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Review Article

Isoflavones Potentials for the Treatment of Osteoporosis: An Update on *In-vivo* Studies

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ABSTRACT

In plant-derived compounds, phytoestrogens are biologically active substances that exhibit various estrogenic and antiestrogenic effects. With the increasing prevalence of osteoporosis among older women caused by estrogen deficiency, identifying natural substances that can potentially treat the disease is of utmost significance. This review study aimed to explore how phytoestrogen metabolites mimic mammalian estrogens and prevent bone loss following menopause. Phytoestrogens derived from plants have gained considerable attention due to their similarity to mammalian estrogens and lower impact on sensitive tissues, such as the uterus and breasts. One well-established approach to simulate postmenopausal conditions is by using ovariectomized rats or mice (OVX). The administration of phytoestrogens in the OVX murine model has inhibited osteoclast differentiation, activation, and Pyridinoline secretion. Furthermore, these compounds have been shown to enhance bone formation and increase bone mineral density and the expression levels of various osteoblast markers, such as alkaline phosphatase, osteocalcin, osteopontin, and alpha-1 collagen. Several natural phytoestrogen compounds in plants possess a chemical structure akin to 17 beta-estradiol, a steroid hormone. In postmenopausal women with osteoporosis, isoflavones, a type of phytoestrogen, can potentially treat the disease by binding to estrogen receptors on the surface of target cells. Mechanistic investigations have demonstrated that phytoestrogens can retard bone resorption and promote bone formation. Novel approaches in phytoestrogen research could involve investigating the synergistic effects of combining different phytoestrogen compounds, exploring their interactions with other signaling pathways, or assessing their effects on various bone types. Furthermore, identifying novel sources of phytoestrogens could lead to the discovery of new compounds with potent osteoprotective effects.

1. Introduction

There are several subtypes of osteoporosis, such as senile osteoporosis and postmenopausal osteoporosis[1]. In older and postmenopausal women, osteoporosis is one of the most prevalent skeletal diseases[2]. In addition to decreased bone mass and deteriorated microarchitecture, osteoporosis also increases the risk of fractures[3]. As one of the most serious issues facing women, postmenopausal osteoporosis is difficult to prevent[4]. It has been shown that hormone replacement therapy and pharmacological supplements of calcium, vitamins D and K can reduce bone loss and hip fracture complications after menopause, as well as select estrogen-receptor modulators, estrogen analogs or bisphosphonates, calcium, and parathyroid hormone[5-7]. Several nonpharmacological interventions are also available, including dietary modification, physical activity, hip protectors, and orthopedic treatment of fractures[8]. Osteoporosis can be treated with estrogen and related compounds[9]. Still, these are primarily aimed at blunting bone resorption, a tightly coupled process involving bone formation and resorption, largely the aim of current treatments[10]. Osteoporosis is currently treated with various drugs, which act as antiresorptive agents to prevent bone loss. These include bisphosphonates, estrogen, selective estrogen receptor modulators, and statins[11,12]. Most osteoporosis cases are caused by estrogen loss[13]. A well-established and reproducible method of simulating postmenopausal conditions is using ovariectomized (OVX) rats or mice[14,15]. Models like these simulate the decline of cancellous bone during postmenopause[16]. A biphasic loss of bone occurs

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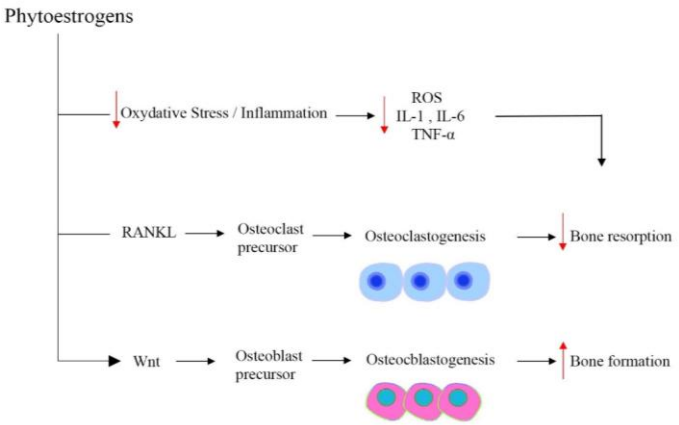

Figure 1. Phytoestrogens mechanism of action

following an ovariectomy. The initial loss of bone occurs up to 100 days after ovariectomy, followed by an intermediate period of relatively stable cancellous bone volume [17,18]. Today, the use of plants in veterinary medicine and medicine has very wide uses[19,20]. Phytoestrogens have attracted increased interest over the past decade as a class of bioactive compounds[21-23]. Phytoestrogens are molecules derived from plants chemically similar to estradiol, an endogenous estrogen in mammals[24]. These compounds exert various estrogenic and antiestrogenic effects when they bind to estrogen receptor [25]. Since phytoestrogens have structural similarities to estrogen, epidemiological studies, and clinical trials indicate that they protect against postmenopausal symptoms, cardiovascular disease, bone health problems, breast, prostate, and colon cancers, and postmenopausal syndrome[26,27] (Figure 1). The present review article aimed to discuss how phytoestrogen metabolites mimic mammalian estrogens and prevent bone loss after menopause by improving their actions.

2. Hormonal regulation of bone growth

Growth hormones, including thyroid hormones, testosterone, and estrogen, are commonly involved in the development of bones during the younger age period[28]. Sex hormones are involved in the closure of epiphyseal plates and the halting of longitudinal bone growth at puberty[29]. Thus, hormonal therapy influences bone growth. Several hormones regulate bone metabolism, including estrogen, progesterone, and androgen[30]. During reproduction, sex hormones maintain bone function and mineral homeostasis. Certain sexual steroids affect metabolism and bone health when lacking at certain levels. When estrogen levels are disturbed, it causes bone loss or osteoporosis in individuals with high estrogen levels[31]. Estrogen is a potent bone resorption inhibitor[32].

3. Isoflavones

Osteoblasts and osteoclasts are controlled by estrogen directly through Estrogen receptors alpha and beta and indirectly through parathyroid glands[33]. Furthermore, estrogen regulates inflammatory cytokines that are involved in bone remodeling[34,35]. Postmenopausal women and those with hypogonadism will likely get osteoporosis when estrogen levels are reduced[36]. Therefore, plant-derived phytoestrogens have been of considerable interest due to their similarity to mammalian estrogens[37]. There are four main types of phytoestrogens, namely isoflavones, lignans, coumestans, and diosgenin, all of which bind to estrogen receptors and exert either agonist or antagonist effects[38,39]. Soy is rich in isoflavones, which act as analogs of mammalian estrogens[40]. There are two dominant soy isoflavones found in plants: genistein and daidzein[41]. Mammals metabolize daidzein to produce equol and Odesmethylangolensin (o-DMA). Several studies have demonstrated that soy isoflavones can prevent bone loss postmenopausal in OVX rats[42-44]. (Figure 2)

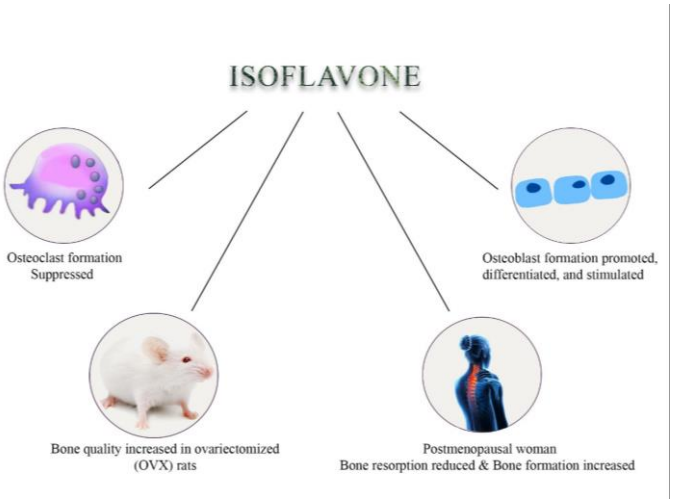

Figure 2. Isoflavone mechanism of action

4. Genistein

In addition to its anti-inflammatory properties, genistein has antiangiogenesis, antiproliferative, antioxidant, immunomodulatory, analgesic properties, and joint protection[45,46]. In addition, Genistein alleviates the symptoms of postmenopausal estrogen deficiency[47]. Wang et al. successfully synthesized compounds that release NO gradually over 5 hours in a biological environment such as blood or cell cultures[48]. It was found that it was possible to enhance osteoblast proliferation, differentiation, and mineralization with the prodrug, NO-donating genistein. Although the prodrug showed better potency than its parent drugs, genistein, glyceryl trinitrate, and their combination, it increased osteoblast formation, alkaline phosphatase activity, and osteocalcin secretion in osteoblast-like cells more efficiently. Genistein has antiosteoporotic activity in OVX rats[49]. A study found that Erythrina variegates (containing genistein derivatives) increased serum ALP levels, maintained serum calcium and phosphorus levels, decreased urinary excretion, and promoted bone growth[50]. A study by Hertrampf et al. compared an isoflavone-rich diet to a low-isoflavone diet enriched with genistein, and a subcutaneous injection of genistein in rats, finding that an isoflavone-rich diet

promoted bone formation and resisted bone loss[51]. In contrast, genistein was administered subcutaneously or dietary only to promote bone growth. After three months, all three diet groups showed increased trabecular bone marrow density (BMD). However, only the isoflavone-rich diet group had reduced serum collagen type I telopeptides and Pyridinoline cross-links. The most commonly prescribed drugs for osteoporosis are alendronate, raloxifene, and estradiol. It was shown that genistein increased BMD and bone mineral content (BMC) more than these conventional drugs in OVX rats[52]. The glucoside form of genistein, genistein aglycone, has also been shown to benefit women after menopause without causing any apparent side effects. In postmenopausal women, osteoporosis significantly increases BMD in the femoral neck and the lumbar spine. New bone formation markers, such as Bone-specific Alkaline Phosphatase, insulin-like growth factor 1, and osteoprotegerin, are increased by genistein aglycone, while thre is a decrease in bone resorption markers, such as PYR, Carboxy-terminal cross-linking telopeptide, and receptor activator of nuclear factor-κB[53].

5. Daidzein and equol

Natural phytoestrogen daidzein (4′, 7-dihydroxy isoflavone, C15H10O4) belongs to the family of diphenolic compounds and is structurally similar to synthetic and natural estrogen [54]. Soy products comprise daidzein, which can be converted into equol through metabolism[55]. It has also been reported that daidzein can reduce intraarticular adhesions around the knee after knee surgery [56]. There is a strong similarity between the chemical structure of daidzein and estrogen. Replacing or interfering with estrogen and the estrogen receptor (ER) works like estrogen[57]. Therefore, Daidzein effectively prevents estrogen-related diseases such as breast cancer, osteoporosis, and cardiovascular disease[58]. As well as being anti-inflammatory, anticancerous, and capable of protecting the skin and nerves from oxidative stress, daidzein positively affects non-estrogen-related diseases[59]. Scavenging oxygen-free radicals is one of the ways that daidzein regulates the immune system. According to a recent research study, daidzein increased body mass, increased trabecular bone density, and decreased bone turnover rate in severely andropause animals[60].

There are only a few metabolites of daidzein that exhibit the same pattern. Soy protein contains equol (C15H14O3), a daidzein metabolite[61]. Equol, an oestrogenic metabolite produced by soybeans, can biotransform into soy phytoestrogens to enhance their effects[62]. The anti-androgenic and antioxidant properties of equol make it especially effective at attaching to estrogen receptors[63,64]. Human intestinal flora metabolizes daidzein to equol in the body. By increasing bone mineral density, decreasing low-density lipoprotein, and improving endothelial dysfunction, this compound has anti-inflammatory and vasomotor effects [65]. Another study examined how daidzein combined with calcium preserved bone mass and biomechanical strength in OVX B6 mice[66]. In this study, daidzein produced equol in all mice and did not produce Uterotrophic effects. Estrogen deficiency increases bone turnover and accelerates bone loss, resulting in increased fracture risk[67]. In addition to being estrogenic, equol, an isoflavone metabolite of daidzein, was found to inhibit bone loss following ovariectomy[68]. O-desmethylangolensin (O-DMA) and equol are the most common metabolic products of daidzein in the animal gastrointestinal tract, and intestinal microflora variability may explain its opposite relationship[69]. In addition, equol administration maintained proximal, distal, and whole femur BMD, but not O-DMA administration[70]. Several studies have shown that resistant starch may increase urinary equol secretion, tibial bone mineral density, and the availability of daidzein in the body[71,72].

6. Challenges and future of osteoporosis treatment in postmenopausal women

Postmenopausal women who have lost bone mass are at risk of developing vertebral and non-vertebral fractures, which can have severe consequences[73]. Therefore, the use of bone replacement therapy is an essential part of treatment for osteoporosis. Hormone replacement therapy, particularly estrogen replacement therapy, is recommended as the primary treatment for menopausal women[74]. Progestins, progesterones, and testosterones are also available in various compositions for effective treatment. In addition, drugs such as alendronate, raloxifene, risedronate, 1-34 fragment of parathyroid hormone, and nasal calcitonin are commonly prescribed to help maintain adequate bone mass and prevent fractures[75]. It is essential to ensure that postmenopausal women receive an adequate supply of calcium and Vitamin D through a healthy diet.

Estrogen replacement therapy is particularly effective in preserving bone mass by inhibiting osteoclastic resorption. Progesterone, an anabolic hormone, promotes the production of osteoblasts, while estrogen and progesterone together reduce the risk of uterine cancers[76]. Replacing androgens with estrogen also increases bone mass. Despite their effectiveness, allopathic drugs may cause several side effects[77]. Therefore, natural treatments for postmenopausal osteoporosis are worth investigating.

Several natural phytoestrogen compounds with a chemical structure similar to 17 beta-estradiol, a steroid hormone, are found in plants[78,79]. Phytoestrogens are available from various sources, including supplements and soy products. In postmenopausal women, soybean isoflavones have a similar structure and function to 17-beta-estradiol. They can efficiently affect bone metabolism, bone turnover markers, and mechanical strength of bones by acting on osteoblasts and osteoclasts using genomic and non-genomic pathways[80,81]. Isoflavones could help treat osteoporosis in postmenopausal women by binding to estrogen receptors on the target cell surface[82,83]. Therefore,

incorporating soy products into the diet may be useful for postmenopausal women with osteoporosis.

In summary, treating osteoporosis in postmenopausal women is a complex process that requires a multifaceted approach. Hormone replacement therapy, bone replacement therapy, and dietary interventions can all play a vital role in preventing and treating osteoporosis. While allopathic drugs are effective, natural treatments, such as phytoestrogens in soy products, are worth investigating. These natural compounds have the potential to provide effective treatment with fewer side effects.

7. Conclusion

A vast array of ingredients, compounds, botanicals, and combinations have been shown to possess bone-preserving properties and prevent bone loss. These nature-derived compounds exhibit diverse therapeutic properties such as estrogen-like, antioxidant, anti-inflammatory, and immunomodulatory effects. Moreover, they have been shown to modulate crucial signaling pathways involved in osteoporosis pathogenesis. The use of nature-derived compounds in combination has garnered significant attention in recent years as they have been shown to offer greater efficacy while minimizing excessive toxicity compared to the use of individual compounds. The combination of these compounds as cocktails holds great promise for the treatment and management of osteoporosis.

Several studies have reported the beneficial effects of nature-derived compounds on bone health. For example, resveratrol, a polyphenol found in grapes, berries, and nuts, has improved bone microstructure and prevented bone loss by increasing osteoblast differentiation and reducing osteoclast formation. Similarly, curcumin, a compound found in turmeric, has been reported to inhibit bone loss and enhance bone mineral density by suppressing osteoclast activity and promoting osteoblast differentiation. Furthermore, botanicals such as black cohosh, and red clover have been shown to exhibit estrogen-like effects and modulate estrogen receptors, thereby reducing bone loss and preventing osteoporosis in menopausal women. These botanicals, in combination with other nature-derived compounds, have been reported to provide synergistic effects and offer improved bone-preserving properties. In conclusion, nature-derived compounds possess diverse therapeutic properties and hold great promise for the prevention and management of osteoporosis. The combination of these compounds as cocktails offers an effective and safe approach to the treatment of osteoporosis. Further research is necessary to investigate the efficacy and safety of these natural compounds and their combinations for the treatment and management of osteoporosis.

Declarations

Competing interests

The authors have declared no conflicts of interest.

Authors' contributions

Hossein Kazemi Mehrjerdi conceptualized the work, while all authors were responsible for the methodology employed in the study. The formal analysis and investigation of the research was also carried out by all authors. The writing process involved both the original draft preparation and subsequent review and editing, which were also undertaken by all authors. Finally, Hassan Borji provided supervision for the project. The manuscript was thoroughly reviewed and approved by all authors before submission for publication in the present journal.

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Ethical considerations

Authors declare that this manuscript is original and has not been submitted elsewhere for possible publication. The authors also declare that the data used/presented in this manuscript has not been fabricated.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

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