Review / Derleme

DOI: 10.4274/tod.galenos.2025.74317
Turk J Osteoporos

Evaluation of the Effects of Menopause on the Musculoskeletal System with Experimental Rat Models

Deneysel Sıçan Modelleri ile Menopozun Kas-iskelet Sistemi Üzerindeki Etkilerinin Değerlendirilmesi

Nesime Hamalı[1], Kezban Kartlaşmış[2], Ayşe Nur Çakır Güngör[3]

[1]Çukurova University Faculty of Medicine, Adana, Türkiye
[2]Çukurova University Institute of Health Sciences, Adana, Türkiye
[3]Çukurova University Faculty of Medicine, Department of Obstetrics and Gynaecology, Adana, Türkiye

Abstract

In pre-menopausal women, low bone mass and trauma fractures may be due to secondary causes such as estrogen deficiency, glucocorticoid exposure, malabsorption or hyperparathyroidism. However, during the menopausal transition and menopause, the major cause is a decrease in the hormone estrogen. The transition to menopause begins about four years before the last menstrual period. Over time, the production and secretion of estradiol, the potent estrogen, decreases in the ovaries. In the skeletal system, this deficiency causes loss of spongiosis and cortical bone; in the muscular system, it reduces muscle strength through muscle mass and contractile proteins. Micro- and macro-level losses in muscle and bone tissues, which are both endocrine and paracrine tissues, begin during perimenopause. This article discusses the evaluation of menopause-induced musculoskeletal changes in experimental rat models and different treatment approaches. The adverse effects of estrogen deficiency on muscle and bone have been investigated with various therapeutic strategies to address this deficiency. Several compounds such as myostatin, irisin, lycopene, nacre, superjami rice and annatto tocotrienol have the potential to improve muscle and bone health after menopause. Furthermore, it has been emphasized that estrogen plays important roles such as supporting muscle satellitic cells, improving mitochondrial function and inhibiting bone destruction. In each study, efforts to reduce the effects of menopause through different pathways and molecules were described. These findings offer significant potential for the protection of musculoskeletal health in the postmenopausal period and the development of treatment alternatives.

Keywords: Menopause, musculoskeletal system, rat models

Öz

Premenopozal dönemdeki kadınlarda düşük kemik kütlesi ve travma kaynaklı kırıklar, östrojen eksikliği, glukokortikoid maruziyeti, malabsorpsiyon veya hiperparatiroidizm gibi ikincil nedenlere bağlı olabilir. Ancak menopoz geçiş dönemi ve menopoz sırasında temel neden, östrojen hormonundaki azalmadır. Menopoza geçiş, son adet döneminden yaklaşık dört yıl önce başlar. Zamanla, güçlü bir östrojen olan estradiolün overlerdeki üretimi ve salınımı azalır. İskelet sisteminde bu eksiklik, süngerimsi ve kortikal kemik kaybına; kas sisteminde ise kas kütlesi ve kasılma proteinleri yoluyla kas gücünde azalmaya neden olur. Endokrin ve parakrin dokular olan kas ve kemik dokularındaki mikro ve makro düzeydeki kayıplar, perimenopoz döneminde başlar. Bu makalede, menopozun neden olduğu kas-iskelet değişikliklerinin deneysel sıçan modellerinde değerlendirilmesi ve farklı tedavi yaklaşımları ele alınmıştır. Östrojen eksikliğinin kas ve kemik üzerindeki olumsuz etkilerinin, bu eksikliği gidermek için çeşitli terapötik stratejilerle araştırıldığı bildirilmiştir. Myostatin, irisin, likopen, sedef, Superjami pirinci ve annatto tokotrienol gibi çeşitli bileşiklerin menopoz sonrası kas ve kemik sağlığını iyileştirme potansiyeline sahip olduğu ifade edilmiştir. Ayrıca, östrojenin kas satelit hücrelerini destekleme, mitokondriyal fonksiyonu iyileştirme ve kemik yıkımını önleme gibi önemli roller oynadığı vurgulanmıştır. Her bir çalışmada, menopozun etkilerini farklı yollar ve moleküller aracılığıyla azaltmaya yönelik çabaların ele alındığı belirtilmiştir. Bu bulguların, postmenopozal dönemde kas-iskelet sağlığının korunması ve tedavi alternatiflerinin geliştirilmesi açısından önemli bir potansiyele sahip olduğu ifade edilmiştir.

Anahtar kelimeler: Menopoz, kas-iskelet sistemi, sıçan modelleri

Corresponding Author/Sorumlu Yazar: Kezban Kartlaşmış MD, Çukurova University Institute of Health Sciences, Adana, Türkiye
E-mail: kzbn.krtlsms@gmail.com **ORCID ID:** orcid.org/0000-0001-5090-0013
Received/Geliş Tarihi: 27.01.2025 **Accepted/Kabul Tarihi:** 04.03.2025 **Epub:** 30.07.2025

Cite this article as/Atıf: Hamalı N, Kartlaşmış K, Çakır Güngör AN. Evaluation of the effects of menopause on the musculoskeletal system with experimental rat models. Turk J Osteoporos. [Epub Ahead of Print]

Introduction

Estrogens are a group of hormones that include E1, E2, E3 and E4 (1). They act through classical nuclear estrogen receptors (ERs) and the membrane-type G protein-coupled ER. ERs are "nuclear steroid receptors" in the NR3 class of the nuclear receptor superfamily. They act through the most familiar ERα (NR3A1) and ERβ (NR3A2) receptors, which have the same structural features but different distribution in tissues. They bind directly to DNA and regulate gene expression like ligands that activate transcription factors. The non-genomic effect of estrogen is to regulate gene expression by regulating protein kinase signaling cascades (2,3). Estrogen stimulates muscle stem cells (satellitic cells) proliferation and differentiation, thereby increasing muscle mass and strength to maintain muscle health. Collins et al. (4) conducted a study to show that estrogen deficiency negatively affects satellitic cells and impairs muscle fiber self-renewal and differentiation. They found that estradiol and ERα promote satellitic cells by downregulating genes associated with mitochondrial caspase-induced apoptosis.

Osteoblasts and osteoclasts are affected by systemic and sex hormones. ERα and ERβ receptors are found in both osteoblasts and osteoblast progenitors. Estrogen deficiency increases osteoblast apoptosis and decreases bone formation time and osteoclast apoptosis. Therefore, bone destruction continues for a longer period of time and thus bone remodelling imbalance occurs. Also during estrogen deficiency, an increase in osteocyte apoptosis is observed in humans and animals (1).

Bone tissue secretes osteokines (osteocalcin, fibroblast growth factor-23 and sclerostin) and muscle tissue secretes cytokines called myokines (interleukin-6, irisin and β-aminoisobutyric acid). These cytokines also produce autocrine and paracrine effects. This effect causes muscle and bone tissue to communicate and influence each other (1,2).

The subchondral bone regularly distributes the load and stress on the bone. Alterations here cause an uneven distribution, initiating or accelerating cartilage degeneration. Yang et al. (3) compared osteochondral changes by studying post-traumatic (PT) and oophorectomized (OVX) osteoarthritis (OA) models (5). Rapid cartilage degeneration and increased bone formation were observed in PT-OA, whereas only mild cartilage erosion, proteoglycan and significant bone loss were observed in OVX-OA. In addition, subchondral bone degradation in OVX-OA occurred 6 weeks before cartilage degeneration. Transforming growth factor (TGF)-β downregulation was observed in the osteochondral unit of OVX rats. Estrogen supplementation prevented subchondral bone loss, cartilage degradation and TGF-β downregulation. TGF-β has been shown to be a regulator of both subchondral bone and cartilage. Developing therapies targeting TGF-β in menopause-induced OA may be considered as a future treatment alternative by acting on both bone and cartilage tissue.

Many experimental studies have been conducted to reduce and stop the negative effects of menopause on the musculoskeletal system. In each of these studies, different active substances were used and effects were monitored through different pathways. Whether these active substances will be a treatment alternative in the future is a matter of debate. This article aims to comprehensively present the studies examining the effects of menopause on the musculoskeletal system in rats.

Active Ingredients and Potential Treatment Alternatives Aiming to Reduce the Effects of Menopause on the Musculoskeletal System

1.1. Exercise

Exercise-induced oxidative stress may differ between genders in humans and animals. In a 2017 study, these differences were evaluated in a male and female rat model that was jogged for 6 weeks (4). Exercise was shown to improve the oxidative capacity of mitochondrial function in both male and female rats, but this was more pronounced in males. In sedentary rats, oxidative stress resistance has been shown to be higher in female rats. It has been suggested that this may be due to the effects of estrogen. In another study, it was shown that estrogen deficiency in rats decreased mitochondrial respiratory complex I activity in muscle and this was corrected by estrogen treatment (6). In these studies, we see that estrogen hormone, which also affects mitochondria, which we know as the power plant; also affects the energy balance.

1.2. Myostatin

Myostatin (MSTN) is one of the key factors involved in communication between muscle and bone tissue. MSTN signaling, a member of the TGF-β superfamily, is thought to accompany aging in musculoskeletal tissues and lead to loss of muscle and bone mass. Tang et al. (7) established an osteoporosis (OP) model in OVX rats. Low-intensity pulsed ultrasound (LIPUS) application in OVX rats has been shown to have positive effects on MSTN expression inhibition and consequently prevention of bone loss and healing of bone damage. It has also been suggested that these effects may be related to MSTN/Wnt/β-catenin signaling pathways. According to the study, LIPUS application may be effective in the treatment of OP.

1.3. Irisin

The effect of exercise on the musculoskeletal system is closely related to Irisin. Irisin is a myokine that affects thermogenesis, energy expenditure and glucose homeostasis. It is released by exercise and targets bone tissue. Irisin's mechanism of action is mediated by peroxisome ɣ and its coactivator-1 α receptors that regulate thermogenesis via mitochondria. Irisin increases energy expenditure, promotes weight loss, and reduces diet-induced insulin resistance. Nyugen et al. (8) evaluated the effect of irisin injection on OP in OVX rats. It was suggested that intermittent treatment with irisin has a role in bone health and may be a valuable target for the treatment of postmenopausal OP.

1.4. Nacre

In the exoskeleton of mollusk's, the part consisting of calcium carbonate ($CaCO_3$) embedded in the organic matrix is called Nacre. The effect of Nacre on bone cells has been documented by *in vitro* studies Lin et al. (9) examined the effect of a 90-day long Nacre-supplemented diet on both bone mass and bone strength using 16-month-old C57BL/6 mice (10). Nacre supplementation increased bone strength by limiting gene expression related to osteoclast activity. It also reduced cortical bone loss by decreasing pore formation in cortical bone. It has also been shown that bone dynamics in trabecular bone, which decreases with aging, is preserved with Nacre diet (10).

1.5. Lycopene

Oxidative stress induced by reactive oxygen species is increased in aging or inflammatory conditions. This may adversely affect bone homeostasis. Carotenoids, which are antioxidant substances, have the potential to reduce these negative effects. Lycopene is one of the compounds in the carotenoid group. Oliveira et al. (11) evaluated the activity of osteoblasts cultured with lycopene from OVX rats for 8 weeks using *in vitro* analyses and also evaluated the femoral epiphyseal osteocytes and trabecular microarchitecture of 8-week-old ovectomised rats receiving lycopene by oral gavage using microtomography and stereological analyses. In addition to biochemical analyses, gene expression was investigated by molecular studies. The data obtained showed an increase in alkaline phosphatase in situ detection and mineralization in the group receiving lycopene. The previous report showed no difference in mineralization with the presence of lycopene after 14 days of culture. In conclusion, further studies are needed to clarify the issue. Quantitative expression of genes encoding important proteins and considered as bone markers was analysed using polymerase chain reaction. Lycopene significantly upregulated Runx2 and Bglap expression. Stereological analysis showed that lycopene treatment caused a significant increase in the number of osteoblasts, but no change in their volume, indicating that lycopene has an effect on osteoblastogenesis. There was also a decrease in the volume and number of osteoclasts. The number of osteocytes was significantly increased compared to the OVX group despite the decrease in their volume. No significant difference was found in microtomographic analyses (12).

1.6. Superjami

Superjami is a dark purple rice variety cultivated in Korea. It is characterized by its strong antioxidant activity as it is particularly rich in cyanidin-3-glucoside and anthocyanins. Chung et al. (13) evaluated the effects of ethanol extracts obtained from dark purple Superjami rice (*Oryza sativa* L. Cv. Superjami) bran on bone metabolism and antioxidant defense systems in OVX rats in an 8-week study. Bone turnover was significantly decreased in rats receiving Superjami rice bran extract supplementation as evidenced by the decrease in Alkaline Phosphatase, osteocalcin and CTx (C terminal telopeptide) amounts. The supplementation was shown to significantly improve bone metabolism and reduce bone loss in OVX rats. In addition, it was also found to significantly suppress oxidative stress and increase the activity of antioxidant enzymes. In another study conducted in 2019 and lasting 8 weeks, the effects of germinated and non-germinated Superjami diets were examined in OVX rats. The bioactive components contained between the two groups were found to be different. Both germinated and ungerminated Superjami rice flour were found to significantly reduce body weight gain, body fat, glucose and insulin levels and adipokine concentrations. In addition, it has been shown to significantly improve the antioxidant defense system and bone metabolism. In addition to all these, germinated Superjami has been shown to reduce body weight more than its ungerminated form and to have a greater effect on glucose homeostasis, antioxidative activities and bone metabolism. It has also been shown that germinated Superjami may be more effective and may be more useful in the treatment of menopausal hyperglycemia, oxidative stress and bone turnover imbalance (14).

1.7. Tocotrienol

Tocotrienol, which shows vitamin E activity, acts as an antioxidant. Since its bioavailability is low in oral administration, it is thought that solubilisation with self-emulsifying systems (SEDDS) facilitates its absorption by the lymphatic system and increases its bioavailability. Assuming that annatto (*Bixa orellana*) tocotrienol formulated with SEDDS has stronger effects on the skeleton than unformulated tocotrienol, the study was conducted in May 2021. The efficacy of SEDDS-formulated annatto-tocotrienol on bone parameters and oxidative stress markers was evaluated in OVX rats. In rats divided into 4 groups: OVX, OVX +unformulated AnTT, OVX +formulated AnTT-SEDDS and OVX +raloxifene (a selective ER modulator known to benefit the skeletal system). After 8 weeks of oral administration, blood levels of delta-tocotrienol and oxidative stress markers were analyzed, and microcomputed tomography, calcium content and biomechanical strength analyses were performed on the femur. Plasma delta-tocotrienol level was significantly higher in the AnTT-SEDDS group than in the AnTT group. AnTT-SEDDS group improved bone microarchitecture in rats by increasing trabecular thickness and trabecular number. However, these two parameters were not found to be dramatically different between OVX and AnTT groups. Both forms of annatto tocotrienol preserved femoral bone calcium content in rats. It is noteworthy that only the AnTT-SEDDS group significantly increased bone stiffness compared to the OVX group. The AnTT and AnTT-SEDDS groups did not attenuate the effects of OVX on bone mineral density (BMD). The group using raloxifene as a positive control showed similar skeletal effects as annatto tocotrienol. In this study, raloxifene treatment preserved bone microarchitecture, calcium content and strength. There was no noticeable change in BMD in this group. AnTT and AnTT-SEDDS groups increased superoxide dismutase and glutathione peroxidase activities in ovariectomised rats. However, it

decreased the level of malonaldehyde, a marker of oxidative stress, only in the AnTT-SEDDS group. In this study, the effects of annatto tocotrienol on the skeletal system were proven and no significant difference was observed between the SEDDS groups. This may be due to the fact that the experiments were not long-term experiments that would create a clear difference between the two groups or oral diet administration (15).

1.8. Nanoparticles

One of the recent studies involving modern technology is the experiment conducted by Guo et al. (16). Using a remote-controlled release system with Nanoparticles (NPs) targeting bone tissue, OP treatment was approached from a different direction. In recent years, with the use of drugs that act systematically on bone tissue (estrogen, calcitonin, bisphosphonates, raloxifene and RANK ligand inhibitors, etc.), new treatment methods have been needed due to serious side effects due to high doses and frequent administration. The aim of this system is to achieve a magnetically remotely controllable drug release under the guidance of an external magnetic field. An OVX rat model was used in a 3-month experiment to investigate the effect of NPs in anti-OP. As a result of the study, NPs showed good stability, good biocompatibility and high encapsulation ability for E2. In addition, the release of these NPs has a temperature-dependent effect. The system used improved OVX-induced bone loss, increased bone strength and enabled new bone formation in extra-skeletal tissues with fewer side effects. The absence of significant toxic side effects was also considered as an advantage (16,17).

1.9. Sirtuin

Many studies have been conducted on the potential role of the Sirtuin (SIRT) gene family, known as NAD^+-dependent class III deacetylase enzymes, in various diseases. However, little is currently known about the effect of mammalian SIRT1 on ageing. In a study, it was found that SIRT1 expression promotes healthy aging but does not improve longevity. In a mouse experiment, SIRT1 was observed to be partially protective against the development of pathologies typically associated with ageing, such as glucose intolerance, OP and wound healing (18).

1.10. Resveratrol

Resveratrol (R) is a phytoestrogen structurally similar to natural and synthetic estrogens. It can bind to ERs in bone and cartilage cells, providing chondroprotective and osteoprotective effects (19). R, a polyphenol compound found in peanuts, blueberries, grapes and other plants, is a natural activator of SIRT1 (20). The effect of R on bone mass in OVX rats and the role of SIRT1 in the maintenance of bone mass during perimenopause and early post-menopause were investigated (21). The rats were divided into 4 groups: 1- control group; 2-OVX; 3-OVX+low dose resveratrol; and 4-OVX + high dose resveratrol. One week after the surgery, rats in groups 3 and 4 were given R orally for 10 weeks. As a result, positive effects on serum osteoprotegrin, SIRT1 protein, Wnt/β-catenin signaling, BMD and bone microarchitecture in rat femurs and negative effects on RANKL were observed. In another study, control group, OVX and OVX groups given intravaginal R gel were formed and the experiment lasted for about 5 weeks. Knee joint tissues (articular cartilage, subchondral plate, subchondral bone) were evaluated by histomorphometry. In addition, mammalian target of rapamycin protein complex (mTOR), protein tyrosine phosphatase and tensin homologue, caspase 3 (cysteine proteinase involved in apoptosis) and [B-cell lymphoma/leukaemia-2 (BCL-2), antiapopitotic agent] expression in articular cartilage and subchondral bone were immunohistochemically evaluated. R treatment prevented weight gain by 17%. Trabecular bone degradation was attenuated due to upregulation of BCL-2 and downregulation of Casp-3. mTOR expression was downregulated. This effect prevented chondrocyte hypertrophy and maintained cartilage homeostasis. It was also found that intravaginal R treatment had systemic effects, decreased weight gain and increased oestradiol levels in OVX rats. Histological examination of joint tissues in OVX rats confirmed the protective effect of R treatment against degenerative changes in articular cartilage and trabecular bone resorption (19).

1.11. Edible Bird's Nest

In recent years, numerous studies have been conducted on the pharmacological effect and mechanism of Edible Bird's Nest (EBN). These studies are on antiviral effect, immune regulation, cognitive functions, neurodegenerative diseases and antioxidant issues. EBN has also been shown to control arthritis and may support the regeneration of chondrocytes (22). In a study conducted in 2019, administration of estrogen and EBN in OVX rats increased estrogen levels and ER expression in bone tissue, causing pro-osteoplastic hormone production and increased bone density (23). At this point, EBN can be considered as an alternative in hormone replacement therapies.

1.12. Opuntia ficus-indica

A study was conducted in 2020 to reveal the effect of Opuntia ficus-indica, which has been shown to have antioxidant and anti-inflammatory effects, on calcium bioavailability. In a 9-week period, the effect of O. ficus-indica in terms of physical, densitometric, biomechanical, microstructural and mineral content in bones in OVX rat model was evaluated. In rats receiving O. ficus-indica diet, calcium bioavailability and absorption, fracture resistance of bones and BMD were found to be high. No dose-based evaluation was performed in this study, but O. ficus-indica may be considered as a future therapeutic hope (24).

1.13. Parathormone (PTH) treatment

The effects of intermittent parathormone (PTH) treatment on vertebral body, tibia, BMD in distal femoral metaphysis, trabecular structures and femoral neck in OVX rats have been investigated in studies. In a 12-week study by Wang et al. (20), fracture resistance of the femoral neck was found to be highly correlated with two parameters, CSA (cross-sectional area) and bone strength index of the cortical bone in the femoral neck.

Using micro-CT, it has been demonstrated that intermittent PTH contributes to the cancellous and cortical bones of the femoral neck in OVX rats. Thus, it was shown that this method may be useful in the treatment of OP (22,24).

1.14. Abeliophyllum Distichum Nakai

Abeliophyllum distichum Nakai (AD), called Miseon, is one of the endemic species in Korea. In an 8-week experiment using OVX rat model, the effects and mechanism of orally administered AD-ethyl acetate fraction (EA) extract were investigated. Femur bone parameters were measured using micro-CT. AD-EA was found to inhibit osteoclast differentiation and bone resorption by inhibiting osteoclast-related gene expression via MAPK and c-fos/NFATc1 pathways. AD-EA also inhibited CTK and TRAcP activation, preventing bone loss caused by estrogen deficiency *in vivo* (25).

1.15. Marantodes pumilum (Blume) Kuntze

Wnt/β-catenin signaling is an important cellular pathway involved in osteoblast activation. This pathway is crucial for the regulation of bone formation and destruction. Marantodes pumilum (Blume) Kuntze (MP), also known as Kacip Fatimah, is a popular herb widely used in Southeast Asia. The protective effect of MP on bone has been attributed to phytoestrogenic compounds found in this plant. In the study, Marantodes pumilum leaf aqueous extract (MPLA) (50 mg/kg/day and 100 mg/kg/day) and estrogen were treated for 28 days. The results of the experiment showed that MPLA was able to ameliorate bone loss by increasing the level and distribution of osteoblastogenesis proteins in bone, and to maintain bone mass and collagen content close to normal in DM associated with estrogen deficiency. MPLA has also been found to prevent osteoblast apoptosis in the presence of sex steroid deficiency and DM. Treatment with 100 mg/kg/day MPLA had a greater effect than estrogen, whereas treatment with 50 mg/kg/day MPLA had less effect than estrogen (26).

1.16. Fructans

Fructans are indigestible carbohydrates found as storage polysaccharides in many higher plants. They also have numerous biological activities, including antioxidant, immunomodulatory, anti-inflammatory, anticancer, anti-hyperglycemic and prebiotic activities, providing many benefits to human health. Fructans also increase calcium absorption to prevent bone loss and OP (26). Estrogen deficiency during menopause as well as decreased dietary calcium intake cause serious problems on bone tissue. In the study by Topolska et al. (21) the effects of diet containing only fructan or enriched with "strawberry matrix" on bone tissue were investigated in calcium hypoalimentated OVX rats. As a result, inulin type fructans improved bone quality. Bone density was also increased in rats fed the given diet.

Conclusions and Future Perspective

The negative effects of menopause on the musculoskeletal system occur through estrogen deficiency and the resulting cellular and molecular mechanisms. Experimental studies focus on different treatment alternatives to reduce or stop these effects. In particular, various agents such as MSTN inhibitors, irisin, lycopene, nacre, superjami rice, and tocotrienol stand out as potential treatment options to improve postmenopausal musculoskeletal health. Each of these agents acts through different biochemical pathways targeting muscle and bone loss caused by estrogen deficiency. However, more research is needed before these substances can be put into clinical use. Although the experimental studies are promising, new studies are needed to fully understand and treat the effects of menopause on the musculoskeletal system.

Footnotes

Authorship Contributions

Concept: K.K., A.N.Ç.G., Design: K.K., A.N.Ç.G., Data Collection or Processing: N.H., Analysis or Interpretation: K.K., A.N.Ç.G., Literature Search: N.H., Writing: N.H., K.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that this study received no financial support.

References

1. Lingyun L, Tian L. Postmenopausal osteoporosis coexisting with sarcopenia: the role and mechanisms of estrogen. J Endocrinol. 2023;259:1-19.
2. Yoh K, Ikeda K, Horie K, Inoue S. Roles of estrogen, estrogen receptors, and estrogen-related receptors in skeletal muscle: regulation of mitochondrial function. Int J Mol Sci. 2023;24:1853.
3. Yang Z, Tan Q, Zhao Z, Niu G, Li S, Li W, et al. Distinct pathological changes of osteochondral units in early OVX-OA involving TGF-β signaling. Front Endocrinol. 2022;13:1074176.
4. Collins BC, Arpke RW, Larson AA, Baumann CW, Xie N, Cabelka CA, et al. Estrogen regulates the satellite cell compartment in females. Cell Rep. 2019;28:368-81.
5. Torres MJ, Ryan TE, Lin CT, Zeczycki TN, Neufer PD. Impact of 17β-estradiol on complex I kinetics and H_2O_2 production in liver and skeletal muscle mitochondria. J Biol Chem. 2018;293:16889-98.
6. Farhat F, Amérand A, Simon B, Guegueniat N, Moisan C. Gender-dependent differences of mitochondrial function and oxidative stress in rat skeletal muscle at rest and after exercise training. Redox Rep. 2017;22:508-14.
7. Tang L, Kang Y, Sun S, Zhao T, Cao W, Fan X, et al. Inhibition of MSTN signal pathway may participate in LIPUS preventing bone loss in ovariectomized rats. J Bone Miner Metab. 2020;38:14-26.
8. Nguyen DK, Vanden-Bossche A, Laroche N, Thomas M, Linossier MT, Peyroche S, et al. Dietary supplementation with nacre reduces cortical bone loss in aged female mice. Exp Gerontol. 2023;184:112337.
9. Lin JB, Wu H, Liu YL, Shaw PC, Li PB. N16 suppresses RANKL-mediated osteoclatogenesis by down-regulating RANK expression. Int J Biol Macromol. 2020;151:1154-62.

10. Semeghini MS, Scalize PH, Coelho MC, Fernandes, RR, Pitol DL, Tavares MS, et al. Lycopene prevents bone loss in ovariectomized rats and increases the number of osteocytes and osteoblasts. J Anat. 2022;241:729-40.
11. Oliveira GR, Vargas-Sanchez PK, Fernandes RR, Ricoldi MST, Semeghini MS, Pitol, DL, et al. Lycopene influences osteoblast functional activity and prevents femur bone loss in female rats submitted to an experimental model of osteoporosis. J Bone Miner Metab. 2019;37:658-67.
12. Chung SI, Ryu SN, Kang MY. Changes in bone metabolism and antioxidant defense systems in menopause-induced rats fed bran extract from dark purple rice (*Oryza sativa* L. Cv. Superjami). Nutrients. 2021;13:2926.
13. Chung SI, Ham TH, Kang MY. Effect of germinated pigmented rice "Superjami" on the glucose level, antioxidant defense system, and bone metabolism in menopausal rat model. Nutrients. 2019;11:2184.
14. Mohamad NV, Ima-Nirwana S, Chin KY. Therapeutic potential of annatto tocotrienol with self-emulsifying drug delivery system in a rat model of postmenopausal bone loss. Biomed Pharmacother. 2021;137:111368.
15. Wang SP, Chen YJ, Hsu CE, Chiu YC, Tsai MT, Hsu JT. Intermittent parathyroid hormone treatment affects the bone structural parameters and mechanical strength of the femoral neck after ovariectomy-induced osteoporosis in rats. BioMed Eng OnLine. 2022;21:6.
16. Guo Y, Liu Y, Shi C, Wu T, Cui Y, Wang S, et al. Remote-controllable bone-targeted delivery of estradiol for the treatment of ovariectomy-induced osteoporosis in rats. J Nanobiotechnol. 2021;19:1-18.
17. Herranz D, Muñoz-Martin M, Cañamero M, Mulero F, Martinez-Pastor B, Fernandez-Capetillo O, et al. Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. Nat Commun. 2010;12:3.
18. Zaychenko G, Belenichev I, Hnatiuk V, Doroshenko A, Sinitsyna O, Sulaieva O, et al. Protective effect of vaginal resveratrol administration on joint tissues in ovariectomized rats: targeting mTOR and caspase 3. Biomed Pharmacother. 2023;165:115176.
19. Koushki M, Amiri-Dashatan N, Ahmadi N, Abbaszadeh HA, Rezaei-Tavirani M. Resveratrol: a miraculous natural compound for diseases treatment. Food Sci Nutr. 2018;6:2473-90.
20. Wang X, Lu C, Chen Y, Wang Q, Bao X, Zhang Z, Huang X. Resveratrol promotes bone mass in ovariectomized rats and the SIRT1 rs7896005 SNP is associated with bone mass in women during perimenopause and early postmenopause. Climacteric. 2023;26:25-33.
21. Topolska K, Radzki RP, Filipiak-Florkiewicz A, Bienko M, Florkiewicz A, Cieslik E. Could fructan sources in strawberry matrix be more effective as a tool for improvement of bone structure than these compounds added to diet alone?-Study on osteopenic rat model. Ann Agric Environ Med. 2020;27:19-28.
22. Hou ZP, Tang SY, Ji HR, He PY, Li YH, Dong XL, et al. Edible bird's nest attenuates menopause-related bone degeneration in rats via increaing bone estrogen-receptor expression. Chin J Integr Med. 2021;27:280-5.
23. Nam DG, Yang HS, Bae UJ, Park E, Choi AJ, Choe JS. The cactus (*Opuntia ficus-indica*) cladodes and callus extracts: a study combined with lc-ms metabolic profiling, in-silico, and in-vitro analyses. Antioxidants (Basel). 2023;12:1329.
24. Kim EY, Kim JH, Kim M, Park JH, Sohn Y, Jung HS. Abeliophyllum distichum Nakai alleviates postmenopausal osteoporosis in ovariectomized rats and prevents RANKL-induced osteoclastogenesis in vitro. J Ethnopharmacol. 2020;257:112828.
25. Karim K, Giribabu N, Salleh N. Marantodes pumilum (blume) Kuntze (Kacip Fatimah) leaves aqueous extract prevents downregulation of Wnt/β-catenin pathway and upregulation of apoptosis in osteoblasts of estrogen-deficient, diabetes-induced rats. J Ethnopharmacol. 2021;280:114236.
26. Wang M, Cheong KL. Preparation, structural characterisation, and bioactivities of fructans: a review. Molecules. 2023;28:1613.