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Zenggu yin decoction alleviates ovariectomy-induced osteoporosis in rats by activating the Wnt/β-catenin pathway

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This study investigated the therapeutic effects of Zenggu Yin Decoction (ZGYD) on ovariectomy-induced osteoporosis in rats and explored its role in modulating the Wnt/β-catenin signaling pathway. Ovariectomy-induced postmenopausal osteoporosis (PMOP) rat models were administered ZGYD for 12 weeks. We measured femur bone mineral density (BMD), osteoporosis-related protein expression, and serum parameters. The mRNA and protein expression levels of runt-related transcription factor 2 (RUNX2), osteoprotegerin (OPG), Wnt1, and β-catenin were determined using qRT-PCR and western blotting, respectively. Our results demonstrated that ZGYD significantly improved bone microstructure, as evidenced by increased BMD, bone volume/total volume (BV/TV), trabecular number (Tb.N), and trabecular thickness (Tb.Th), while reducing trabecular separation (Tb.Sp) in ovariectomized (OVX) rats. Additionally, ZGYD treatment restored serum calcium (Ca), phosphorus (P), estradiol (E2), osteocalcin, and bone-specific alkaline phosphatase (BALP) levels while decreasing procollagen type I N-terminal propeptide (PINP) levels, indicating enhanced bone formation and reduced resorption. Furthermore, ZGYD attenuated oxidative stress by lowering malondialdehyde (MDA) and elevating superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities. Mechanistically, ZGYD upregulated runt-related transcription factor 2 (RUNX2) and osteoprotegerin (OPG) while suppressing receptor activator of nuclear factor-κB ligand (RANKL) and RANK, thus inhibiting osteoclastogenesis. Importantly, ZGYD activated the Wnt/β-catenin pathway, as evidenced by increased Wnt1 and β-catenin expression at both the mRNA and protein levels. ZGYD alleviated osteoporosis in OVX rats by enhancing bone formation, reducing oxidative stress, and activating the Wnt/β-catenin pathway, suggesting its potential as a therapeutic agent for postmenopausal osteoporosis.

Keywords: Bone mineral density, Osteoporosis, Postmenopausal osteoporosis, Wnt/β-catenin pathway, Zenggu Yin Decoction

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Postmenopausal osteoporosis (PMOP) is a prevalent metabolic bone disorder characterized by decreased bone mineral density (BMD) and deterioration of bone microarchitecture, resulting in an increased risk of fractures[1]. The decline in estrogen levels following menopause disrupts bone remodeling, favoring bone resorption over formation, which contributes to progressive bone loss[2]. The pathogenesis of PMOP involves an imbalance between bone resorption and formation driven by estrogen deficiency, oxidative stress, and chronic low-grade inflammation[3]. Conventional treatments, including bisphosphonates and hormone replacement therapy (HRT), exhibit limitations, such as side effects and long-term safety concerns[4]. Therefore, the exploration of alternative therapeutic strategies, particularly traditional herbal medicines, has gained increasing attention.

In China, Traditional Chinese Medicine (TCM) has demonstrated distinct advantages in the prevention and treatment of PMOP[5-8]. According to TCM theory, the development of PMOP is closely associated with kidney essence deficiency, liver blood insufficiency, splenic-stomach weakness, and blood stasis. Kidney deficiency is regarded as the fundamental pathogenesis, as the kidney governs bones and generates marrow[9,10]. Consequently, tonifying the kidney represents a core therapeutic principle in the management of PMOP and has shown favorable clinical outcomes in improving BMD and skeletal strength[7,11].

The Wnt/β-catenin signaling pathway is a key regulator of bone formation and remodeling, promoting osteoblast differentiation and inhibiting osteoclastogenesis through RUNX2 and OPG/RANKL regulation[12,13]. Activation of this pathway enhances bone mass and strength, whereas dysregulation contributes to the development of

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PMOP[14,15]. Modulation of Wnt/β-catenin represents a critical mechanism for therapeutic interventions targeting osteoporosis.

Within the TCM framework, postmenopausal osteoporosis is commonly attributed to kidney essence deficiency accompanied by inadequate nourishment of bones and marrow. Zenggu Yin Decoction (ZGYD) was formulated according to the classical therapeutic principle of "tonifying the kidney and strengthening bone," which has long been applied in the treatment of age-related skeletal disorders[16,17]. The constituent herbs-*Cuscuta chinensis* (kidney-liver tonic), deerhorn glue (essence- and marrow-nourishing agent), *Eucommia ulmoides* (bone- and tendon-strengthening herb), *Cinnamomum cassia* (Yang-warming and circulation-promoting agent), and *Lycium chinense* (liver-kidney Yin-nourishing antioxidant) possess a long history of ethnomedicinal use in East Asian traditional practice for managing musculoskeletal weakness, aging-associated degeneration, and fracture susceptibility[18-20]. Collectively, ZGYD represents a synergistic ethnopharmacological formula designed to restore skeletal homeostasis by reinforcing kidney essence, enhancing circulation, and counteracting degenerative processes, thereby providing a strong traditional rationale for its application in PMOP[17,20].

ZGYD comprises five Chinese herbal medicines, including *Cuscuta chinensis* Lam, deerhorn glue, *Eucommia ulmoides*, *Cinnamomum cassia*, and *Lycium chinense* Miller. *Cuscuta chinensis* Lam., commonly referred to as Chinese dodder or "tu-si-zi," is a parasitic plant utilized in traditional Chinese medicine (TCM) for its ability to tonify the liver and kidneys and promote reproductive health[19]. Deerhorn glue, derived from cervids such as *Cervus nippon* and *Cervus elaphus*, is used in TCM to enhance kidney function, fortify bones, and boost vitality[21]. The bark of *Eucommia ulmoides*, known as "Du Zhong," is recognized for its ability to support kidney function, reduce blood pressure, and improve musculoskeletal health[22]. *Cinnamomum cassia* is noted for its role in aiding circulation and digestion, and its essential oils exhibit anti-inflammatory properties[23]. *Lycium chinense* Miller is used to nourish the liver and kidneys, enhance vision, and strengthen immunity owing to its antioxidant content, including zeaxanthin[24]. However, the therapeutic effects of ZGYD have not yet been documented.

This study aimed to investigate the therapeutic potential of ZGYD in alleviating PMOP and elucidate its underlying mechanism, focusing on the activation of the Wnt/β-catenin pathway. We hypothesized that ZGYD enhances bone formation and reduces bone loss by upregulating Wnt/β-catenin signaling, thereby offering a novel complementary approach for the management of PMOP.

Materials and Methods

Preparation of ZGYD

The basic formula for ZGYD is as follows: 15 g *Cuscuta chinensis* Lam, 10 g deerhorn glue, 15 g *Eucommia ulmoides*, 10 g *Cinnamomum cassia*, and 10 g *Lycium chinense* Miller. The above herbs were washed and boiled with water to concentrate the juice to approximately 1 g/mL (each mL contained 1 g of raw medicine). The final decoction was stored in a refrigerator at 4°C for future use.

Establishment of animal models

A total of 24 healthy and clean grade female Sprague-Dawley rats (6-month-old, weight 240±10 g) were purchased from Shanghai Slack Laboratory Animal Company. They are typically housed in animal breeding rooms. These rats were maintained under standard conditions with a constant temperature of 22-25°C, 50%-65% humidity, 12 h/12 h light and dark cycles, and free access to water. After adaptive feeding for 1 week, ovariectomized surgery was performed on 16 female rats randomly selected from the 24 rats. Briefly, rats were anesthetized by intraperitoneal injection of 30 mg/kg pentobarbital sodium, and the bilateral ovaries were removed. After 4 weeks, the 16 rats were randomly assigned to two groups: the ovariectomized (OVX) and ZGYD groups. The sham group underwent a sham operation and served as the control group ($n = 8$). Rats in the sham and OVX groups were administered saline (10 mL/kg/d) via gavage for 12 weeks. The rats in the ZGYD group were administered ZGYD (1 mL/100 g body weight) via gavage for 12 weeks. At the end of the treatment period, all rats were fasted overnight for 12 h with free access to water and then anesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg). Blood samples were collected for subsequent biochemical and molecular analyses. All experimental procedures were approved by the Animal Ethics Committee of our hospital and conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Micro CT scanning measurement

After treatment with ZGYD for 12 weeks, the rats were anesthetized by intraperitoneal injection of 30 mg/kg pentobarbital sodium. The femur specimens were obtained by removing all surrounding muscles and connective tissues and soaking them in 4% paraformaldehyde for 24 h. The Skyscan Micro CT system was used to perform a cross-sectional scan of the femur, with a voltage of 88 KV, a current of 80 μA, a resolution of 18 μM, a scanning method of 360° rotation, and an imaging field of 9 × 9 × 9 mm. Investigators conducting the Micro-CT scanning and subsequent quantitative analyses were blinded to the experimental group assignments. Following image acquisition, the distal femoral region was selected for three-dimensional reconstruction and quantitative analysis. Trabecular bone parameters, including bone mineral density (BMD), bone volume/total volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp), were calculated using dedicated image analysis software.

Detection of serum indicators

Serum Ca and P levels were detected using an Automatic biochemical analyzer. ELISA kits were used to measure the serum levels of estradiol (E2, ab108667, Abcam), osteocalcin (E-EL-R3070, Elabscience), bone-specific alkaline phosphatase (BALP; DECO2457, Beijing Zhongke Quality Inspection Biotechnology Co., Ltd), procollagen type I N-terminal propeptide (PINP, mL105054, Shanghai Enzyme-linked Biotechnology), RUNX2 (mL106179, Shanghai Enzyme-linked Biotechnology), osteoprotegerin (OPG, E-EL-R3005, Elabscience), RANKL (mL003065, Shanghai Enzyme-linked Biotechnology), and RANK (mL106802, Shanghai Enzyme-linked Biotechnology). All serum biochemical and ELISA analyses were conducted by investigators who were blinded to the treatment group assignments.

Measurement of oxidative stress indicators in serum of rats

Blood serum samples were collected and stored at -80°C. A commercial assay kit was used to quantify the activities of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (S0131S, S0109, S0051, and S0056, respectively; Beyotime, Shanghai, China). All serum oxidative stress measurements were conducted by investigators blinded to the treatment group allocations. Oxidative stress levels were estimated according to a previously established protocol[25].

Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Total RNA was extracted from bone tissues using TRIzol reagent (Invitrogen, USA). Following reverse transcription, complementary DNA (cDNA) was synthesized from total RNA. For mRNA amplification via Real-Time Quantitative PCR (RT-qPCR), SYBR Green reagent (TaKaRa, Japan) was employed in an ABI Prism 7700 Real-Time PCR system (Applied Biosystems, USA). Relative gene expression was normalized to the internal control, GADPH[25], using the $2^{-\Delta\Delta Ct}$ method. Primers for Rat RUNX2, Rat OPG, Rat Wnt1, Rat β-catenin, and Rat GAPDH were designed using the NCBI Primer-Blast Tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), as specified in (Table 1).

Western blotting

Protein samples were obtained by breaking down bone tissues using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China). Protein concentration was determined using a BCA kit (Beyotime, Shanghai, China). Equal volumes of protein (40 μg) were mixed with loading buffer (Beyotime) and denatured in a boiling water bath for 3 min. Once bromophenol blue reached the separation gel, electrophoresis was conducted at 80 V for 30 min, followed by 1-2 h at 120 V. Proteins were transferred onto membranes in an ice bath at 300 mA for 60 min. The membranes were rinsed for 1-2 min with a washing solution and then either inactivated for 1 h at room temperature or sealed overnight at 4°C. Subsequently, the membranes were incubated with primary antibodies against RUNX2 (1:500, ab192256, rabbit monoclonal, Abcam), OPG (1:500, sc-390518, mouse monoclonal, Santa Cruz), Wnt1 (1:500, ab316738, rabbit monoclonal, Abcam), β-

Table 1 — List of primer sequences used in this research

Genes	Forward primer (5′-3′)	Reverse primer (5′-3′)
Rat RUNX2	GGAGGGCCGTGGGTTCT	TTTAGGGCGCATTCCTCATC
Rat OPG	GTTCTTGCACAGCTTCACCA	AAACAGCCCAGTGACCATTC
Rat Wnt1	GGGACCTACGCTTCCTCATG	ACATCCCGTGGCATTTGCA
Rat β-catenin	GAGTGCTGAAGGTGCTGTCTGTC	CAGATGGCAGGCTCGGTAATGTC
Rat GAPDH	TGCTGGTGCTGAGTATGTCG	TTGAGAGCAATGCCAGCC

catenin (1:500, ab246504, rabbit monoclonal, Abcam), and GAPDH (1:2000, ab8245, mouse monoclonal, Abcam) on a shaking table for 1 h at room temperature. The membranes underwent a series of washing steps using a washing solution three times within a 10-min interval before and after 1 h of exposure to the secondary antibody at ambient temperature. Finally, the membranes were introduced into the developing solution, and observations were conducted using a chemiluminescence imaging analysis equipment (Gel Doc XR, Bio-Rad).

Statistical analysis

All data are presented as the mean ± standard deviation (SD) from a minimum of three independent experiments. Statistical analyses were conducted using GraphPad Prism software (version 9.0). For comparisons between two groups, two-tailed unpaired Student's t-tests were used. For analyses involving three or more groups, one-way ANOVA was performed, followed by post hoc multiple comparisons using Tukey's honestly significant difference (HSD) test to control for family-wise error, consistent with IJTK guidelines. Statistical significance was defined as #/p<0.05, ##/* p<0.01, and ###/*** p<0.001.

Results

ZGYD improves bone microstructure and density in rats with osteoporosis

Osteoporotic rats were established by ovariectomy and treated with ZGYD. The body weights of the osteoporosis rats were recorded at 3, 6, and 12 weeks after ZGYD treatment. The results showed that body weight was significantly increased in ovariectomized rats compared with that in sham rats after 6 weeks. ZGYD treatment significantly reduced body weight (Fig. 1a). Micro-CT imaging was performed on the cortical and cancellous bones of the distal femur in rats at the end of the animal experiment. Our results revealed that bone mineral density (BMD), bone volume/total volume (BV/TV), trabecular number (Tb.N), and trabecular thickness (Tb.Th) were significantly decreased, whereas increased trabecular separation (Tb.Sp) was observed in ovariectomized rats. However, ZGYD treatment significantly reversed these effects (Fig. 1b-f).

ZGYD increases serum calcium and phosphorus and ameliorates osteoporosis in rats

Enzyme-linked immunosorbent assay (ELISA) was performed on rat serum to quantify the levels of

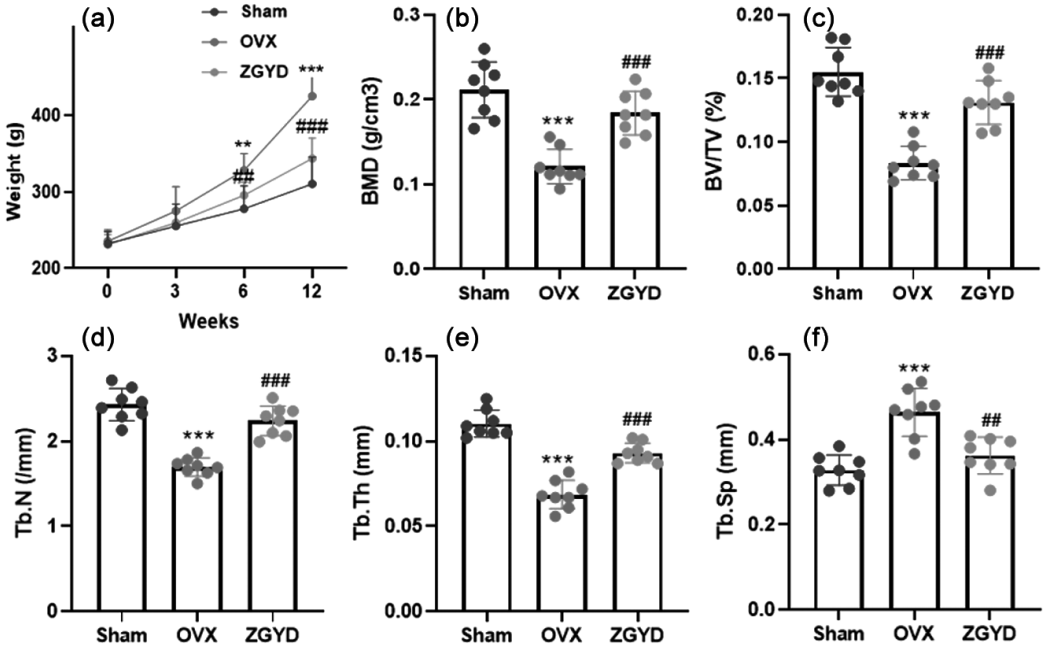

Fig. 1 — Zenggu yin decoction (ZGYD) promotes the bone microstructure and density of osteoporosis rats. Osteoporosis rats were established by ovariectomy and treated with ZGYD. (a) The body weight of the osteoporosis rats was recorded at the 3, 6 and 12 week after ZGYD treatment. Micro CT imaging was performed on the cortical and cancellous bones of the distal femur in rats at the end day of the animal experiment. (b) The bone mineral density (BMD) of rat femur. (c) Bone Volume/Total Volume (BV/TV). (d) Trabecular number (Tb.N). (e) Trabecular thickness (Tb.Th). (f) Trabecular separation (Tb.Sp). Data were presented as mean ± standard deviation (SD) (n = 8). **p<0.01, ***p<0.001 vs. Sham group; ##p<0.01, ###p<0.001 vs. OVX group

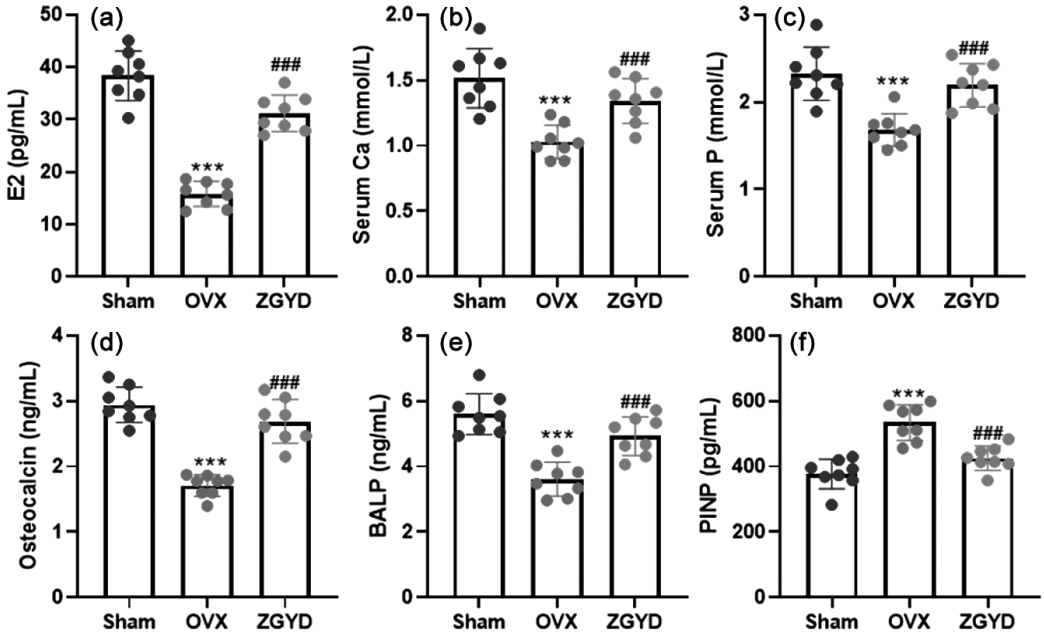

Fig. 2 — ZGYD increases serum calcium and phosphorus and promotes osteoporosis in rats. ELISA was performed on serum of rats to measure (a) Estradiol (E2). (b) Serum calcium (Ca). (c) Serum phosphorus (P). (d) Osteocalcin. (e) Bone specific alkaline phosphatase (BALP). (f) Procollagen type I N-terminal propeptide (PINP). Data were presented as mean ± standard deviation (SD) (n = 8). ***p<0.001 vs. Sham group; ###p<0.001 vs. OVX group

estradiol (E2), serum calcium (Ca), serum phosphorus (P), osteocalcin, BALP, and PINP. The findings revealed a reduction in E2, serum Ca, serum P, osteocalcin, and BALP levels, whereas the PINP levels were elevated in OVX rats. Notably, treatment with ZGYD significantly ameliorated these alterations (Fig. 2a-f).

ZGYD inhibits the oxidative stress in osteoporosis rats

In this study, the serum levels of oxidative stress markers were quantified in osteoporosis-induced rats, specifically assessing MDA concentration, SOD activity, CAT activity, and GPX activity. Our findings indicated elevated MDA levels, accompanied by reduced SOD, CAT, and GPX activities in OVX rats. Importantly, ZGYD treatment significantly mitigated these changes (Fig. 3a-d).

ZGYD modulates RUNX2 and OPG/RANKL/RANK signaling pathways in rats with osteoporosis

The serum concentrations of proteins associated with osteoclast differentiation, including runt-related transcription factor 2 (RUNX2), osteoprotegerin (OPG), receptor activator of nuclear factor-κB ligand (RANKL), and receptor activator of nuclear factor-kappa B (RANK), were quantified in the serum of osteoporotic rats. Our results showed that RUNX2 and OPG levels were reduced, whereas RANKL and

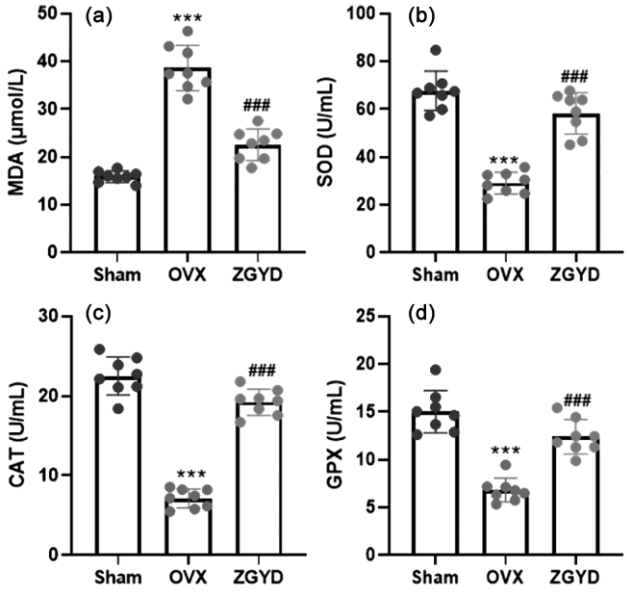

Fig. 3 — ZGYD inhibits oxidative stress of osteoporosis rats. Serum levels of oxidative stress indicators were measured in serum of osteoporosis rats, including (a) MDA concentration, (b) SOD activity, (c) CAT activity and (d) GPX activity. Data were presented as mean ± standard deviation (n = 8). ***p<0.001 vs. Sham group; ###p<0.001 vs. OVX group

RANK levels were elevated in the OVX rats. Notably, treatment with ZGYD significantly ameliorated these alterations (Fig. 4a-d).

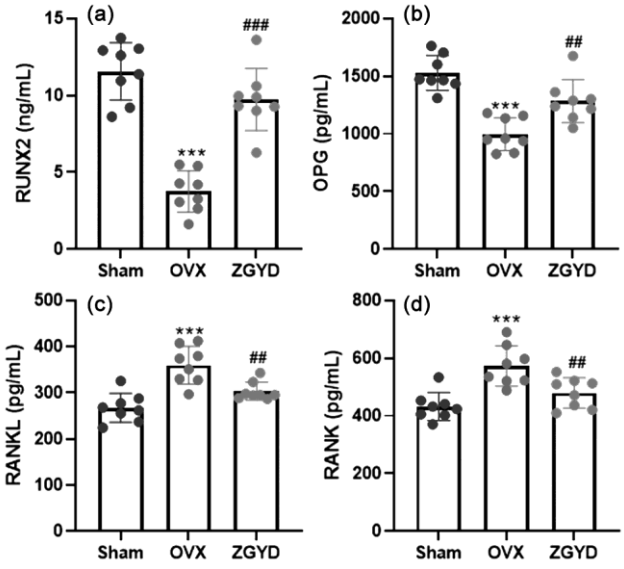

Fig. 4 — ZGYD modulates RUNX2 and OPG/RANKL/RANK signaling pathway of osteoporosis rats. Serum levels of osteoclast differentiation-related proteins were measured in serum of osteoporosis rats, including (a) RUNX2, (b) Osteoprotegerin (OPG), (c) RANKL and (d) RANK. Data were presented as mean ± standard deviation (SD) (n = 8). ***p<0.001 vs. Sham group; ##p<0.01, ###p<0.001 vs. OVX group

ZGYD modulates RUNX2/OPG and Wnt/β-catenin signaling pathway in rats with osteoporosis

The mRNA levels of RUNX2, OPG, Wnt1, and β-catenin in the femurs of the three groups were quantified using RT-qPCR. These findings indicate reduced RUNX2, OPG, Wnt1, and β-catenin levels in OVX rats. Notably, ZGYD treatment significantly ameliorated these effects (Fig. 5a-d). Western blotting was performed to evaluate the protein expression of RUNX2, OPG, Wnt1, and β-catenin. Quantification of representative bands revealed diminished expression of RUNX2, OPG, Wnt1, and β-catenin in OVX rats. However, ZGYD treatment substantially enhanced the expression levels of RUNX2, OPG, Wnt1, and β-catenin (Fig. 5e-h).

Discussion

The present study demonstrates that ZGYD (a traditional Chinese herbal formula) effectively ameliorates osteoporosis in OVX rats by improving bone microstructure, enhancing bone mineral density (BMD), modulating serum calcium and phosphorus levels, reducing oxidative stress, and regulating key osteogenic signaling pathways, including

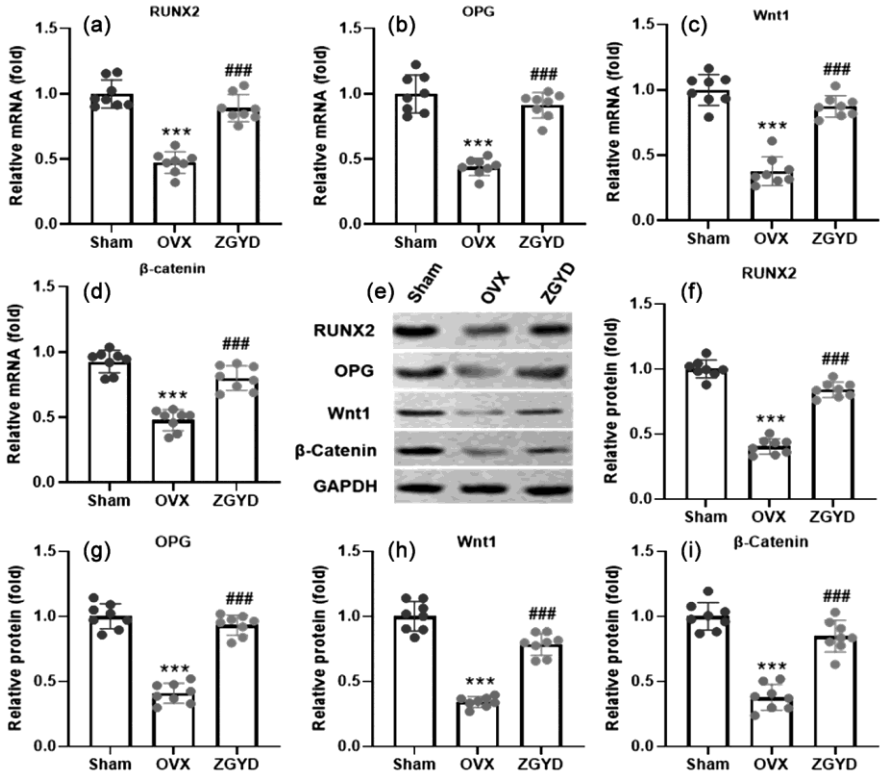

Fig. 5 — ZGYD modulates RUNX2/OPG and Wnt/β-catenin signaling pathway of osteoporosis rats. The mRNA levels of (a) RUNX2, (b) OPG, (c) Wnt1 and (d) β-catenin in the femur of the three groups were determined by RT-qPCR. (e) Representative bands of proteins by Western blot in the femur of rats. Quantification of protein bands of (f) RUNX2, (g) OPG, (h) Wnt1 and (i) β-catenin. Data were presented as mean ± standard deviation (SD) (n = 8). ***p<0.001 vs. Sham group; ###p<0.001 vs. OVX group

RUNX2/OPG/RANKL/RANK and Wnt/β-catenin. These findings align with and extend previous research on herbal interventions for osteoporosis, providing mechanistic insights into the potential therapeutic applications of ZGYD.

Our results show that ZGYD treatment significantly increased BMD, BV/TV, Tb.N, and Tb.Th while reducing Tb.Sp in OVX rats, indicating improved trabecular bone structure. These findings are consistent with those of previous studies on other herbal compounds, such as Epimedium extracts, which enhance BMD by stimulating osteoblast activity[26]. Similarly, berberine has been reported to improve bone microstructure in OVX rats by suppressing osteoclastogenesis[27]. The observed reduction in body weight following ZGYD treatment may also contribute to bone preservation, as excessive weight in postmenopausal osteoporosis exacerbates bone loss due to increased mechanical stress[19].

ZGYD significantly increased serum calcium, phosphorus, E2, and osteocalcin levels, while reducing PINP, suggesting a beneficial effect on bone formation and resorption. These results are comparable to those of studies on *Drynaria fortunei*, which enhances calcium absorption and osteoblast activity[21]. The elevation of BALP, a marker of osteoblast function, further supports the anabolic effects of ZGYD, similar to those reported for *Rehmannia glutinosa*[22].

Oxidative stress plays a critical role in osteoporosis by ameliorating osteoclast activity and suppressing osteoblast function[23]. Our study found that ZGYD reduced MDA levels and restored SOD, CAT, and GPX activities, which is in accordance with previous findings on *Astragalus membranaceus*, which exerts antioxidant effects in OVX rats[24]. The reduction in oxidative stress may contribute to the bone-protective effects of ZGYD by preserving osteoblast viability.

The upregulation of RUNX2 and OPG, along with the downregulation of RANKL/RANK, suggests that ZGYD inhibits osteoclast differentiation while promoting osteogenesis (Fig. 6). These findings are supported by previous studies on *Salvia miltiorrhiza*, which suppresses RANKL-induced osteoclastogenesis[25]. Additionally, ZGYD activated the Wnt/β-catenin pathway, a crucial regulator of bone formation, consistent with the effects of icariin in enhancing osteoblast differentiation via Wnt signaling[28].

Although previous studies have demonstrated the efficacy of individual herbs in osteoporosis

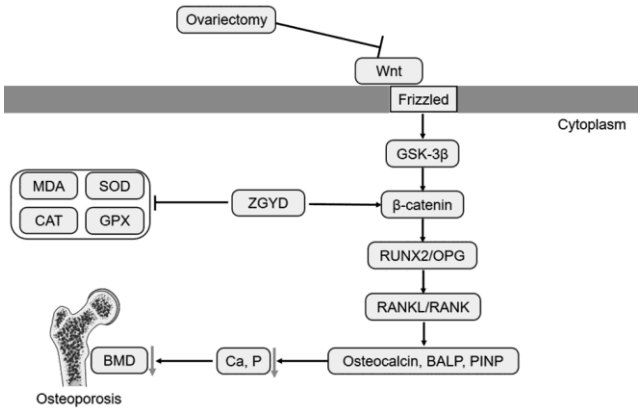

Fig. 6 — Schematic diagram of effect of ZGYD on postmenopausal osteoporosis in PMOP rats

management, ZGYD's multi-component formulation appears to offer synergistic benefits by targeting multiple pathways simultaneously. Unlike bisphosphonates, which primarily inhibit bone resorption, ZGYD promotes both bone formation and mineralization while mitigating oxidative stress, a dual mechanism akin to the *Herba Epimedii* and *Fructus Ligustri* Lucidi combinations[29]. However, further preclinical and clinical trials are needed to validate the efficacy of ZGYD in humans.

Study Limitations

This study has several limitations that should be acknowledged. First, only a single dose of ZGYD was evaluated, and no dose-response analysis was performed; thus, the optimal therapeutic dose and exposure-effect relationship remain undefined. Second, although ZGYD treatment was associated with activation of the Wnt/β-catenin signaling pathway, no pathway inhibition or loss-of-function experiments were conducted, which limits definitive mechanistic conclusions. Third, ZGYD was prepared using standardized traditional methods; however, detailed phytochemical profiling and quantification of bioactive constituents were not performed, which may affect reproducibility and hinder the correlation between specific compounds and observed pharmacological effects. Fourth, the absence of a positive control drug (e.g., bisphosphonates or selective estrogen receptor modulators) restricts direct comparison with established anti-osteoporotic therapies. Additionally, although no overt adverse effects were observed during treatment, systematic toxicity and safety assessments were not conducted, and a comprehensive toxicological evaluation is required prior to clinical translation.

Finally, the study was limited to ovariectomized rat models, and extrapolation of these findings to human postmenopausal osteoporosis requires further preclinical and clinical validation.

Conclusion

In summary, ZGYD exerts anti-osteoporotic effects by improving bone density, regulating bone metabolism, reducing oxidative stress, and modulating key osteogenic pathways. These findings support its potential as a complementary therapy for osteoporosis, warranting further investigation in the clinical setting.

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Author Contributions

FS: Conceptualization, methodology, investigation, formal analysis, data curation. JL: conceptualization, methodology, supervision. All authors contributed to the writing of this manuscript.

Conflict of Interest

All authors confirmed that they don't have any conflict of interest.

Ethics Statement

The ethics committee of Nantong Second People's Hospital approved this study (2023-038). The authors envisaged all standard protocols in accordance with the 1964 Declaration of Helsinki. All methods carried out in this study were in accordance with ARRIVE guidelines.

Data Availability

The data from this study is available upon a reasonable request from the corresponding author

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