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REVIEW

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Ejiao as a preventive agent for osteoporosis - a scoping review of current evidence

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Abstract

Ejiao, a traditional Chinese medicinal product derived from donkey's hide, has long been used to promote blood formation and treat various ailments. Recently, growing pharmacological evidence has suggested that Ejiao and its formulations may also possess bone-protecting properties, making it a potential candidate for preventing and treating osteoporosis. This scoping review aims to summarise the current scientific evidence on the anti-osteoporosis potential of Ejiao and its formulations in osteoporosis prevention. A comprehensive literature search was conducted using PubMed, Web of Science, Scopus, and China National Knowledge Infrastructure up to October 2024. Primary studies published in English or Mandarin, regardless of study design, that investigated the effects of Ejiao on bone in vivo or bone cells in vitro were included. A total of 22 studies were included, comprising five studies on Ejiao alone and 17 studies on Ejiao-based formulations. The findings indicated that Ejiao alone enhanced osteoblast differentiation by increasing alkaline phosphatase synthesis and reducing bone remodelling markers in ovariectomised rats. However, its direct effect on bone mineralisation and density remains uncertain due to the absence of an exogenous mineral source. In contrast, Ejiao-based formulations, such as calcium-Ejiao oral liquid and Donkey-hide glue reinforcing bone oral solution, demonstrated more pronounced bone-protective effects, including improving bone density, enhancing bone repair, and supporting vitamin D metabolism in both animal models and clinical studies. These findings suggest that while Ejiao alone may promote osteoblast activity, its role in osteoporosis management may be more effective when combined with essential minerals. Further long-term studies and human clinical trials are needed to clarify its therapeutic potential and underlying molecular mechanisms.

Keywords Bone, E'jiao, Fracture, Osteoblast, Traditional Chinese medicine

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Introduction

Osteoporosis and haematopoietic disorders are significant health concerns that contribute substantially to morbidity and mortality globally. Osteoporosis is characterised by decreased bone mass and microarchitectural deterioration of bone tissue, leading to an increased risk of fragility fractures, particularly in older adults [1–3]. The pathogenesis of osteoporosis involves an imbalance between bone resorption and bone formation, driven by the dysregulation of osteoclast and osteoblast activity. Osteoclasts, responsible for bone resorption, are activated by factors such as receptor activator of nuclear factor kappa-B ligand (RANKL). In contrast, osteoblasts, which mediate bone formation, are regulated by pathways including Wnt/β-catenin and bone morphogenetic protein (BMP) signalling [4–6]. These processes are further influenced by systemic factors such as hormonal changes, inflammation, and oxidative stress, which disrupt bone remodelling homeostasis [7–9]. Haematopoietic disorders, such as anaemia and leukaemia, affect the production and function of blood cells, thereby impacting overall health and quality of life. These conditions are intricately linked, as the deterioration of haematopoietic autophagy contributes to bone loss and disrupts osteocyte homeostasis, highlighting the profound impact of impaired blood cell production on bone health [10]. For instance, abnormal haematopoiesis can lead to the overproduction of pro-inflammatory cytokines, such as interleukin (IL)-6 and tumour necrosis factor (TNF)-α, which promote osteoclastogenesis and bone resorption [11, 12]. Abnormal proliferation of bone marrow cells is linked to significant bone density loss observed in various conditions, indicating a strong association between bone marrow cell overgrowth and bone degradation [13].

Ejiao, also known as donkey-hide gelatin, is a traditional Chinese medicine (TCM) highly regarded for its blood-nourishing properties [14, 15], its anti-inflammatory properties [16], improving skin health [17], and potentially supporting bone health [18]. Ejiao is often combined with other herbs to enhance its effects [19–21]. Modern pharmacology studies have validated the traditional medical uses of Ejiao [22]. As evidenced in previous studies, it plays a role in regulating bone metabolism and hormonal balance, and exerts anti-inflammatory and antioxidant effects [18]. TCM formations containing Ejiao have been shown to modulate the RANKL/OPG (osteoprotegerin) ratio, thereby inhibiting excessive osteoclast activity and reducing bone resorption [23]. Additionally, Ejiao has been reported to influence Wnt/β-catenin signalling [24], which is implicated in bone formation. These beneficial effects of Ejiao on bone are believed to be contributed by collagen peptides, glycosaminoglycans, amino acids, and essential minerals, such as calcium and phosphorus [25–27]. However, the mineral content of Ejiao is relatively low, with calcium levels reported at only 0.07–0.13 ppm [27], which is negligible as a dietary calcium source. Therefore, the skeletal beneficial effects of Ejiao are likely independent of its calcium content, but rather its bioactive compounds that influence bone metabolism.

Given its medicinal effects, Ejiao is poised to be a TCM product with dual effects on the skeletal and haematopoietic systems. It offers a holistic approach to treating conditions that involve disruptions in the bone marrow microenvironment. Understanding the molecular and cellular pathways involved can enhance the development of integrated therapies targeting both bone and blood health. While the effects of Ejiao on the haematological system have been extensively reported and summarised in previous studies [28–30], reports on its effects on bone health are relatively scarce.

This scoping review aims to summarise the existing evidence on Ejiao's skeletal effects, explore its underlying mechanisms, and suggest future research directions. It seeks to provide a comprehensive understanding of Ejiao's role in bone health and its potential benefits in preventing osteoporosis.

Methods

This scoping review was developed following Arksey and O'Malley's framework and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines [31, 32]. The methodology encompassed the following steps: (1) formulating the research question; (2) identifying pertinent studies; (3) selecting the studies; (4) charting the data; and (5) collating, summarising, and reporting the findings.

Formulating the research question

The research question was "What is the current evidence on the effectiveness of Ejiao in preventing osteoporosis?" It is recognised that various Ejiao-containing formulations are available on the market, and they contain additional ingredients which enhance bone health. These products were also included in the review [19, 33]. Osteoporosis prevention encompasses various aspects, including the prevention of bone density loss, reduction in fracture risk, and enhancement of bone strength.

Identifying pertinent studies

A comprehensive literature search was performed using electronic databases, including PubMed, Scopus, and China National Knowledge Infrastructure (CNKI), in October 2024. The search string used was, ("Ejiao" OR "donkey-hide gelatin" OR "Colla corii asini" OR "ā' jiao") AND ("bone" OR "osteoporosis" OR "gǔ"). Studies published from database inception through October

2024 were considered. Studies that examined the effects of Ejiao or its formulations on bone health, its skeletal mechanisms of action, or clinical outcomes related to osteoporosis were included. All study types (in vitro, in vivo and clinical trials) were considered. Articles not written in English or Mandarin were excluded. Publications not containing primary data, such as reviews, editorials, letters, books and book chapters, were excluded. Conference abstracts were excluded due to incomplete data and redundancy. No additional filters were applied during the search to ensure inclusivity.

Selecting the studies

The search results of each database were downloaded and merged using Endnote (version 21.4, Clarivate, London, UK). The selection process began with the removal of duplicate records from different databases using Endnote [34]. Titles and abstracts were then screened to evaluate their eligibility, followed by a thorough review of complete texts by three researchers (YW, GC, XJ). The references of articles included in full-text screening were examined to prevent omissions. Disagreements regarding inclusion or exclusion were resolved through consensus, with the assistance of another researcher (KYC). The article selection process is summarised in the PRISMA flow chart (Fig. 1) [32].

Charting the data

Two authors (YW, GC) used a standard Excel table to extract relevant information from the selected studies, including researchers, publication years, study design, subjects or disease models used, type of Ejiao, dosage, treatment period and major findings.

Collating, summarising and reporting the results

Due to the heterogeneity of the studies involved and the variability in reported outcomes, the scoping review approach was adopted instead of a systematic review, as it allowed for a broad examination of available evidence without imposing strict methodological constraints. The study types, disease models, Ejiao formulations (dose and treatment period), and major outcomes were summarised and reported. Quality appraisal was not performed on the studies included, as the objective of this scoping review was to map the existing literature rather than selecting the best evidence. The role of Ejiao in preventing osteoporosis and the research gaps identified were discussed.

Results

Results of the literature search

The literature search yielded 108 results (17 from PubMed, 8 from Web of Science, 23 from Scopus, and 60 from CKNI). After duplicate removal, 89 articles were screened by title and abstract. A total of 71 articles were excluded (9 non-original research articles, 10 conference abstracts, 3 theses/dissertations, 3 newspaper articles, and 46 outside the study scope), leaving 18 articles for full-text review, along with 4 additional reports identified through reference tracing. No further exclusions were made, resulting in 22 articles included in the review. This final selection comprised 2 in vitro studies, 15 in vivo studies, and 4 clinical trials, with 5 articles focused on the skeletal effects of Ejiao alone and 17 on Ejiao formulations.

Skeletal effects of Ejiao

In vitro research showed that Ejiao did not promote the proliferation of primary osteoblasts from Wistar rats but significantly enhanced their alkaline phosphatase (ALP) synthesis, suggesting it supported bone health by promoting differentiation rather than proliferation. Using an in vitro osteoblast culture model, serum containing Ejiao at low (1 g/kg), medium (2 g/kg), and high (4 g/kg) concentrations was administered twice daily for three days, resulting in enhanced ALP synthesis and osteoblast differentiation [35].

The effects of Ejiao in promoting tibial bone defect repair have been investigated. In the study, rats were divided into three groups, normal, model, and Ejiao (50 rats per group). Ejiao was administered orally at 0.45 g/2 mL per rat, twice daily, starting two days after surgery, for 28 days. The findings showed that Ejiao upregulated the expression of procollagen mRNA types I, II, and III, as well as transforming growth factor-β (TGF-β) mRNA, particularly during the early and intermediate stages of bone repair. However, Ejiao had no significant effect on BMP-2 or vascular endothelial growth factor (VEGF) mRNA expression. Ejiao enhanced the activity of cartilage cells and osteoblasts, facilitating ossification and accelerating bone repair. These results highlight Ejiao's role in supporting bone health by stimulating key cellular activities involved in bone repair and regeneration [36].

The osteoporosis-preventing effects of Ejiao were studied in ovariectomised rats. Ejiao treatment (low, 0.26 g/kg; medium, 0.53 g/kg; high, 1.06 g/kg) administered orally for eight weeks suppressed high bone remodelling, as evidenced by reduced levels of circulating bone formation and resorption markers, as well as a normalised mineralising surface/bone surface ratio. However, no significant effects were observed on bone structural, cellular, or biomechanical parameters. These findings suggest that more time may be required for osteoporosis to develop fully in the model. Nevertheless, Ejiao prevented high bone remodelling associated with early-stage oestrogen deficiency, but long-term studies are needed to evaluate its impact on bone structure and strength [18].

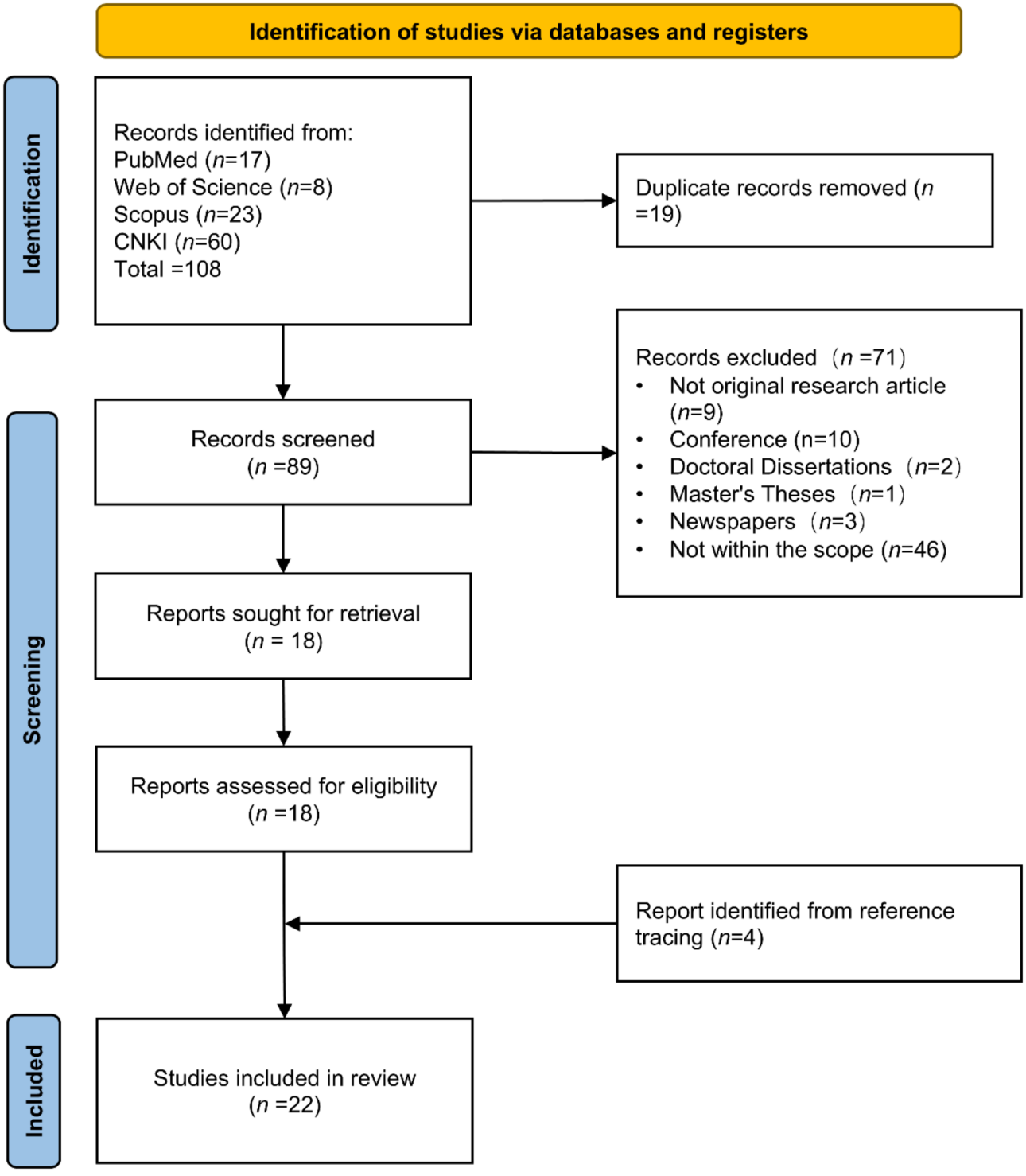

Fig. 1 PRISMA flow chart of the article selection process

Ejiao has been shown to influence the Wnt pathway in promoting hematopoiesis [24]. At the same time, Wnt signalling plays a key role in the pathogenesis of osteoporosis, particularly in bone formation [37]. Therefore, the effects of Ejiao on skeletal mineralisation, osteocytes, and Wnt signalling inhibitors in an ovariectomised rat model have been investigated. Female rats were treated with Ejiao at doses of 0.26 g/kg, 0.53 g/kg, and 1.06 g/kg for two months. The results revealed that Ejiao did not significantly alter the skeletal mineral/matrix ratio, osteocyte numbers, or the levels of Wnt signalling inhibitors, including Dickkopf-1 and sclerostin [38]. These

findings suggest that Ejiao's effects on bone health may not be mediated through osteocytes or the Wnt signalling pathway.

Oestrogen deficiency has been shown to promote overall and bone marrow adiposity, which can further aggravate bone loss [39]. One study investigated the effects of Ejiao on body composition, bone marrow adiposity, and skeletal redox status using an ovariectomised rat model. Female Sprague Dawley rats were treated with low (0.26 g/kg), medium (0.53 g/kg), or high (1.06 g/kg) doses of Ejiao or calcium carbonate for 8 weeks. The results showed that ovariectomised rats exhibited increased total mass, lean mass, and bone marrow adipocyte numbers compared to the sham group. Ejiao supplementation, particularly at the low dose, effectively counteracted these changes. Additionally, Ejiao treatment enhanced skeletal catalase and superoxide dismutase activities and reduced skeletal malondialdehyde levels, indicating antioxidant effects. However, there was no change in the expression of peroxisome proliferator-activated receptor gamma protein. The study concluded that Ejiao, particularly at lower doses, could prevent body composition changes and bone marrow adiposity caused by ovariectomy, likely due to its antioxidant properties [40].

Table 1 summarises studies investigating the effects of Ejiao on osteoporosis models, and Fig. 2 presents its mechanism.

Skeletal effects of Ejiao Qianggu Oral Liquid

Donkey-hide glue reinforcing bone oral solution (DGRBOS), also known as Ejiao Qianggu Oral Liquid (EJQG), is composed of prepared Rehmannia root, Ejiao (donkey-hide gelatin), goji berries, oyster, Astragalus root, and Codonopsis root. It has shown promising potential in the treatment of osteoporosis by promoting osteoblast proliferation in a dose-dependent manner, with effects comparable to tibolone, a hormone replacement therapy. A study aimed at exploring the molecular

Table 1 Evidence on the skeletal effects of Ejiao

Researchers	Study Design	Findings
Chang et al. (2009) [35]	Model: Osteoblasts from one-day-old Wistar rat calvaria Treatment: Serum from Wistar rats fed with Ejiao (1, 2, 4 g/kg) to Wistar rats for 3 days	Osteoblast proliferation: NS Intracellular ALP level: ↑ vs. blank control
Gao et al. (2004) [36]	Animals: Female Sprague-Dawley rats (13–14 weeks old) Disease model: Bone defect with three 0.8 mm holes on left tibia Treatment: Ejiao oral solution (0.45 g/2 mL, p.o., twice daily) for 4, 7, 14, 21 and 28 days Negative control: Distilled water Positive control: NA	Osteoblast proliferation: NS vs. negative control Intracellular ALP level: ↑ vs. negative control Procollagen mRNA expression (types I, II, III): ↑ vs. negative control TGF-β1 mRNA expression: ↑ vs. negative control BMP-2 mRNA expression: NS vs. negative control VEGF mRNA expression: NS vs. negative control
Chin et al. (2023) [38]	Animals: Female Sprague-Dawley rats (3 months old) Disease model: OVX-induced osteoporosis Treatment: Ejiao (0.26, 0.53 and 1.06 g/kg, p.o., 8 weeks) Negative control: distilled water Positive control: 1% calcium carbonate (oral)	Osteocyte number: NS vs. negative control Skeletal mineral/matrix ratio: NS vs. negative control Skeletal DKK1 and sclerostin level: NS vs. negative control
Ekeuku et al. (2023) [18]	Animals: Female Sprague-Dawley rats (3 months old) Disease model: OVX-induced osteoporosis Treatment: Ejiao (0.26, 0.53 and 1.06 g/kg, p.o., 8 weeks) Negative control: distilled water Positive control: 1% calcium carbonate (oral)	Bone mineral density and content, NS vs. negative control Bone histomorphometry Bone structural indices, NS vs. negative control Bone dynamic indices, ↓ mineralising surface/bone surface ratio vs. negative control Bone cellular indices, NS vs. negative control Bone mechanical strength, NS vs. negative control Bone remodelling markers, ↓circulating osteocalcin and CTX-1 with high-dose Ejiao
Ekeuku et al. (2023) [40]	Animals: Female Sprague-Dawley rats (3 months old) Disease model: OVX-induced osteoporosis Treatment: Ejiao (0.26, 0.53 and 1.06 g/kg, p.o., 8 weeks) Negative control: distilled water Positive control: 1% calcium carbonate (oral)	Total body mass: ↑ in all groups except control and calcium Fat mass and fat percentage: ↑ in the OVX groups, regardless of treatments Lean mass: ↑ in the OVX groups, regardless of treatments Bone marrow adiposity: ↓ in low and medium-dose Ejiao and calcium groups vs. negative control PPAR-γ levels: ↓ in Ejiao and calcium groups vs. negative control Catalase, superoxide dismutase, glutathione peroxidase: ↑ vs. negative control Glutathione: ↓ vs. negative control Malondialdehyde: NS vs. negative control

Abbreviations: ALP, Alkaline phosphatase; BMP-2, Bone morphogenetic protein-2; CTX-1, C-terminal telopeptide of type I collagen; DKK1, Dickkopf-1; NA, Not available; NS, Not significant; OVX, Ovariectomy; p.o., per-oral; PPAR-γ, Peroxisome proliferator-activated receptor gamma; TGF-β1, Transforming growth factor beta-1; VEGF, Vascular endothelial growth factor; ↑, increase or upregulate↓, decrease or downregulate

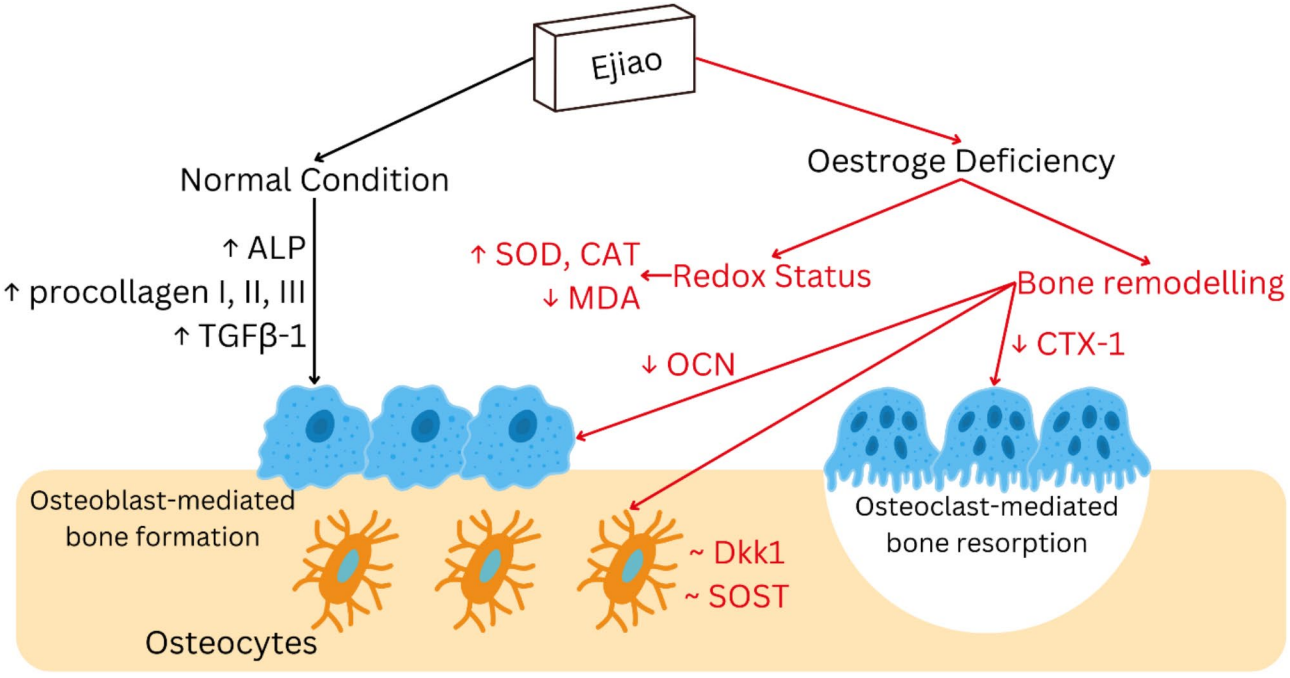

Fig. 2 Mechanism of action of Ejio on bone. The red arrows indicate processes under oestrogen deficiency while the black arrows indicate processes under normal condition. Abbreviations: ↑, increase or upregulate; ↓, decrease or downregulate; ~, no significant change; ALP, alkaline phosphatase; CAT, catalase; CTX-1, type I collagen cross-linked C-telopeptide; Dkk1, Dickkopf-1; MDA, malondialdehyde; OCN, osteocalcin; SOD, superoxide dismutase; SOST, sclerostin; TGF-β, transforming growth factor beta (Drawn using Canva)

mechanisms behind EJQG's therapeutic effects in osteoporosis investigated its impact on the osteoprotegerin (OPG)/RANKL pathway. In this study, 3-month-old Wistar rats were divided into three groups: EJQG, oestrogen, and saline control. After 7 days of treatment, medicated serum was collected from the rats and applied to osteoblasts isolated from newborn Sprague-Dawley rats. The results revealed that EJQG significantly promoted osteoblast proliferation, with a prominent increase in OPG mRNA expression observed at a concentration of 100 mL/L, similar to the effects seen in the oestrogen group. Furthermore, RANKL mRNA expression was notably lower at 1000 mL/L compared to the control group [23, 41]. These findings suggest that EJQG works by enhancing osteoblast activity, increasing OPG expression, and decreasing RANKL expression, thereby promoting bone formation through a balanced modulation of osteoclastogenesis inhibition and osteoblast activation. Additional findings also suggest that EJQG promotes osteoblast proliferation, calcium uptake, and mineralisation at various concentrations, aiding in bone regeneration and repair [42, 43].

In ovariectomised rats, EJQG has been shown to improve bone mineral density, mechanical strength, and vitamin D levels ($25(OH)D_3$ and $1,25(OH)D_3$), producing results comparable to sham-operated controls [44]. It also elevates $25OHD_3$ and $1,25(OH)D_3$ levels and upregulates vitamin D receptor expression in the liver, indicating enhanced vitamin D metabolism [45]. It also increases type I collagen protein levels and mRNA expression in ovariectomised rats, indicating enhanced bone matrix production [46]. EJQG has been shown to significantly enhance the expression of vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2), which play critical roles in fracture healing. VEGF is a key regulator of angiogenesis, promoting the formation of new blood vessels essential for delivering nutrients and oxygen to the healing bone. On the other hand, FGF-2 stimulates the proliferation and differentiation of osteoblasts and chondrocytes, supporting bone formation and cartilage repair. By upregulating VEGF and FGF-2, EJQG promotes fracture healing through improved angiogenesis, osteogenesis, and chondrogenesis, ultimately enhancing the overall bone repair process [47, 48]. Clinical studies have shown that combining EJQG with calcitriol significantly enhances clinical outcomes in elderly patients with osteoporosis. This combination therapy provides faster symptom relief and leads to more significant improvements in bone mineral density and bone metabolism markers compared to calcitriol alone, indicating its potential for clinical use [49].

Table 2 summarises studies investigating the effects of EJQG on osteoporosis models, and Fig. 3 presents its mechanism of action.

Table 2 Evidence on the skeletal effects of Ejiao Qianggu oral liquid (EJQG)

Researchers	Composition of prescription	Study Design	Findings
Shen et al. (2005) [41]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Animals: 3-month-old Wistar rats (male & female $n=30$) Cells: Osteoblasts from newborn Sprague-Dawley rats Treatment: EJQG-medicated serum (100, 500, 1000 g/L) applied to osteoblasts Negative control: Standard culture medium (untreated) Positive control: tibolone-medicated serum (100, 1000 g/L)	Osteoblast proliferation: ↑ (vs. untreated control) Osteocalcin levels: ↑ (vs. untreated control) OPG mRNA expression: ↑ (vs. untreated control) RANKL mRNA expression: ↓ (vs. untreated control)
Shen et al. (2005) [23]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Animals: 3-month-old Wistar rats (male & female) Cells: Osteoblasts from newborn Sprague-Dawley rats Treatment: Serum from Wistar rats supplemented with EJQG (100, 500, 1000 g/L) for 7 days was applied to primary osteoblasts Untreated control: culture medium Positive control: tibolone (100, 1000 g/L)	Osteoblast proliferation: ↑ vs. untreated control Bone Gla protein levels: ↑ vs. untreated control OPG mRNA expression: ↑ at 1000 g/L vs. untreated control RANKL mRNA expression: ↓ at 1000 g/L vs. untreated control
Li et al. (2006) [47]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Animals: Sprague-Dawley rats (3 months old) Model: Closed mid-shaft tibial fracture model without fixation Treatment: EJQG (2 mL/rat, p.o.) twice daily Negative control: Normal saline, twice daily Positive control: Jiegu Qili tablets (0.45 g/2 mL, p.o.) twice daily Evaluation periods: 4, 7, 14, 21 & 28 days post-fracture	VEGF Expression: EJQG Group: ↑ vs. negative control on day 7 FGF-2 Expression: EJQG Group: ↑ vs. negative control on day 14
Li et al. (2007) [48]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Animals: Female Sprague-Dawley rats (3 months old) Model: Tibial fracture induced via three-point bending Treatment: EJQG (2 mL twice daily, p.o.) Negative control: Normal saline Positive control: Qili Linking Bone Pill (225 g/L, 2 mL, p.o. twice daily) Evaluation: VEGF and FGF-2 expression on days 4, 7, 14, 21, 28 post-fracture via immunohistochemistry	VEGF Expression: EJQG Group ↑ on day 14 vs. negative control FGF-2 Expression: EJQG Group ↑ on day 7 vs. negative control
Wu et al. 2007 [43]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Osteoblasts cells. Negative control: NA Positive control: NA	Proliferation: EJQG serum:↑ S-phase cells, ↑ proliferation index. Calcium Uptake: EJQG serum: ↑ intracellular Ca^{2+} Mineralisation: High-dose serum: ↑ calcified nodule formation ($P<0.05$).
Wu et al. (2007) [42]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Cells: 24-h-old Sprague-Dawley rats' calvarial osteoblasts Treatment: EJQG (low, 100 mL, medium: 500 mL, high, 1000 mL, p.o.) Untreated control: normal saline Positive control: NA	Osteoblast proliferation: ↑ vs. untreated control Alkaline Phosphatase Activity: ↑ vs. untreated control Mineralisation: medium group ↑ vs. untreated control
Shuai et al. (2008) [44]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Animals: 4-month-old female Sprague-Dawley rats Disease Model; OVX-induced osteoporosis Treatment: EJQG 2 mL, p.o., twice daily Negative control: Normal saline Positive control: NA	BMD: ↑ vs. negative control Biomechanical strength: ↑ vs. negative control $25(OH)D_3$ and $1,25(OH)2D_3$ levels: ↑ vs. negative control
Guo et al. (2009) [45]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Animals: 6-month-old female Sprague-Dawley rats Disease Model: OVX-induced osteoporosis Treatment: 2 mL of EJQG, p.o., twice daily for 6 months Negative control: Normal saline Positive control: NA	25-OH-VD3 levels: ↑ vs. negative control 1,25-(OH)2-VD3 levels: ↑ vs. negative control VDR gene expression: ↑ vs. negative control

Table 2 (continued)

Researchers	Composition of prescription	Study Design	Findings
Guo et al. (2009) [46]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Animals: 6-month-old Female Sprague-Dawley rats Disease Model, OVX Treatment: 2 mL of EJQG p.o., twice daily for 6 months Negative control: Normal saline Positive control: NA	Type I collagen protein and mRNA levels: ↑ vs. negative control
Wang et al. (2021) [49]	Ejiao, cooked ground Radix Rehmanniae Preparata, *Codonopsis pilosula*, *Astragalus membranaceus*, *Lycium barbarum*, *Ostrea gigas*	Patients: 128 older adults with osteoporosis (mean age: 69.52 ± 1.31 years) Groups: Treatment group: *n* = 64 (36 men, 28 women) Mean age: 71.22 ± 1.01 years old Treatment: EJQG + Calcitriol (10 mL, 3 times daily), p.o. Control group: *n* = 64 (35 men, 29 females) Mean age: 68.60 ± 1.03 years old Treatment: Calcitriol (1 capsule of 0.25 μg, twice daily), p.o. Duration: 3 months	Effective rate: Treatment group: ↑ vs. control group Symptom improvement time: ↑ vs. control group Low back pain: ↓ vs. control group Bone pain: ↓ vs. control group Lower limb weakness: ↓ vs. control group Muscle cramps: ↓vs control group BMD: ↑ vs. control group

Abbreviations: 1,25(OH)2D_3, 1,25-Dihydroxyvitamin D_3; 25(OH)D_3, 25-Hydroxyvitamin D_3; BGP, Bone Gla protein; BMD, Bone mineral density; DGRBOS, Donkey-hide glue reinforcing bone oral solution; FGF-2, Fibroblast growth factor 2; p.o., per-oral; OPG, Osteoprotegerin; OVX, Ovariectomised; RANKL, Receptor activator of nuclear factor kappa-B ligand; VEGF, Vascular endothelial growth factor; VDR, Vitamin D Receptor; ↑, increase or upregulate↓, decrease or downregulate

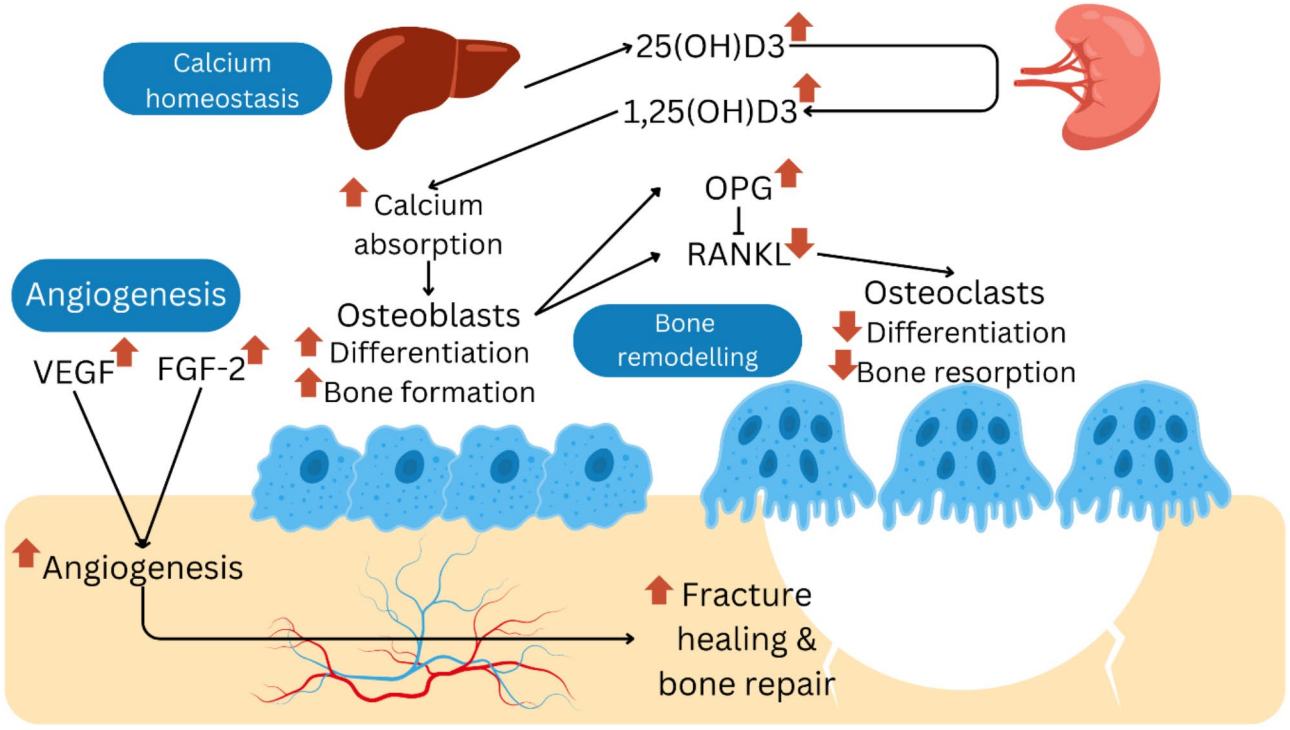

Fig. 3 Mechanism of action of Ejiao Qianggu Oral Liquid on bone. The red arrows indicate effects of Ejiao Qianggu Oral Liquid. Abbreviations: ↑, increase or upregulate; ↓, decrease or downregulate; 1,25(OH)D_3, 1,25-dihydroxyvitamin D3; 25(OH)D_3, 25-hydroxy vitamin D3; FGF-2, fibroblast growth factor 2; OCN, osteocalcin; OPG, osteoprotegerin; RANKL, receptor activator of NF-κB ligand; VDR, vitamin D receptor; VEGF, vascular endothelial growth factor (Drawn using Canva)

Skeletal effects of other Ejiao formulations

Excessive intake of retinoic acid is known to cause bone loss [50, 51]. Calcium Ejiao liquid oral formulation (CCA), with its composition detailed in Table 3, has shown promise in preventing bone loss related to retinoic acid-induced osteoporosis in rats. In studies using both retinoic acid-induced osteoporosis and vitamin D deficiency-induced rickets models, CCA treatment significantly increased serum calcium levels, improved femoral calcium and phosphorus content, and reduced alkaline phosphatase activity. In the vitamin D deficiency-induced

Table 3 Evidence on the skeletal effects of Ejiao formulations

Researchers	Composition of prescription	Study Design	Findings
Xiong et al. (1999) [52]	Ejiao, *Astragalus membranaceus*, cooked *Rehmannia glutinosa*, *Ostrea gigas*	Animals: 20-d-old male Wistar rats Disease model: bone loss induced by retinoic acid (70 mg/kg/day for 20 days) Treatment: Calcium Ejiao oral solution (CCA) at 0.79, 1.58 and 2.37 g/kg/day, p.o., 6 weeks Negative control: normal saline Positive control: Longmu Zhuanggu granules (5 g/kg, p.o.)	Serum calcium level: ↑ vs. negative control Serum phosphorus level: ↑ vs. negative control Serum ALP level: ↓ vs. negative control Femoral calcium and phosphorus content: ↑ vs. negative control X-ray imaging: ↓ signs of bone loss (thinner cortical bone, reduced trabecular bone density) in treatment groups vs. negative control
		Animals: 20-day-old male & female Wistar rats Disease model: vitamin D deficiency-induced rickets model Treatment: Oral calcium CCA at 0.79, 1.58 and 2.37 g/kg/day, p.o., 60 days Negative control: normal saline Positive control: Longmu Zhuanggu granules (5 g/kg, p.o.) Negative control: NA	Serum calcium level: ↑ vs. negative control Serum phosphorus level: NS Serum ALP level: ↓ vs. negative control Femoral calcium and phosphorus content: ↑ vs. negative control X-ray imaging: ↓ signs of osteomalacia vs. negative control
Gu et al. (2019) [53]	Radix Rehmanniae Praeparata: *Rehmannia glutinosa* Yam: *Dioscorea opposita* Dodder: *Cuscuta chinensis* Cornel: *Cornus officinalis* Lycium: *Lycium chinense* Eucommia: *Eucommia ulmoides* Radix Angelicae Sinensis: *Angelica sinensis* Cinnamon: *Cinnamomum cassia* Processed Radix Aconiti Lateralis: *Aconitum carmichaelii* Placenta: Placenta hominis Ejiao: Asini Corii Colla Radix Codonopsis: *Codonopsis pilosula* Rhizoma Atractylodis Macrocephalae: *Atractylodes macrocephala* Semen Coicis: *Coix lacryma-jobi* Fructus Amomi: *Amomum villosum* Baked tortoise's shell: Testudinis Carapax et Plastrum Antlers plastic: Cervi Cornu Pantotrichum	Animals: 4-month-old Female Wistar rats Disease model: OVX-induced osteoporosis Treatment: AOD 10 g/kg, p.o., 12 months Negative control: normal saline Positive control: Yougui Pill 10 g/kg, p.o.	BMD: ↑ vs. negative control Histology: ↑ trabecular bone area vs. negative control Serum PINP and β-CTX: ↓ vs. negative control Skeletal ALP, BMP2, Runx2, collagen I, and osteopontin mRNA expression: ↑ vs. negative control
Jia et al. (2013) [55]	Ejiao Yin Yang Huo: *Epimedium sagittatum* Tu Si Zi: *Cuscuta chinensis* Bu Guo Zi: *Psoralea corylifolia* Huai Niu Xi: *Achyranthes bidentata* Oyster: *Ostrea gigas* Su Mu: *Caesalpinia sappan*	Animals: 8-month-old Sprague-Dawley rats Disease Model: OVX - osteoporosis Treatment: Ejiao Kidney-Tonifying Bone Nourishing Formula (10 g/kg daily, p.o., 3 months) Negative control: NA Positive control: Alendronate sodium (8.2 mg/kg, p.o., weekly)	Body weight: ↑ vs. negative control Uterus and kidney indices: ↑ vs. negative control BMD: ↑ vs. negative control

Table 3 (continued)

Researchers	Composition of prescription	Study Design	Findings
Jia et al. (2013) [54]	Ejiao Yin Yang Huo: *Epimedium sagittatum* Tu Si Zi: *Cuscuta chinensis* Bu Guo Zi: *Psoralea corylifolia* Huai Niu Xi: *Achyranthes bidentata* Oyster: *Ostrea gigas* Su Mu: *Caesalpinia sappan*	Animals: 8-month-old Sprague-Dawley rats Disease Model: OVX - osteoporosis Treatment: Experimental Groups: Ejiao Kidney-Tonifying Bone Nourishing Formula (10 g/kg daily, p.o., 3 months) Negative control: NA Positive control: Alendronate sodium (8.2 mg/kg, p.o., weekly)	Body weight changes: ↑ vs. Control group Uterus and kidney indices: ↑ vs. control group Serum OCN and PICP levels: ↑ vs. Control group
Pan et al. (2023) [58]	Ejiao Wolfberry: *Lycium barbarum* Spatholobus: *Spatholobus suberectus* Codonopsis: *Codonopsis pilosula* Deer's antler gelatin Tuckahoe: *Poria cocos* Mulberry: *Morus alba* Cooked rehmannia: *Rehmannia glutinosa* Achyranthes: *Achyranthes bidentata* Red sage: *Salvia miltiorrhiza* Wulingxian: *Lindera aggregate* Tortoiseshell gum *Atractylodes macrocephala* *Rheum palmatum* Shenji Herba Epimedii Licorice: *Glycyrrhiza uralensis*	Patients: 120 patients with OVCF treated with PVP Treatment group: 35 men and 25 women Mean age: 66.27 ± 2.15 years old Treatment: Ejiao paste (10 g, p.o., 2 times/day) + Baduanjin exercise Positive control group: 33 men and 27 women Mean age: 66.18 ± 2.20 years old Control: Calcium carbonate (1.25 g) + Vitamin D3 (200 IU) + guidance (consume 1.20–1.40 g/kg of high-quality protein daily, engage in walking exercises for over 30 min, and get regular sun exposure Duration: 6 months Follow-Up: 3 years Negative control: NA	BMD: ↑ vs. positive control AVBH: ↑ vs. control Serum B-Cross I: N-MID Ost, PTH, ↓ vs. control Functional improvement: ↑ vs. control
Jia et al. (2018) [59]	Ejiao, *Ostrea gigas*	Patients: 80 children with Vitamin D-deficiency-induced rickets Treatment group: *n* = 40 children (23 males and 17 females) Mean age: 15.3 ± 5.3 month Treatment: Ejiao-Mu Li Oral Solution (≤ 6 months: 10 mL daily, thrice a day; > 6 months: 10 mL/day, twice a day) combined with zinc-iron-calcium composite preparation (10 mL, 3 times a day) for 12 weeks Positive control: *n* = 40 children (21 males and 19 females) Mean age: 14.7 ± 5.3 month Treatment: Zinc-iron-calcium composite preparation (10 mL, 3 times a day) for 12 weeks *Both groups received intramuscular vitamin D 300,000 IU, followed by 4-week oral vitamin D drops for 8 weeks (< 1 year: 500 IU/day, ≥ 1 year: 700 IU/day)	Total effective rate: ↑ vs. positive control Bone metabolism indicators: ALP↑ vs. positive control Bone density (radius, ulna) and 25-(OH) D3 levels: ↑ vs. positive control
Chen et al. (2014) [60]	Ejiao: Colla Corii Asini Jujube: *Ziziphus jujuba* Goji Berry: *Lycium barbarum* Astragalus: *Astragalus membranaceus* Curculigo: *Curculigo orchioides* Amomum: *Amomum villosum*	Participants: 73 patients with closed bone fractures Treatment group: *n* = 37 patients Mean age: 46.21 ± 6.24 years old Treatment: Ejiao and Astragalus, 10 mL, p.o., twice daily, alongside a self-made bone healing ointment applied 6–9 times daily for one week Positive control: *n* = 36 patients Mean age: 45.93 ± 5.71 years old Treatment: Calcium gluconate oral solution (600 mg/d) Negative control: NA	Total effective rate: ↑ vs. positive control Healing outcomes after 12 weeks: ↑ vs. positive control

Abbreviations: 25-(OH) D3, 25-hydroxyvitamin D_3; β-CTX: β-C-terminal telopeptide of type I collagen; ALP, Alkaline phosphatase; AVBH, Anterior vertebral height; BMD, Bone mineral density; BMP2, Bone morphogenetic protein 2; NA, Not available; N-MID Ost, n-terminal middle osteocalcin; NS, Not significant; OCN, Osteocalcin; OVX, Ovariectomised; PICP, Procollagen type I C-terminal propeptide; PINP, N-terminal propeptide of type I procollagen; p.o., per-oral; PTH, Parathyroid hormone; PVP, Percutaneous vertebroplasty; Runx2, Runt-related transcription factor 2; ↑, increase or upregulate;↓, decrease or downregulate

rickets model, CCA also demonstrated a marked improvement in bone density and mineral content. X-ray imaging further confirmed these benefits, showing enhanced bone structure across both models and highlighting CCA's potential protective effects on bone health [52].

Anti-Osteoporosis Decoction (AOD) (composition detailed in Table 3) has shown significant therapeutic effects in ovariectomy-induced osteoporosis in Wistar rats. Administered at 10 g/kg daily for 12 weeks, AOD effectively reduced trabecular bone damage and increased bone mineral density. It notably elevated ALP levels and enhanced the expression of key bone metabolism proteins, including BMP-2, runt-related factor 2 (Runx2), collagen I, and osteopontin. Furthermore, AOD lowered serum levels of procollagen type I N-propeptide (a bone formation marker) and β-C-terminal telopeptide of type I collagen (a bone resorption marker). These results highlight AOD's potential as an anabolic therapy for osteoporosis by mitigating high bone remodelling and promoting bone formation [53].

Studies on the Ejiao Bushen Jiangu Formula (EJBGF) (composition detailed in Table 3) in ovariectomised rats have demonstrated significant improvements in body weight, serum osteocalcin (OCN), and procollagen type I C-terminal propeptide (PICP) levels. EJBGF treatment, initiated 7 days post-surgery, enhanced bone mineral density and improved bone structure. These effects were superior to alendronate sodium, a standard first-line treatment for osteoporosis. Additionally, EJBGF improved uterine and kidney indices, suggesting its potential to regulate lipid metabolism and mitigate bone loss. By increasing serum OCN and PICP levels, EJBGF enhanced bone turnover, highlighting its ability to support bone health and demonstrating its potential for preventing and treating osteoporosis [54, 55].

Vertebra fracture is a debilitating and painful condition that affects patient's quality of life and daily living [56, 57]. A clinical study demonstrated that combining Ejiao paste (composition illustrated in Table 3) and Baduanjin exercise—an ancient Qigong practice designed to enhance flexibility, balance, and strength—significantly improved outcomes for patients with osteoporotic vertebral compression fractures (OVCF) following percutaneous vertebroplasty (PVP). The integrative therapy group showed better clinical recovery, greater pain relief, and improved bone mineral density and anterior vertebral body height (AVBH), while also reducing Cobb angles and serum levels of bone metabolism markers. Furthermore, the re-fracture rate was lower in the integrative group, underscoring the therapeutic benefits of this combined approach in managing OVCF and promoting recovery [58].

A study evaluating the efficacy of combining Ejiao Muli Oral Liquid (composition illustrated in Table 3) with a zinc, iron, and calcium complex in treating vitamin D deficiency rickets in children found that this combination therapy significantly improved both clinical outcomes and bone metabolism indicators. After 12 weeks, the observation group showed a markedly higher clinical effectiveness rate compared to the control group. Additionally, the observation group experienced enhanced bone mineral density, increased 25(OH)D3 levels, and reduced alkaline phosphatase levels, demonstrating the therapy's effectiveness in improving bone health and clinical outcomes [59].

Research on Ejiao Huangqi Oral Liquid (composition illustrated in Table 3) for bone healing in patients with closed fractures showed a significantly higher total effective rate compared to calcium gluconate. After 12 weeks of treatment, several patients in the Ejiao Huangqi group achieved complete fracture healing, while none in the control group did [60]. The findings suggest that the Ejiao and Astragalus oral solution is more effective than calcium gluconate in improving bone pain, accelerating callus formation, and enhancing the overall recovery speed of injured limbs, making it a promising option for clinical application.

Table 3 summarises studies investigating the effects of Ejiao's compound formulations on osteoporosis models, and Fig. 4 presents their mechanisms of action.

Discussion

This scoping review examines the potential of Ejiao as a preventive agent against osteoporosis. The findings from the included studies consistently demonstrate Ejiao's beneficial effects on bone health, including enhanced bone density, improved fracture healing, and suppression of excessive bone remodelling, as evidenced in various animal models and clinical investigations. Mechanistic studies highlight its role in promoting osteoblastic bone formation, reducing the RANKL/OPG ratio to create an anti-osteoclastic environment, and activating VEGF and FGF signalling pathways. Furthermore, Ejiao-based formulations exhibit diverse mechanisms of action. CCA enhances bone mineralisation and structure, while AOD improves bone density and upregulates osteogenic markers. EJBGF surpasses standard treatments in regulating bone turnover and lipid metabolism. Clinical evidence supports the combined use of Ejiao paste with Baduanjin exercises for vertebral compression fractures and Ejiao Muli Oral Liquid with zinc, iron, and calcium for improving bone health in children with vitamin D deficiency rickets. EJQG further demonstrates efficacy in modulating the OPG/RANKL pathway, enhancing osteoblast activity, promoting angiogenesis, and supporting fracture healing. Collectively, these findings underscore Ejiao's

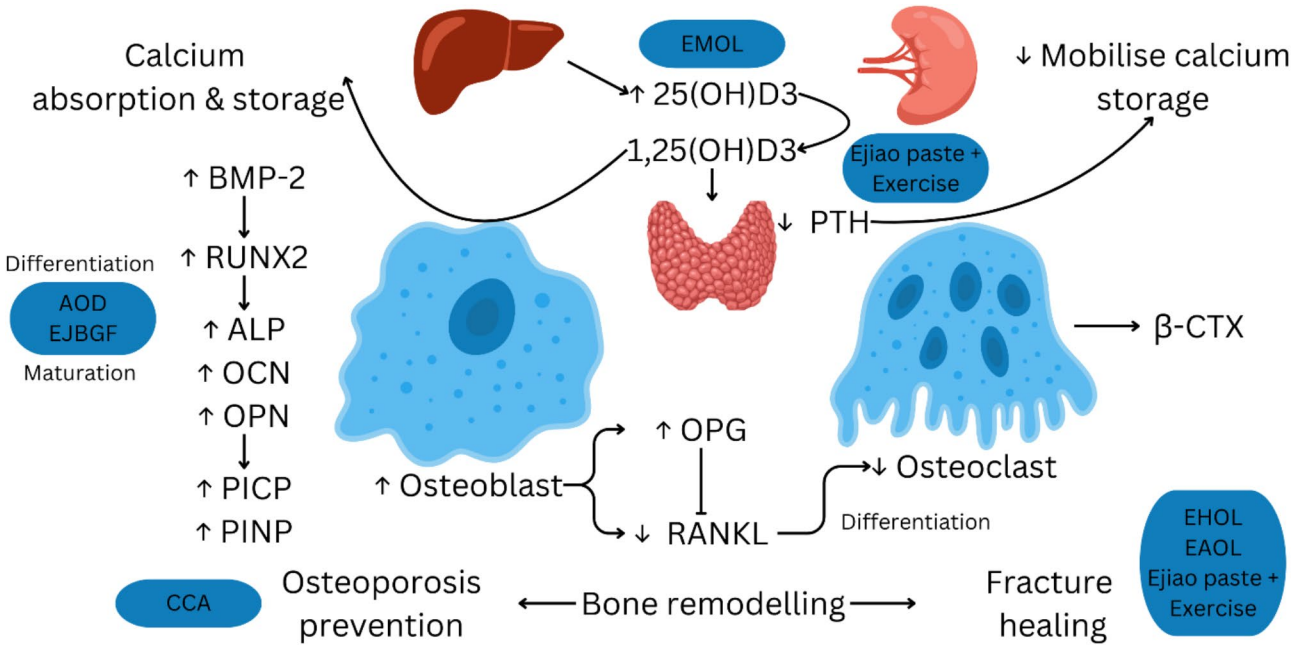

Fig. 4 Mechanism of action of Ejiao formulations on bone. Abbreviations: ↑, increase or upregulate; ↓, decrease or downregulate; ⊤, inhibit or suppress; β-CTX, beta C-terminal telopeptide; 1,25(OH)D3, 1,25-Dihydroxyvitamin D3; 25(OH)D3, 25-hydroxy vitamin D3; ALP, alkaline phosphatase; AOD, Anti-Osteoporosis Decoction; BMP-2, Bone morphogenetic protein 2; CCA, Calcium Ejiao liquid oral formulation; EAOL, Ejiao and Astragalus oral solution; EJBGF, Ejiao Bushen Jiangu Formula; EHOL, Ejiao Huangqi Oral Liquid; EMOL, Ejiao Muli Oral Liquid; RUNX-2, Runt-related transcription factor 2; OPN, osteopontin; OCN, osteocalcin; PICP, procollagen 1 carboxyterminal propeptide; PINP, procollagen type I N-propeptide; PTH, parathyroid hormone (Drawn using Canva)

multifaceted potential in osteoporosis prevention and treatment, while emphasising the need for further clinical validation to confirm its long-term efficacy and safety.

As reported by previous literature, Ejiao does not significantly promote osteoblast proliferation but significantly enhances osteoblast differentiation, as indicated by increased alkaline phosphatase synthesis [35]. This finding suggests that Ejiao supports bone health by fostering the differentiation of osteoblasts rather than merely increasing their number. Since osteoblasts are responsible for the bone formation process, Ejiao's compound formulations have been shown to upregulate procollagen mRNA and TGF-β mRNA expression, which are crucial for bone repair and ossification [61]. At the end of the bone formation process, osteoblasts are encased in the matrix they synthesised and differentiated terminally into osteocytes [62]. Osteocytes play multiple roles in regulating the bone remodelling process. One of the roles of osteocytes is acting as a regulator of bone formation through Wnt signalling pathway [63]. Biomarkers such as bone alkaline phosphatase (bALP) and procollagen type I N propeptide (PINP) are essential for monitoring bone turnover, aligning with Ejiao's reported effects on enhancing osteoblast activity and bone formation markers [64, 65]. However, Ejiao did not significantly influence osteocyte numbers or Wnt signalling pathways in ovariectomised rats [38]. These findings highlight Ejiao's role in supporting bone health through its effects on critical cellular activities involved in bone repair and regeneration. However, it remains a question which pathway is implicated.

Osteoblasts and adipocytes originate from mesenchymal lineage in the bone marrow. Increased adipogenesis in the bone marrow could impair osteoblastogenesis, leading to reduced bone formation and subsequently osteoporosis. Ejiao was reported to counteract bone marrow adiposity in ovariectomised rats, probably due to its ability to improve the skeletal redox environment [40]. However, in this study, no significant reduction in peroxisome proliferator-activated receptors-γ expression (transcription factor for adipogenesis) was observed. Thus, the exact mechanism of how Ejiao achieves this effect remains elusive.

The RANKL-OPG axis governs the differentiation of osteoclasts, whereby the binding of RANKL to RANK receptors stimulates osteoclastic differentiation and OPG, acting as the decoy receptor of RANKL, prevents this binding [66, 67]. Inhibiting RANKL has been shown to significantly improve bone mineral density and reduce fracture risk in postmenopausal osteoporosis, underscoring the therapeutic potential of targeting this pathway [68]. Similarly, EJQG was reported to reduce RANKL/OPG ratio, favouring an anti-osteoclastic environment. Thus bone resorption is suppressed. This finding is further evidenced by the suppression of high bone remodelling by multiple studies using Ejiao [23, 41]. High bone

remodelling is typically observed during osteoporosis due to increased bone formation to compensate for the excessive bone resorption, resulting in elevation of bone formation and resorption markers [69]. Inhibition of this excessive bone turnover process will preserve the bone mass.

The anabolic effects of Ejiao should translate into bone density, structural and mechanical improvements for osteoporosis prevention, but previous studies yielded mixed results. In ovariectomised rats, Ejiao did not significantly alter the structural or biomechanical parameters of the bones despite the suppression of bone remodelling markers [18]. Long-term supplementation of Ejiao might be required to demonstrate positive changes in these aspects. The combination of Ejiao with other compounds, as in the case of AOD and Ejiao Bushen Jiangu Formula, demonstrated better effects in these aspects [53–55].

Compared to standard osteoporosis treatments [70], Ejiao showed both strengths and limitations. Studies on compound formulations like EJQG indicate that it could promote osteoblast proliferation and enhance bone health through the modulation of the OPG/RANKL pathway, similar to hormone replacement therapy [41]. Clinical evidence supports the beneficial effects of combining Ejiao with other TCM therapies and physical exercises for treating OVCF [58]. However, the difference in the impact of Ejiao on bone mineral density and structural parameters, compared to calcium carbonate, may not be as pronounced [38].

The existing evidence highlights the potential of Ejiao as a complementary approach to osteoporosis prevention and management. However, further research is crucial to clarify its efficacy and underlying mechanisms fully. Most existing studies predominantly focus on animal or in vitro models, which may not directly translate to human outcomes. Double-blind, randomised, placebo-controlled studies on osteoporosis treatments are necessary to establish evidence-based guidelines and enhance the reliability of therapeutic recommendations [56]. Standardising the outcome measures in related studies are remains critical to ensuring more robust and reproducible findings [57]. Furthermore, the variability in Ejiao formulations and dosages across different studies complicates comparisons and hinders the establishment of consistent conclusions. There is also a notable lack of comprehensive research on the specific components of Ejiao and their corresponding effects, making it challenging to correlate ingredient efficacy with clinical outcomes.

Future studies should delve into Ejiao's impact on cellular and molecular mechanisms related to bone formation and resorption, especially its effects on the Wnt/β-catenin pathway and the RANK/RANKL/OPG axis. Additionally, exploring its influence on inflammatory markers and oxidative stress levels is vital, as both factors are closely linked to bone deterioration in osteoporosis [71]. Some studies have shown compounds with modulator effects on nuclear factor erythroid 2-related factor 2 pathway critical in oxidative stress defence can prevent osteoporosis [72]. Research comparing Ejiao's efficacy with established treatments, such as bisphosphonates and denosumab [73], could offer valuable insights into its role as a supportive or alternative therapy. Establishing reliable data on optimal dosage, long-term safety, and broader applicability is crucial for solidifying evidence-based recommendations for Ejiao in osteoporosis prevention and treatment.

This review has several limitations, including the predominance of animal and in vitro studies that may not accurately reflect human clinical outcomes, small sample sizes, and a lack of standardised formulations. The heterogeneity of study designs and the limited exploration of the mechanisms by which Ejiao influences bone health further weaken the evidence. Additionally, publication bias may skew perceptions of Ejiao's efficacy in osteoporosis management, as studies with positive results are more likely to be published.

Conclusion

In summary, Ejiao and its formulations show promise in the prevention and management of osteoporosis. Ejiao itself primarily exerts its effects by promoting osteoblast differentiation, regulating the RANKL/OPG axis, and enhancing antioxidant defences. However, its direct impact on bone structure and biomechanical properties remains inconclusive. Meanwhile, Ejiao-based formulations, such as Ejiao Qianggu Oral Liquid and herbal compounds containing Ejiao, offer additional benefits, including enhanced osteoblast proliferation, improvements in bone mineral density, modulation of vitamin D metabolism, and acceleration of fracture healing. While these findings underscore the therapeutic potential of Ejiao and its formulations, further long-term studies and clinical trials are needed to establish their efficacy, mechanisms, and optimal application in osteoporosis management. The integration of Ejiao-based therapies with conventional treatments may provide a more comprehensive strategy for preserving bone health.

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Author contributions

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Use of artificial intelligence tools
The authors used ChatGPT version 3.5 (OpenAI, San Francisco, California) to polish the language of the manuscript but they are responsible for the content.

Competing interests
The authors declare no competing interests.

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