issues with substantial global impact. Epidemiological data have revealed the widespread development of these bone-related conditions across various age groups (1–3). OP is defined as bone brittleness, which is associated with fracture susceptibility (4). OA is characterized by cartilage degradation, leading to pain and impaired mobility (5). RA is a condition causing joint deformities and other organ pathologies (6). Current pharmacological therapies (such as bisphosphonates for OP, non-steroidal anti-inflammatory drugs for OA, and disease-modifying antirheumatic drugs for RA) do not provide a fundamental solution to issues with significant safety risks (7–9). Therefore, safer and more promising alternatives should be investigated.

Tea (*Camellia sinensis*), a traditional beverage, has several benefits for the human body (10, 11). In a randomized placebo-controlled trial, postmenopausal women with osteopenia who received green tea polyphenols exhibited better bone health (12). In another randomized controlled trial involving 50 participants, individuals who supplemented their diclofenac tablets with green tea had significantly lower pain scores, as measured using the visual analog scale, and better OA physical function scores compared with controls (13). In addition, a case–control study has revealed that high tea consumption can have a protective effect on smokers and individuals with anti-citrullinated protein autoantibody-positive RA (14). Nonetheless, the comprehensive roles of tea and its extracts in OP, OA, and RA must be systematically elucidated.

This review aimed to examine the current therapeutic mechanisms of tea and its extracts against OP, OA, and RA. In addition, the key active components and target proteins of tea were identified via computer simulations, thereby providing a theoretical foundation for its potential medical and healthcare applications.

2 Effects of tea against OP, OA, and RA

Tea has remarkable performance due to its antioxidant and anti-inflammatory properties. Therefore, it can be a promising candidate when used as a novel anti-inflammatory or antioxidant agent (15, 16). Furthermore, contemporary research can provide substantial evidence supporting the role of tea in preventing various diseases, particularly joint diseases, inhibiting disease progression, and promoting pain relief (17, 18). Catechins, which encompass (+)-catechin (C), (−)-epigallocatechin (EC), (−)-gallocatechin (GC), (−)-epigallocatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin-3-gallate (EGCG), are the primary components of tea (19, 20). These compounds, which are naturally consumed via tea consumption, play an essential role in maintaining bodily health. Current research focuses on the anti-inflammatory and antioxidant activities of tea and its components. That is, they promote osteoblast growth and inhibit osteoclast formation, thereby counteracting OP. In addition, these activities inhibit chondrocyte damage and synovial inflammation, which then promote resistance to OA and RA.

In this context, the current study primarily aimed to provide an overview of the potential mechanisms underlying the effects of tea against OP, OA, and RA.

2.1 Studies of tea treating osteoporosis

OP, characterized by low bone mass (osteopenia) and deterioration of bone microarchitecture, leads to compromised bone strength and an increased risk of fractures (21). There are several effective strategies against OP. These include the maintenance of bone homeostasis, which enhances bone density, microarchitecture, and strength and reduces the risk of OP and associated fractures (22–25). We gathered clinical studies of tea-treating OP, which are summarized in Table 1. A study showed that in postmenopausal women, additional intake of green tea polyphenol supplementation can improve the serum and urinary levels of oxidative damage biomarkers (31). Furthermore, it can increase the production of bone formation markers and improve bone turnover rates (12). In particular, the intake of active ingredients such as tea water extracts and tea polyphenols leads to significant improvements in bone mineral density, microstructure deterioration, and biological properties in ovariectomized or orchiectomized rats (32–35).

Maintaining bone homeostasis is important for addressing OP by regulating osteoblasts and mesenchymal stem/stromal cells to achieve a balance between bone formation and resorption (36–38). Tea extracts have antioxidant effects that enhance osteoclastogenesis, improve cell survival, and mitigate inflammation (39). Moreover, tea extracts exhibit potent phytoestrogenic effects by upregulating ESR1 expression (40, 41). EGC significantly upregulated the expression of key markers of bone formation, including Runt-related transcription factor 2 (RUNX2), alkaline phosphatase, osteonectin, and osteopontin (42). (−)-Epiafzelechin and (−)-epicatechin promote osteoblast proliferation and differentiation via their antioxidant properties (43). In addition, (−)-epicatechin gallate stimulates osteoblast differentiation by activating the PDZ-binding motif (TAZ) and RUNX2 (44). EGCG has antioxidant effects via the Nrf2 pathway, thereby protecting osteoblasts from apoptosis and attenuating bone microstructure deterioration (45). Theaflavin-3,3′-digallate activates several signaling pathways, including the tumor necrosis factor-α (TNF-α)-inhibited mitogen-activated protein kinase (MAPK), Wnt/β-catenin, and BMP/Smad pathways. This mechanism ultimately promotes the transcription of osteogenesis-associated factors such as RUNX2 and Osterix, leading to osteoblast differentiation and maturation (46). Furthermore, various tea extracts and tea polyphenols inhibit osteoclast formation, with EGCG being the most effective (47–51). Notably, EGCG downregulates the expression of NFATc1, directly binds to RANK, blocks the interaction between RANK and RANKL, and inhibits multiple pathways, including the HO-1-HMGB1-AGE pathway, nuclear factor kappa B (NF-κB) pathway, MAPK signaling pathway, and RANK/RANKL/OPG pathway, ultimately reducing osteoclast formation (52–54). In addition, tea extracts and EGCG enhance the osteogenic differentiation capacity of stem cells (55–57), thereby underscoring the anti-osteoporotic potential of tea and its compounds.

2.2 Studies of tea treating osteoarthritis

OA is a prevalent chronic joint disorder primarily characterized by joint cartilage degeneration, synovial inflammation, and pain. Moreover, it is often associated with the aging process (63, 64). We gathered clinical studies of tea treating OA and summarized in Table 2. Several studies have reported that high green tea intake is associated with a low incidence of OA (65, 66). In a short-term randomized, double-blind pilot study, a mixture of baicalin and catechin was as effective as naproxen in controlling the signs and symptoms of knee OA (67). Green tea has anti-inflammatory properties (58). Hence, tea and its extracts can mitigate the pathological progression of OA by decreasing the expression of inflammatory factors, including interleukins and matrix metalloproteinases, in the articular cartilage and synovium, thereby suppressing the inflammatory response.

Research on the effects of tea beverages on OA dates has been conducted since 1991 (68). Haqqi et al. have made significant contributions by focusing on the pharmacodynamic mechanisms of tea and its components for treating OA. They discovered that tea polyphenols when added to water, can be effective in preventing the development and progression of arthritis (69). Furthermore, they found that EGCG can reduce the expression and activity of various factors, including cyclooxygenase-2 (COX-2), nitric oxide synthase-2

TABLE 1 Clinical trial of tea or components treating osteoporosis.

Tea/Compounds	Subject	Mounts	Age range	Effect	Source
Green tea polyphenols	Women with osteoporosis	171	/	Effective	(12)
Green tea, oolong tea, and black tea	Men and women	25,045	Aged 38–86 years	Effective in women but not in men.	(23)
Tea	Men and women	42,742	Aged 45–74 years	Effective	(25)
Tea	Women	1,377	Aged <80 years	Effective	(26)
Tea	Women with osteoporosis	91,465	Aged 50–79 years	Effective	(27)
Tea	Postmenopausal women	724	Mean age was 57.6 ± 9.6 years	May have a positive effect on BMD but was not found to be a statistically significant factor.	(28)
Oolong tea	Postmenopausal women	476	Aged 40 to 88 years	Effective	(29)
Tea and flavonoid	Women	1,188	Aged >75 years	Effective	(30)

TABLE 2 Clinical trial of tea or components treating osteoarthritis.

Tea/Compounds	Subject	Mounts	Age range	Effect	Source
Green tea	Men and women	50	Aged 40 to 75	Effective	(13)
Anti-inflammatory diet with green tea	Men and women	18	Between 20 and 80	Effective	(58)

TABLE 3 Clinical trial of tea or components treating rheumatoid arthritis.

Tea/Compounds	Subject	Mounts	Age range	Effect	Source
Green tea	Subjects with rheumatoid arthritis	120	Mean age of (60.7 ± 2.53 years)	Effective	(59)
Aqueous green tea extract	Subjects with/without rheumatoid arthritis	130	Aged >40 years	Effective	(60)
Epigallocatechin gallate	Subjects with/without rheumatoid arthritis	50	Aged between 25 and 60 years	Effective	(61)
Tea	Subjects with rheumatoid arthritis	662	/	Effective	(62)

(NOS-2) (70), matrix metalloproteinase (MMP)-1, MMP-13 (71), and TNF-α (72). In addition, EGCG can globally suppress the inflammatory response in human chondrocytes, possibly via the inhibition of NF-κB and c-Jun N-terminal kinase (JNK)-MAPK activation (73–75). A previous study has consistently revealed the protective effect of EGCG against OA (76). Furthermore, its mechanisms have been found to be involved in various processes such as microRNA regulation (e.g., microRNA-140-3p, microRNA-199a-3p, and microRNA-29b) (77–79) and oxidative stress (77, 78). In addition to its chondroprotective effects, EGCG alleviates synovial inflammation (80). Another study has explored the role of other tea components, such as theaflavin-3,3′-digallate (81) and theaflavin (82), both of which have the ability to inhibit cartilage damage. In recent years, previous studies have focused on enhancing the anti-inflammatory effects of tea and its components. Several studies have improved the efficacy of EGCG in the cartilage and synovium by introducing novel materials or altering the mode of application. These mechanisms involve the modulation of autophagy, the production of reactive oxygen species, mitochondrial repair, and synovial macrophage polarization (83–86).

2.3 Studies of tea treating rheumatoid arthritis

In a review published in 2001, the authors proposed that green tea can be a prophylactic agent against chronic inflammatory diseases, including RA (87). We gathered Clinical trial of tea or components treating rheumatoid arthritis in Table 3. A case–control study showed that consuming more than one cup of green tea per month can have a preventive effect against RA (88). Maintaining a daily intake of 4–6 cups of green tea over a period of up to 6 months has a positive effect on RA disease activity in patients with RA (59). In addition, a Swedish case–control study showed that heavy tea consumption has a protective effect against RA in smokers and anti-citrullinated protein autoantibody-positive individuals (14). Numerous experiments have revealed that tea water extracts or the polyphenolic components of tea can reduce RA in experimental animals. The investigation of its mechanism has predominantly revolved around its antioxidant and anti-inflammatory properties.

Various tea-related ingredients, including tea aqueous extract (89), catechin (90), EGCG, and gallic acid (91), are significantly effective in alleviating RA symptoms. The imbalance between oxidation and reduction is an important mechanism in the development of RA (92). Reactive species oxidize cellular biomolecules, leading to DNA damage (93). Therefore, reducing oxidative stress in RA is an effective therapeutic strategy (94). Physiological antioxidant enzymes such as superoxide dismutase (SOD), glutathione (GSH), and peroxiredoxins counteract the possible damaging effects of these reactive species by scavenging or neutralizing free radicals and oxidizing substances. Research has shown that green tea extract can increase the SOD and GSH levels while decreasing the levels of lipid peroxides (LPO), nitric

oxide (NO), and PGE2 in a rat RA model. Hence, it can be beneficial in both the liver and brain (95, 96). EGCG-fed mice exhibited higher levels of heme oxygenase-1 (HO-1) and nuclear factor erythroid2-related factor 2 (Nrf2) (97, 98), and the significant activation of HO-1 and Nrf2 has anti-arthritic effects (99). In addition to their antioxidant effects, tea and its compounds have significant anti-inflammatory effects. Sabrina Fechtner has revealed that EGCG, epigallocatechin (EGC), and EC occupy the active site of the TAK1 kinase domain, with EGCG being the most dominant, interfering with the IL-1β signaling pathway that regulates the expression of IL-6, IL-8, and Cox-2 in primary human RA synovial fibroblasts (100). Another study revealed that EGCG targets TAK1 for treating RA by inhibiting TAK1 phosphorylation at Thr (184/187), suppressing K(63)-linked autoubiquitination of TRAF6, and enhancing proteasome-associated deubiquitinase expression to rescue proteins from proteasomal degradation (101). In addition, green tea extract and EGCG modulate the production of chemokine (102) and immune cells (97), leading to RA improvements.

3 Assessment of the potential active components of tea in OP, OA, and RA

Since tea performed well not only in OP but also in OA and RA, searching for targets in OP, OA, and RA may have a practical meaning in providing guidance for the prevention and control of OP, OA, and RA. With the help of bioinformatics analysis methods, we summarized the ingredients of tea (Table 4) and discovered the relationship between tea ingredients and diseases. We screened the genes of OP, OA, and RA related to tea ingredients and then performed Gene Ontology (GO) analysis and enrichment analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

3.1 Oxidative stress mitigation: unraveling pathways

Due to aging, traumatic injury, or immune dysfunction, various tissue cells are exposed to a range of pathophysiological mediators, including reactive oxygen species (ROS) and reactive nitrogen species (NOS). ROS-mediated stress, by inducing functional impairments in osteoblasts, osteoclasts, chondrocytes, and synovial cells, contributes to the pathological progression of OP, OA, and RA.

Tea exhibits robust antioxidant effects, a perspective supported by network pharmacology results. The active ingredient–gene target networks of the active ingredients for each disease were mapped (Figure 1). Gene Ontology (GO) enrichment analysis reveals that genes associated with OP, targeted by active components of tea, are primarily enriched in response to oxidative stress (Figure 2A). In the GO analysis results of networks between tea and OA and between tea and RA, genes linked to response to oxidative stress occupy the top positions (Figures 2B,C). The frequency ranking of responses to oxidative stress across the three diseases is detailed in Table 5. Among the 37 tea components targeting genes related to this biological process, EGCG, caffeine, ursolic acid, beta-carotene, and (−)-epicatechin emerge as the top five components. KEGG pathway analysis indicates enrichment in pathways such as lipid and atherosclerosis and the AGE-RAGE signaling pathway in diabetic complications (Figure 3 and Table 6). Although direct evidence of oxidative stress is not explicitly shown in the KEGG results, the enrichment of pathways closely related to oxidative stress responses underscores the pivotal role of tea's antioxidant action in the treatment of OP, OA, and RA.

3.2 Inflammatory modulation: an alternate pathway for tea impact

To further investigate the therapeutic effects of tea in treating OP, OA, and RA, network pharmacology was applied to analyze the targeted relationship between tea and commonly associated genes. The analysis results not only confirm the involvement of oxidative stress in line with previous findings but also reveal enrichment in pathways such as lipid and atherosclerosis, the AGE-RAGE signaling pathway in diabetic complications, the IL-17 signaling pathway, and the TNF signaling pathway through KEGG analysis (Figure 4 and Table 7). This demonstrates the anti-inflammatory effects of tea, with core genes such as PTGS2, PTGS1, CASP3, JUN, and IL-6 remaining central in these pathways. A total of 36 tea components target genes related to these pathways, with (−)-epigallocatechin-3-gallate, caffeine, ursolic acid, beta-carotene, and (−)-epicatechin ranking as the top five components targeting the highest number of genes. This suggests that these components play a core role in anti-inflammatory action. Importantly, these components also play a significant role in the previously mentioned antioxidative effects. Therefore, we utilized computer-simulated molecular docking to further validate the relationships between these components and core proteins.

We conducted molecular docking for the aforementioned components (EGCG, ursolic acid, beta-carotene, (−)-epicatechin, and caffeine) and proteins (PTGS2, PTGS1, CASP3, IL-6, and JUN) with established targeting relationships. The affinity for each combination was below −5 kCal • mol-1. Thus, it has favorable binding activity. Furthermore, most combinations exhibited an affinity below −7 kCal • mol-1, which indicated a robust binding activity (Table 8). Figure 5 shows the combinations featuring hydrogen bonds whose affinity is below −9 kCal•mol-1.

4 Discussion

The Chinese have been drinking tea for hundreds of years. Therefore, most people believe that tea can reduce the risk of various diseases. With the development of modern medicine, the efficacy and mechanism of action of tea have been comprehensively explored. However, the results of clinical and animal studies are still inconclusive. This review aimed to explore the possibility and mechanism of action of tea for treating OP, OA, and RA by evaluating previous studies and constructing a network for the association between tea and different diseases. Current studies have focused on the anti-inflammatory and antioxidant effects of tea. Tea and its

TABLE 4 Summary of potential active components from tea.

Name	InChIKey	Source
(−)-Catechin	PFTAWBLQPZVEMU-HIFRSBDPSA-N	(90–93, 95)
(−)-Catechin gallate	LSHVYAFMTMFKBA-PZJWPPBQSA-N	(91, 92, 94, 96)
(−)-Epicatechin	PFTAWBLQPZVEMU-UKRRQHHQSA-N	(14, 90–97)
(−)-Epicatechin-pentaacetate	BKYWAYNSDFXIPL-JWQCQUIFSA-N	(14, 91, 92)
(−)-Epigallocatechin-3-gallate	WMBWREPUVVBILR-WIYYLYMNSA-N	(14, 90–95, 97)
(−)-Gallocatechin gallate	WMBWREPUVVBILR-GHTZIAJQSA-N	(14, 91, 92, 96, 97)
(+)-Catechin	PFTAWBLQPZVEMU-DZGCQCFKSA-N	(14, 90–96)
(+)-Cycloolivil	KCIQZCNOUZCRGH-VOBQZIQPSA-N	(90, 91)
(+)-Epicatechin	PFTAWBLQPZVEMU-ZFWWWQNUSA-N	(90–93, 95, 97)
2-Formylpyrrole	ZSKGQVFRTSEPJT-UHFFFAOYSA-N	(14, 91, 92)
2-Phenylbutenal	DYAOGZLLMZQVHY-MBXJOHMKSA-N	(14, 92)
Aids214634	CICMVLOHBZPXIT-WNISUXOKSA-N	(14)
alpha-Cadinol	LHYHMMRYTDARSZ-BYNSBNAKSA-N	(14)
Astragalin	JPUKWEQWGBDDQB-QSOFNFLRSA-N	(92)
beta-Carotene	OENHQHLEOONYIE-JLTXGRSLSA-N	(14, 97)
Betulinic acid	QGJZLNKBHJESQX-FZFNOLFKSA-N	(14)
Caffeine	RYYVLZVUVIJVGH-UHFFFAOYSA-N	(14, 90–92, 96)
cis-Jasmone	XMLSXPIVAXONDL-PLNGDYQASA-N	(94)
Citral	WTEVQBCEXWBHNA-JXMROGBWSA-N	(14, 95)
Citric acid	KRKNYBCHXYNGOX-UHFFFAOYSA-N	(89, 94, 95)
delta-Terpineol	SQIFACVGCPWBQZ-UHFFFAOYSA-N	(14)
Diosmetin	MBNGWHIJMBWFHU-UHFFFAOYSA-N	(14)
Ellagic acid	AFSDNFLWKVMVRB-UHFFFAOYSA-N	(90, 91, 95, 97)
Epiafzelechin	RSYUFYQTACJFML-UKRRQHHQSA-N	(94, 95)
Epicatechin gallate	LSHVYAFMTMFKBA-FPOVZHCZSA-N	(89, 92, 95–97)
Epigallocatechin	XMOCLSLCDHWDHP-IUODEOHRSA-N	(14, 90, 92, 95–97)
Folic acid	OVBPIULPVIDEAO-LBPRGKRZSA-N	(14, 90–94)
Gallic acid	LNTHITQWFMADLM-UHFFFAOYSA-N	(90, 94–96)
Gallocatechin	XMOCLSLCDHWDHP-SWLSCSKDSA-N	(14, 92, 95–97)
Geraniin	JQQBXPCJFAKSPG-SVYIMCMUSA-N	(14, 92)
Hirsutrin	OVSQVDMCBVZWGM-QSOFNFLRSA-N	(92, 94, 95)
Indole	SIKJAQJRHWYJAI-UHFFFAOYSA-N	(87, 94)
Isomyricitrin	FOHXFLPXBUAOJM-LIBJPBHASA-N	(14)
Isovitexin	MYXNWGACZJSMBT-VJXVFPJBSA-N	(94, 95)
Kaempferitrin	PUPKKEQDLNREIM-SLVXTXDOSA-N	(90, 94–96)
Kaempferol 3-O-glucorhamnoside	SOSLMHZOJATCCP-FPRKOELSSA-N	(90)
Kaempferol 3-O-rhamnoside	SOSLMHZOJATCCP-LYHQQHOMSA-N	(90, 93)
Kaempferol-3-galactoside	JPUKWEQWGBDDQB-DTGCRPNFSA-O	(90)
Kaempferol-3-O-glucuronide	FNTJVYCFNVUBOL-VFKUPZNOSA-N	(90)
L-Epicatechin gallate	LSHVYAFMTMFKBA-TZIWHRDSSA-N	(14, 90, 95)
L-Phenylalanine	COLNVLDHVKWLRT-QMMMGPOBSA-N	(9, 39, 96)
Myricetin-3-O-beta-D-galactopyranoside	FOHXFLPXBUAOJM-MRBQYWCKSA-N	(90, 93, 95)
Myricetin-3-O-beta-D-glucopyranoside	FOHXFLPXBUAOJM-FVNGHLGHSA-O	(90, 93)
Naringin	DFPMSGMNTNDNHN-ZPHOTFPESA-N	(93)

(Continued)

TABLE 4 (Continued)

Name	InChIKey	Source
Nicotiflorin	RTATXGUCZHCSNG-QHWHWDPRSA-N	(14)
Nicotinic acid	PVNIIMVLHYAWGP-UHFFFAOYSA-N	(14)
Oleanolic acid	MIJYXULNPSFWEK-GTOFXWBISA-N	(14)
Petunidin	BLBZAMLPGFAHFX-UHFFFAOYSA-N	(90, 91, 93)
Phenethyl isothiocyanate	IZJDOKYDEWTZSO-UHFFFAOYSA-N	(14, 92)
Procyanidin B1	XFZJEEAOWLFHDH-UKWJTHFESA-N	(92–95)
Procyanidin B2	XFZJEEAOWLFHDH-NFJBMHMQSA-N	(92–95)
Quercetin,3-O-rutinoside	IKGXIBQEEMLURG-BKUODXTLSA-O	(90, 95)
Quercitrin	OXGUCUVFOIWWQJ-HQBVPOQASA-N	(90, 93, 95, 96)
Quinic acid	AAWZDTNXLSGCEK-WYWMIBKRSA-N	(90–93)
Rutin	IKGXIBQEEMLURG-NVPNHPEKSA-N	(93–96)
Theobromine	YAPQBXQYLJRXSA-UHFFFAOYSA-N	(14, 90, 92, 94–96)
Tricin	HRGUSFBJBOKSML-UHFFFAOYSA-N	(14, 92)
Trifolin	JPUKWEQWGBDDQB-DTGCRPNFSA-N	(14, 92)
Tryptophan	QIVBCDIJIAJPQS-VIFPVBQESA-N	(92, 96)
Ursolic acid	WCGUUGGRBIKTOS-GPOJBZKASA-N	(14, 92)
Vicenin-2	FIAAVMJLAGNUKW-VQVVXJKKSA-N	(92, 95, 97)
Xanthine	LRFVTYWOQMYALW-UHFFFAOYSA-N	(14, 92, 97)

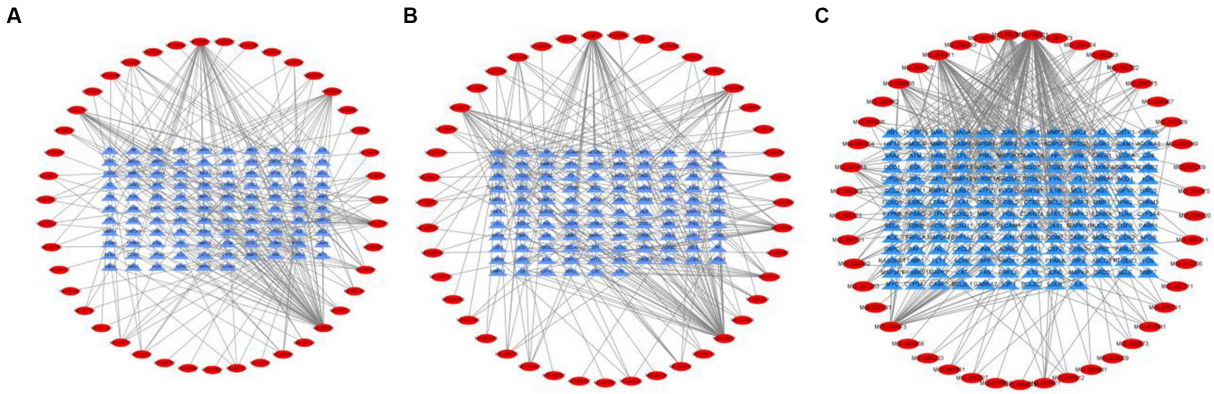

FIGURE 1
Networks of tea components targeting OP, OA and RA. **(A)** Network of tea treating OP; **(B)** Network of tea treating OA; **(C)** Network of tea treating RA.

components affect the activation of various enzymes, transcription of inflammation-related genes, and release of inflammatory factors in bone and joint tissues via the Nrf2-related pathway, MAPK pathway, and NF-κB pathway. Moreover, they regulate oxidative stress and inflammation in tissues and cells in OP, OA, and RA.

Network pharmacology results show that PTGS2, PTGS1, CASP3, IL-6, and JUN are the potential targets of tea when regulating OP, OA, and RA. PTGS2 (also referred to as COX-2) and PTGS1 (also known as COX-1) have been extensively and intensively evaluated. COX inhibitors, or non-steroidal anti-inflammatory drugs, inhibit the production of COX-2 and COX-1 to achieve anti-inflammatory, analgesic, and antipyretic effects. In addition, they are commonly used in the treatment of OA and RA (103). The anti-inflammatory and analgesic efficacies of COX inhibitors are significant. However, they also increase the risk of gastrointestinal ulcers, hemorrhage, and renal and cardiovascular adverse events (104). In the molecular target regulatory network of tea and disease (OP, OA, and RA), 31 molecules can bind to PTGS2 targets, and 18 molecules can bind to PTGS1 targets. Therefore, tea can possibly play a role in COX inhibition. However, epidemiological studies have shown that tea consumption reduces the risk of cardiovascular mortality through mechanisms associated with the lowering of lipid levels, mitigation of ischemia/reperfusion injury (105, 106), inhibition of oxidative stress, enhancement of endothelial function, attenuation of inflammation, and protection of cardiomyocyte function (107). The tea polyphenol EGCG exerted a protective effect on patients with 5-aminosalicylic acid and/or azathioprine-refractory ulcerative colitis (108). According to the report, TIMP1, PTGS2, ICAM1, MMP9, IL1B,

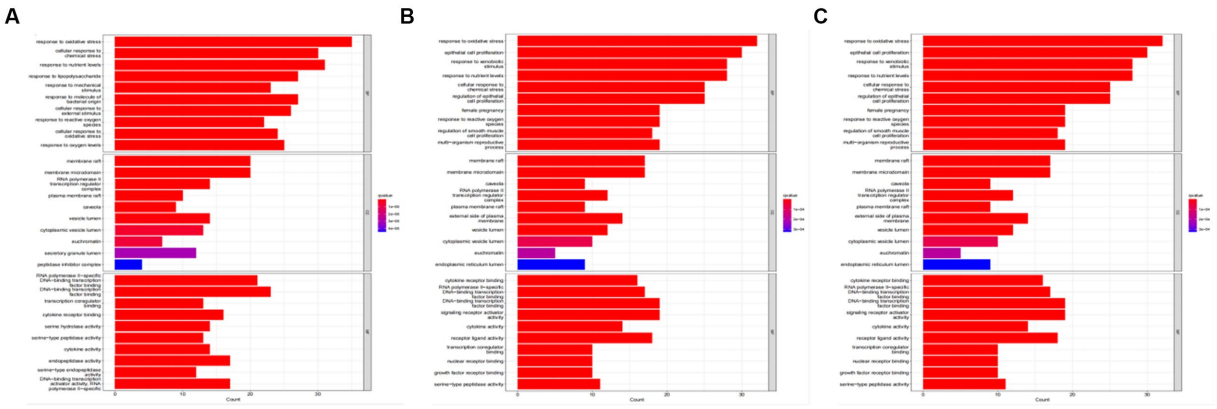

FIGURE 2
GO analysis of tea targeting OP, OA, and RA. **(A)** GO analysis of tea targeting OP; **(B)** GO analysis of tea targeting OA; **(C)** GO analysis of tea targeting RA.

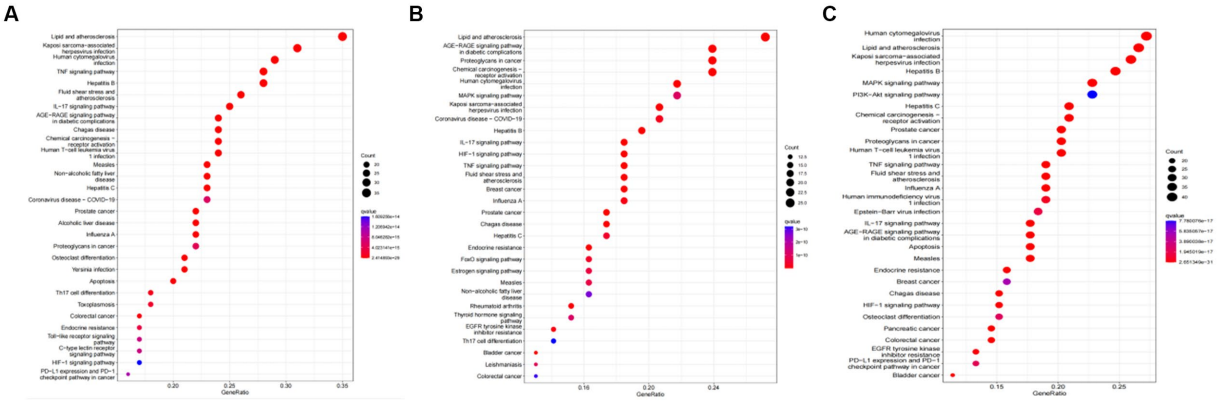

FIGURE 3
KEGG analysis of tea targeting OP, OA, and RA. **(A)** KEGG analysis of tea targeting OP; **(B)** KEGG analysis of tea targeting OA; **(C)** KEGG analysis of tea targeting RA.

TABLE 5 Gene Ontology (GO) enrichment analysis.

Disease	ID	Description	GeneRatio	BgRatio	*p*-value	p.adjust	*q*-value
Osteoporosis	GO:0006979	Response to oxidative stress	32/95	434/18903	6.00E-29	2.21E-25	9.89E-26
Osteoarthritis	GO:0006979	Response to oxidative stress	35/106	434/18903	3.23E-31	1.22E-27	5.36E-28
Rheumatoid arthritis	GO:0006979	Response to oxidative stress	45/165	434/18903	1.01E-35	4.33E-32	1.80E-32

CXCL8, IL-6, and RELA were identified as hub genes in ulcerative colitis (109), which had been found in the target collection of tea components. A new study, processed by integrating network pharmacology and metabolomics, demonstrated that *Jasminum elongatum* reverses ulcerative colitis in mice via the IκB/p65/COX-2/arachidonic acid pathway (110). The tea aqueous extract inhibited experimentally induced colitis and liver injury in mice (111). Tea and its extracts confer protective effects against alcoholic liver disease, non-alcoholic fatty liver disease, CCL4-induced liver injury, and inflammatory liver damage. The mechanisms underlying these protective effects involve modulation of signaling pathways such as the NF-κB signal pathway, TGFβ/p-ERK/p-Smad1/2 signal pathway, Nrf2 signaling activation, and autophagy restoration (112–117). Tea consumption has been associated with a reduced risk of renal cell carcinoma (118) and improved kidney function in diabetic patients (119). Studies indicate that effective components such as L-theanine, tea polyphenols, and EGCG can ameliorate renal cell damage through the modulation of related pathways, including the AGEs/RAGE signaling pathway (120), CYP450s/ROS/MAPK/NF-κB pathway (121), TGFβ/Smad3 signaling pathway (122), and ferroptosis (123). The abovementioned studies have revealed the potential COX inhibitory effects of tea and its ability to fight diseases such as OP, OA, and RA with minimal cardiovascular, gastrointestinal, hepatic, and renal damage.

Via a network pharmacological analysis, a number of tea ingredients target disease genes in OP, OA, and RA, proving that tea

TABLE 6 KEGG pathway analysis of tea treating OP, OA, and RA.

Disease	ID	Description	GeneRatio	*p*-value	p.adjust	*q*-value
Osteoporosis	hsa05417	Lipid and atherosclerosis	25/92	2.06E-19	2.45E-17	7.91E-18
	hsa04933	AGE-RAGE signaling pathway in diabetic complications	22/92	2.04E-23	4.86E-21	1.57E-21
	hsa05205	Proteoglycans in cancer	22/92	2.36E-16	1.41E-14	4.54E-15
	hsa05207	Chemical carcinogenesis - receptor activation	22/92	4.86E-16	2.31E-14	7.47E-15
	hsa05163	Human cytomegalovirus infection	20/92	2.47E-13	3.78E-12	1.22E-12
Osteoarthritis	hsa05417	Lipid and atherosclerosis	35/100	3.32E-31	7.98E-29	2.41E-29
	hsa05167	Kaposi sarcoma-associated herpesvirus infection	31/100	2.98E-27	1.79E-25	5.41E-26
	hsa05163	Human cytomegalovirus infection	29/100	1.06E-22	2.55E-21	7.72E-22
	hsa04668	TNF signaling pathway	28/100	3.77E-30	4.53E-28	1.37E-28
	hsa05161	Hepatitis B	28/100	1.48E-25	5.91E-24	1.79E-24
Rheumatoid arthritis	hsa05163	Human cytomegalovirus infection	43/158	3.68E-32	2.59E-30	8.49E-31
	hsa05417	Lipid and atherosclerosis	42/158	8.35E-32	4.29E-30	1.41E-30
	hsa05167	Kaposi sarcoma-associated herpesvirus infection	41/158	1.71E-32	2.20E-30	7.21E-31
	hsa05161	Hepatitis B	39/158	3.15E-33	8.09E-31	2.65E-31
	hsa04010	MAPK signaling pathway	36/158	5.79E-20	7.09E-19	2.32E-19

TABLE 7 GO and KEGG analyses for tea targeting common genes of OP, OA, and RA.

Analysis type	ID	Description	GeneRatio	BgRatio	*p*-value	p.adjust	*q*-value
GO	GO:0006979	Response to oxidative stress	29/69	434/18903	1.18E-29	3.83E-26	1.50E-26
KEGG	hsa04933	AGE-RAGE signaling pathway in diabetic complications	20/67	100/8644	9.38E-24	2.10E-21	6.12E-22
	hsa05417	Lipid and atherosclerosis	24/67	215/8644	3.30E-22	3.69E-20	1.08E-20
	hsa04657	IL-17 signaling pathway	17/67	94/8644	2.00E-19	1.49E-17	4.35E-18
	hsa04668	TNF signaling pathway	17/67	114/8644	6.32E-18	3.54E-16	1.03E-16
	hsa05142	Chagas disease	16/67	102/8644	2.88E-17	1.29E-15	3.75E-16

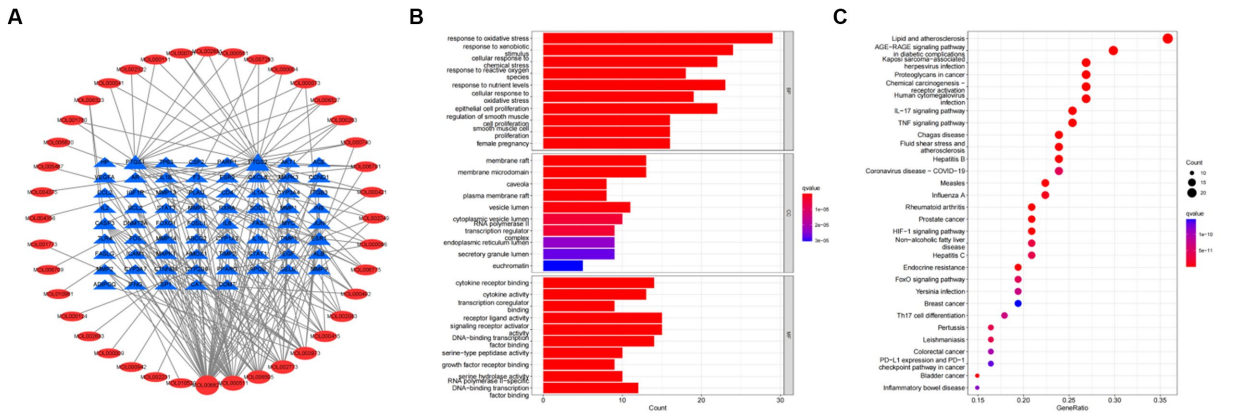

FIGURE 4
Bioinformatics analysis for tea targeting common genes of OP, OA, and RA. **(A)** Network of tea treating common genes, **(B)** Gene ontology (GO) enrichment analysis; **(C)** Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis.

has therapeutic or adjunctive therapeutic effects against OP, OA, and RA. Current clinical studies do not provide clear conclusions. Some reports have shown that tea can be effective in treating OP, OA, or RA. However, there are limitations in terms of the study population, the size of the population, or the quality of the data. Meanwhile, some clinical studies or meta-analyses have revealed that tea consumption does not improve the clinical performance of patients or reduce the risk of OP, OA, or RA (124). Considering the diversity of active

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TABLE 8 Summary of affinity of each combination.

Affinity (kcal/mol)	EGCG	Ursolic acid	Beta-Carotene	(−)-Epicatechin	Caffeine
PTGS2	−10.4	−9.8	−7.9	−9.1	−6.7
PTGS1	/	−7.6	/	−8.0	−6.9
CASP3	−8.7	−8.4	−8.7	−7.7	−5.3
IL-6	−7.2	−8.7	/	−7.3	−5.2
JUN	−11.0	−7.5	−7.7	−9.1	−6.8

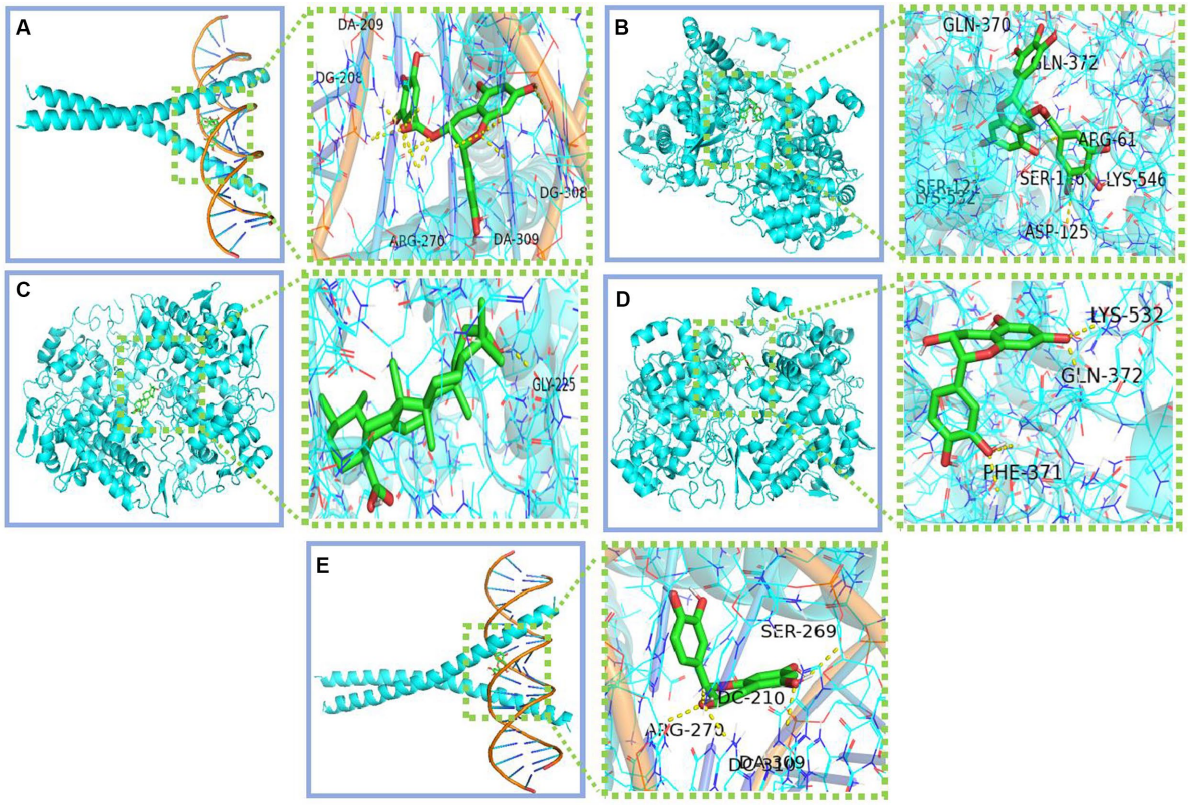

FIGURE 5
Combinations with affinity below −9 kCal•mol-1 **(A)** Jun-EGCG; **(B)** PTGS2-EGCG; **(C)** PTGS2-Ursolic acid; **(D)** PTGS2-Epicatechin; **(E)** Jun-Epicatechin.

ingredients in tea, in addition to ingredients such as EGCG and EC, which play a positive role, other ingredients, such as caffeine, increase the risk of fracture, OP, or OA and can be an influencing factor in the therapeutic effects of tea. Therefore, further studies on the role of tea must be performed.

Studies on effective treatment strategies against OP, OA, and RA are still conducted by the medical community. Tea is rich in various natural compounds that can be used for disease treatment. This study aimed to evaluate the potential mechanisms of action of tea and its related components for treating OP, OA, and RA. These mechanisms of action mainly focus on the antioxidant and anti-inflammatory responses of tea components. In previous experimental studies, tea and tea extracts and their active ingredients mainly acted on OA inflammatory factors to alleviate OA cartilage degeneration. Furthermore, they are mainly used to promote osteoblast growth, inhibit osteoclast formation in OP, and inhibit inflammation mainly via their antioxidant effects in RA. The network pharmacological results revealed targets and pathways not covered by existing experimental studies. Moreover, they were validated by molecular docking. The network pharmacology results showed that tea has an anti-COX capacity, hormone-like properties, and cardiovascular, gastrointestinal, hepatic, and renal protective effects. This is because tea has various components that synergize or antagonize each other, which has a more pronounced dual effect than a single component. In the network pharmacology analysis, we comprehensively collected data on the compounds of tea and did not screen the compounds for bioavailability and drug-like properties so that we could analyze the mechanism of action of tea against OP, OA, and RA analyzed without omission. However, different kinds of tea have different compound compositions; for example, black tea contains theaflavins, thearubigins, and other components, and lower levels of polyphenols compared with green tea, which cannot be represented in network pharmacological analysis results. The network pharmacological analysis results may conceal the specific effects of tea on certain disease genes.

Nevertheless, there is still a need for future research on the composition of tea and the development of standardized tea beverages, which will lead to efficacy studies. Research on the relationship between the use of standardized tea beverages and disease would be helpful to clarify the efficacy of tea. In conclusion, the use of tea has great potential in the medical and healthcare fields.

Author contributions

XX: Data curation, Formal analysis, Writing – original draft. JF: Investigation, Methodology, Writing – original draft. WG: Conceptualization, Investigation, Writing – original draft. YQ: Investigation, Writing – original draft. DW: Methodology, Writing – original draft. ZH: Formal analysis, Validation, Writing – original draft. LW: Supervision, Writing – review & editing. XL: Funding acquisition, Project administration, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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