

## ROLE OF PHYTOESTROGENS IN PREVENTION OF OSTEOPOROSIS

STEFKA PANKOVA<sup>1</sup>, DOBRINA TSVETKOVA<sup>2</sup>

<sup>1</sup>Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy Medical University – Plovdiv, Bulgaria, <sup>2</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy Medical University – Sofia, Bulgaria  
Email: dobrinka30@mail.bg

Received: 04 Feb 2015, Revised and Accepted: 28 Feb 2015

## ABSTRACT

Osteoporosis is a disease characterized with the disbalance between the bone-building activity of osteoblasts and the reabsorptive activity of osteoclasts. Estrogen deficiency occurring after menopause leads to enhanced risk of sudden fractures due to reduced bone mass.

Phytoestrogens are polyphenolic plant metabolites: isoflavonoids, flavonoids (flavones, flavanones, chalcones), lignans, stilbenes. Phytoestrogens possess estrogenic activity and display the higher affinity for  $\beta$ -estrogen receptor due to their structural similarity to  $17\beta$ -Estradiol. It is reported that bone loss is suppressed by Genistein and Daidzein from soy products, Quercetin and Rutin, Resveratrol from grapes and red wine, Kaempferol and Apigenin, Hesperidin, (+)-Catechin and Epigallocatechin gallate from green tea and Oleuropein.

The most effective isoflavone in preserving bone health appears to be Genistein, which stimulates osteoblast and inhibits osteoclast function. Osteo protective effect of Formononetin on skeletal system results from the inhibition of osteoclastogenesis. Effects of Coumestrol on osteoblasts and osteoclasts are in increasing the bone density and preventing of bone resorption.

Bioactive flavonoids enhance bone formation and inhibit bone resorption through their action on cell signaling pathways that influence osteoblast and osteoclast differentiation. 8-Prenylnaringenin promotes osteoblastic differentiation and inhibits bone resorption activity of osteoclasts by inducing of their apoptosis.

Plant lignans have been shown to provide estrogenic support, antioxidant and anti-inflammatory activity. Stilbene antioxidant Resveratrol exerts potential to attenuate bone loss due to multiple actions on both osteoblasts and osteoclasts. Monoterpene components from essential oils protect from bone loss due to inhibitory of the osteoclast activity.

**Keywords:** Phytoestrogens, Osteoporosis, Bone, Prevention.

## INTRODUCTION

Osteoporosis is a disease characterized by an enhanced risk of sudden fractures due to reduced bone mass, structural deterioration of bone tissue and weakness of skeletal strength. The disbalance between the bone-building activity of osteoblasts and the reabsorptive activity of osteoclasts causes osteoporosis, resulting in increased bone loss and decreased bone replacement.

Some people are more susceptible to osteoporosis than others. Risk factors for disease are controllable and uncontrollable. Uncontrollable risk factors include: age, gender, genetic factors, race, diseases, medications, oxidative stress, family history, insufficient bone growth, history of broken bone, body type [1].

Bone resorption is a natural phenomenon and can occur due to old age [2]. Osteoporosis can affect both men and women at any age, but it becomes more common after age 50. Gender is the most dominant risk factor. Osteoporosis is more prevalent in women: the disease affects women four-fold compared to men. 50 % of women and 25 % of men, over the age of 50 have an osteoporosis-related fracture [3].

The primary cause of osteoporosis in women is diminished estrogen levels, which in postmenopausal women are about one-tenth of the levels in premenopausal women. Estrone stimulates the development of the osteoblasts. Lower levels of Estrogen in menopause decrease the ability of absorption and assimilation of calcium in bones and lead osteoclasts to live longer than osteoblasts. Testosterone is the hormone responsible for bone strength and breakdown in men. Osteoporosis affects all races, but european and asian races are more likely to develop the disease than those of african heritage [3].

Diseases which are risk factor for osteoporosis are: AIDS, anorexia, asthma, blood, bone and eating disorders, Cushing syndrome (increased production of cortisol from adrenal glands), colitis, diabetes mellitus, emphysema, gastrectomy, gastrointestinal bypass

procedures, hyperparathyroidism, hyperthyroidism, inflammatory bowel disease, kidney and liver failure, Lupus, lymphoma and leukemia, malabsorption syndromes, multiple sclerosis, organ transplants, Parkinson's disease, premature menopause, prostate cancer, rheumatoid arthritis, spinal cord injuries, stroke, thalassemia, weight loss [3]. Medications that can cause osteoporosis by reducing the bone ability to rebuild themselves are Cortisone, Dexamethasone, Prednisone, Prednisolone, excess thyroid hormone, diuretics, heparin, anticonvulsants, tranquilizers, sedatives, selective serotonin reuptake inhibitors [4].

The controllable risk factors for osteoporosis are: deficiency of calcium, magnesium, vitamins B<sub>12</sub>, D, K, high intake of alcohol, Caffeine, cigarette, sodium, environmental pollutants, heavy metals (especially cadmium). The chemicals in cigarettes and alcohol intake can reduce bone formation [3]. Foods that contain salt [5], Caffeine [6] and soda [7] promote calcium loss. High Caffeine intake is associated with increased bone loss and osteoporotic fractures due to promoting the excretion of calcium in the urine [6]. Soda consumption increases parathyroid hormone in the blood, which lead to high excretion in the urine of increased blood calcium concentrations by stimulating bone breakdown [7].

The standard treatment for osteoporosis is with drugs which reduce the risk of fractures, by slowing bone loss: 1) bisphosphonates: Clodronate, Fosamax, Minodronate, Risedronate (Actonel), Zoledronic acid (Reclast); 2) Calcitonin-works by inhibiting the work of osteoclasts; 3) estrogen replacement therapy; 4) selective estrogen receptor modulators: Raloxifene (Evista) [8]. Bisphosphonates cause serious health risks effects: cancer of oesophagus, stomach and colorectum [9]. Hormone replacement therapy for reducing menopausal symptoms, including osteoporosis, has also been proven to have negative side effects: breast cancer, abnormal uterus bleedings or cardiovascular diseases in postmenopausal women [10].

Very important for prevention of osteoporosis loss in both men and women is avoiding of sodium, alcohol, Caffeine and smoking and using diets rich in the following foods and nutrients containing minerals and vitamins: calcium (broccoli, cheese, milk, orange juice, sesame seeds); magnesium (avocado, banana, milk, nectarine, nuts, orange juice, potato, spinach); potassium (nuts, potato, spinach); phosphorous (beans, nuts, sesame seeds, sunflower seeds) [11], boron (almond, apricots, avocado, hazelnuts); copper (avocado, apricots, goat cheese, hazelnuts); manganese (beans, hazelnuts, spinach); strontium (beans, carrots, potatoes, spinach); zinc (beans, cocoa, chocolate, spinach, sunflower seeds); Vitamins C (broccoli, blackberries, grapefruit, kiwi, lemon, mango, peppers, pineapple, spinach, tomatoes); Vitamin B<sub>6</sub> (apricots, avocado, banana, sesame seeds, spinach); Vitamin K (broccoli, blueberries, cabbage, pears, peaches spinach); beta carotene (broccoli, carrots, pepper, peaches) [12].

Magnesium promotes calcium transport and absorption in a ratio as close as possible to 1:2. Zinc stimulates bone formation and inhibits bone loss. Strontium contributes to bone health and strength by supporting the differentiation of osteoblasts and helping for a balance with the activity of osteoclasts and osteoblasts. Strontium ranelate increases bone formation by promoting the growth of osteoblasts. Vitamin D<sub>3</sub> is essential for good absorption of calcium and its resorption by bones. Vitamin K prevents bone loss caused by Estrogen deficiency and with interaction with Vitamin D can increase the formation of new bone. Vitamin C may limit bone loss in early years of menopause. Carotenoids protect bone mineral density

in older men and women. Omega-3 fatty acids found in fish oil increase bone mass, improve bone strength and enhance bone growth and diminish the amount of calcium loss in urine [12].

Plant polyphenols present in the human diet are of great interest as they possess potential antioxidant activity and anti-osteoporosis properties in their function as free radical scavengers. Food supplements and bone health-promoting effects of flavonoids [13], isoflavones, polyphenols and additional phytochemicals are now being investigated [14].

Phytoestrogens are polyphenolic, nonsteroidal secondary plant metabolites which can be divided into the following classes: isoflavonoids, flavonoids (flavones, flavanones, chalcones), lignans, stilbenes (Resveratrol) [11].

Isoflavonoids are isoflavones (Daidzein, Genistein, Glycitein) [15] (fig. 1) and coumestans (coumestrol, 4'-O-methylcoumestrol) (fig. 2.). Isoflavones Daidzein, Genistein and Glycitein occur in plants as their inactive glycozides (Dadzin, Glycitin, Genistin) [15] (fig. 3.) and as their respective 4-methyl ether derivatives Formononetin and Biochanin A (fig. 1) [16].

It is reported that bone loss is suppressed by Genistein and Daidzein from soy products [17], Quercetin [18] and Rutin [19], Resveratrol from grapes and red wine [20], Kaempferol and Apigenin, Hesperidin, (+)-Catechin and Epigallocatechin gallate from green tea and Oleuropein [21].

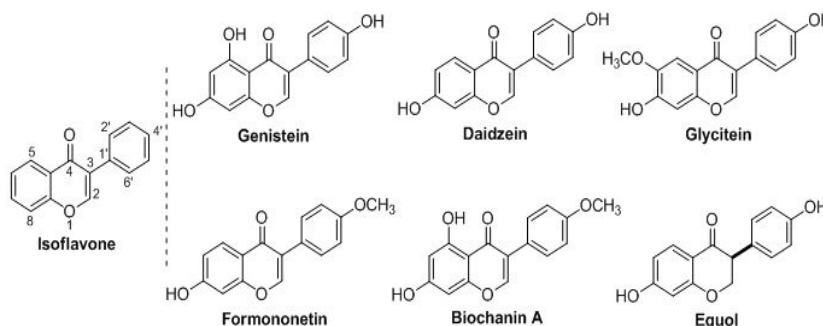


Fig. 1: Structures of Dadzin, Glycitin and Genistin

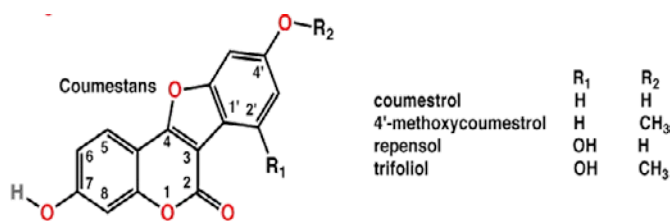


Fig. 2: Structures of coumestans

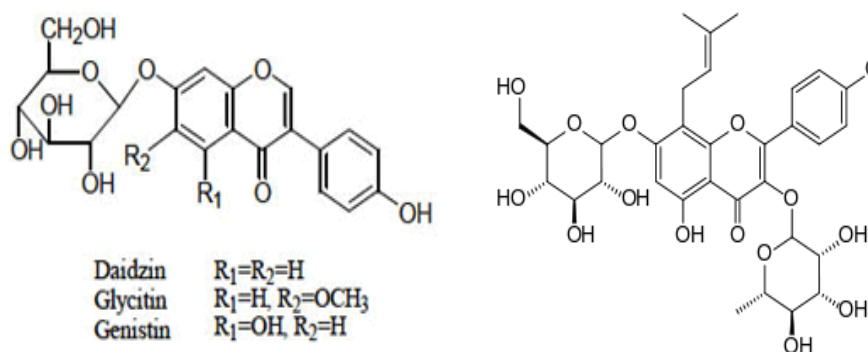


Fig. 3: Structures of Dadzin, Glycitin, Genistin and Icarin

It is known that bone always undergoing two opposite processes: break down and formation. Estrogens are signaling molecules that exert their effects by binding to estrogen receptors within cells. The estrogen-receptor complex interacts with DNA to change the expression of estrogen-responsive genes. Estrogen receptors are present in numerous tissues associated with reproduction, including bone, liver, heart and brain. Estrogen acts on the first of these processes by inhibiting bone breakdown. Estrogen suppresses bone resorption by down regulating the expression of RANK ligand (RANKL) and up regulating the Osteoprotegerin in osteoblasts and bone marrow stromal cells [22]. Estrogen decreases the expression of interleukin 1, interleukin 6 and  $\beta$ -tumor necrosis factor from bone cells and osteoblast, which play a crucial role in osteoclast stimulation and bone resorption [23].

Phytoestrogens exert their effects through binding to estrogen receptors  $\alpha$  and  $\beta$ . Many phytoestrogens display the higher affinity for  $\beta$ -estrogen receptor. Due to their structural similarity to  $17\beta$ -Estradiol, phytoestrogens possess estrogenic activity and are selective estrogen receptor modulators. They may bind to estrogen receptors and mimic the effect of Estradiol, but their activity is  $10^{-2}$ – $10^{-3}$ -fold lower [24].

It is founded that the important structural elements that enable phytoestrogens to bind with high affinity to estrogen receptors are: the ring of isoflavones mimicking a ring of estrogens at the receptors binding site; distance between two hydroxyl groups at the isoflavones nucleus similar to that occurring in Estradiol; low molecular weight similar to estrogens site [25].

Phytoestrogens modulate the concentration of endogenous estrogens by binding some enzymes and by affecting the bioavailability of sex hormones by depressing or stimulating the synthesis of sex hormone-binding globulin [24].

It is reported that the metabolic balance of bone formation and resorption can be restored by high isoflavone intake. Isoflavones not only suppress bone breakdown but at the same time enhance the new bone formation [17].

Isoflavones exhibit weak estrogenic activity and affect the balance of reproductive hormones in women before and after menopause. Isoflavones significantly increase bone mineral content and reduce the number of osteoclasts [26] leading to restoring the balance of bone formation and resorption [27].

Phytoestrogen isoflavones are found in soybeans [28], *Equisetum arvense L.*, *Trifolium pratense L.* [29]. Soy is one of the best-known sources of phytoestrogens [30], providing the isoflavones Genistein, Daidzein and Glycitein, their glycosides (Genistin, Daidzin and Glycitin). Glycosides are converted by intestinal bacteria in biologically active isoflavones Genistein and Daidzein [31].

The most effective isoflavone in preserving bone health appears to be Genistein, which stimulates osteoblast and inhibits osteoclast function through inducing osteoclastogenic inhibitor Osteoprotegerin. Genistein reacts with  $\beta$ -estrogen receptors, possess antiplatelet effect and increases the activity of an antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase [27].

Genistein exhibit more potent estrogenic effects than other substances from this group [32]. Genistein decreases osteoclast formation and bone resorption through inducing osteoclastogenic inhibitor Osteoprotegerin and blocking nuclear factor  $\kappa$ B signaling [33].

It is reported that in postmenopausal women after 2 years 54 mg Genistein plus calcium and Vitamin D improve bone density to a greater extent than calcium and Vitamin D alone [17].

Icariin is a flavonol glycoside-the 8-prenyl derivative of kaempferol 3, 7-O-diglucoside. Genistein and Icariin inhibit bone resorption activity of osteoclasts and stimulate osteogenic differentiation and of bone marrow stromal progenitor cells and osteoblasts. Icariin is more potent than Genistein in promoting osteogenic differentiation of osteoblasts due to the existence of a prenyl group on C<sub>8</sub> [33].

Icariin enhances bone healing and reduces osteoporosis occurrence because possesses a bone anabolic effect by induction of bone morphogenetic protein-2 and stimulation of the osteoblasts proliferation and differentiation, resulting in bone formation [34].

Comparative effects of Genistein, Icariin and Hispidulin prove that they possess estrogen-like and antiosteoporotic activity and can be potentially used for the treatment of osteoporosis. Results showed that in comparison with Icariin and Hispidulin, Genistein significantly contributes to increasing of bone density and possesses superior bone protective effect [35].

Isoflavone supplement made from *Trifolium pratense L.* (containing 6 mg Biochanin A, 16 mg Formononetin, 1 mg Genistein and 0.5 mg Daidzein) inhibits bone loss [36].

Glycitein is an O-methylated isoflavone which accounts for 5-10% of the total isoflavones in soy food products. Glycitein is a phytoestrogen with weak estrogenic activity, comparable to that of the other soy isoflavones [16].

The differentiation of osteoblasts requires a osteogenesis-related factors, including alkaline phosphatase, osteocalcin, Osteoprotegerin and runt-related transcription factor 2. It is reported that medullary adipocytes are secretory cells from the bone marrow stroma that may influence osteogenesis by impairing osteoblast proliferation, differentiation and mineralization and by promoting osteoclast formation and activation. Biochanin A induces preosteoblasts to differentiate into osteoblasts, increases osteoblast mineralization and effectively prevents the increase of bone loss. Biochanin A promotes osteogenesis, induces preosteoblasts to differentiate into osteoblasts, increases osteoblast mineralization, enhances osteoblast number, inhibits adipogenesis by decreasing of adipocyte numbers. Due to its structure similarity to Estradiol, Biochanin A may act as potential replacement for estrogen deficiency and may be useful in prevention and treatment of postmenopausal osteoporosis. Biochanin A is preferentially bound to  $\beta$ -estrogen receptor rather than  $\alpha$ -estrogen receptor [37].

The greatest sources of a naturally occurring isoflavone Formononetin (7-hydroxy-3-(4-methoxyphenyl)chromone) are roots of *Astragalus membranaceus L.* and *Astragalus mongholicus L.*, leaves and flowers of *Trifolium pratense L.*, roots of *Glycyrrhiza glabra L.*, *Glycyrrhiza uralensis L.* and *Pueraria lobata L.* This isoflavonoid could also occur in dietary products like beans, carrot, cauliflower, green peas, red potatoes. Formononetin has structural similarity to  $17\beta$ -Estradiol and can bind to estrogen receptors in bone tissue and can mimic Estradiol's effect. Osteoprotective effect of Formononetin on skeletal system result from the inhibition of osteoclastogenesis and bone turnover rate, which is increased under estrogen deficiency [38].

Free radicals are responsible for osteoblasts apoptosis induction and inhibition of osteoblastogenesis and for activation of osteoclasts differentiation. For this reason, administration of antioxidants may have the beneficial effect on the bone tissue altered by osteoporosis. Beneficial effect of Formononetin result from its antioxidant effect due to increasing of activity of superoxide dismutase, catalase and glutathione peroxidase [39].

Effects of Coumestrol on osteoblasts and osteoclasts are in increasing bone density and preventing bone resorption. Treatment with Coumestrol increases alkaline phosphatase, type I collagen and osteocalcin activity. These results demonstrate that phytoestrogen Coumestrol has a direct enhancing effect on the proliferation and osteogenic differentiation of bone marrow stromal cells, which would lead to stimulation of bone formation [40].

Flavonoids from fruits and vegetables, herbs and spices, essential oils and beverages have the most potential of dietary components for promotion of bone health. Bioactive flavonoids enhance bone formation and inhibit bone resorption through their action on cell signaling pathways that influence osteoblast and osteoclast differentiation [13].

Equol (7-hydroxy-3-(4-hydroxyphenyl)-chroman) is an isoflavon and is a metabolite of the soy isoflavone Daidzein in humans and

animals. Equol is produced by intestinal bacteria and has a stronger affinity for the  $\beta$ -estrogen receptor [41]. It is reported that receptor activator of nuclear factor  $\kappa$ B ligand and reactive oxygen species stimulate expression of tumor necrosis factor- $\alpha$  in osteoclast precursor cells, which lead to increased bone resorption. Rutin, a glycoside of flavonol, inhibits osteoclast formation by reducing of reactive oxygen species and by decreasing of levels of tumor-necrosis factor- $\alpha$  [19].

Monomer flavanols catechin, epicatechin, gallic acid and epigallocatechin gallate from dried leaves of *Camellia sinensis* L. increase the values of serum bone specific alkaline phosphatase (bone formation biomarker) [42]. Grape seed polymer flavanols proanthocyanidins in skeletal protection increase bone formation and bone strength [11].

Citrus flavanone Hesperidin protects against the development of osteoporosis in both women and men by slowing down the bone resorption and by modulating the production of inflammatory products [21]. Olive polyphenols Oleuropein, Oleocanin, Tyrosol and Hydroxytyrosol both stimulates osteoblasts and inhibits the formation of osteoclasts [43].

Dried fruits of *Prunus domestica* L. contain several phenolic compounds: chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid and protect against the deterioration of bone structure by exerting positive effects on bone mass, bone microarchitecture and bone strength. [44].

The found in *Humulus lupulus* L. [45] 8-Prenylnaringenin (fig. 4.) is a prenylflavonoid and is the most phytoestrogen important for treatment of menopause [46] and osteoporosis [47].

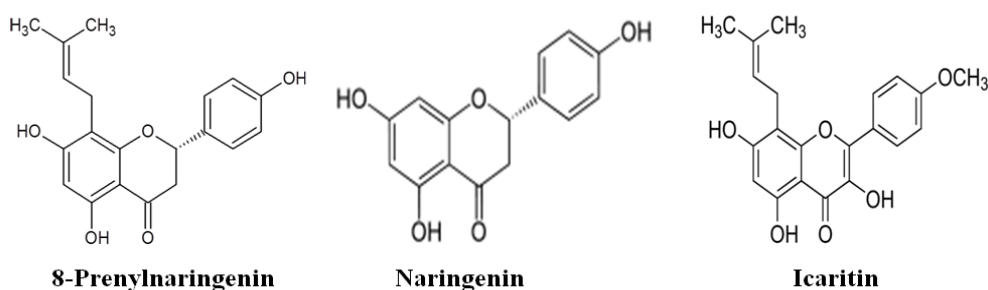


Fig. 4: Structure of 8-Prenylnaringenin, Naringenin and Icaritin

8-Prenylnaringenin is a phytoestrogen with estrogenic activity similar to Estradiol, but with weaker activity [48]. It promotes osteoblastic differentiation, inhibits osteoclast differentiation and also indirectly controls osteoclasts by regulating the expression and secretion of Osteoprotegerin [49]. 8-Prenylnaringenin inhibits bone resorption activity of osteoclasts by inducing osteoclast apoptosis [50].

These effects of 8-Prenylnaringenin are mediated by  $\alpha$  estrogenic receptor and were stronger than those of Genistein and Daidzein [49]. 8-Prenylnaringenin is much more active than Naringenin in inhibiting the resorption of osteoclasts and inducing apoptosis of

osteoclasts. Their only difference lies in 8-prenyl group, which is proved to be able to enhance the anti-bone resorption activity of 8-Prenylnaringenin [51]. 8-Prenylflavonoids have a higher osteogenic activity than do flavonoids, which suggested that the 8-prenyl group may plays an important role and contributes to the higher bone-protective activity [52].

Other prenylflavonoids is Icaritin and exerts anti-resorptive effect on osteoporotic bone and significantly increases the number of osteoblasts [53]. Lignan precursors are Lariciresinol, Matairesinol, Pinoresinol and Secoisolariciresinol (fig. 5).

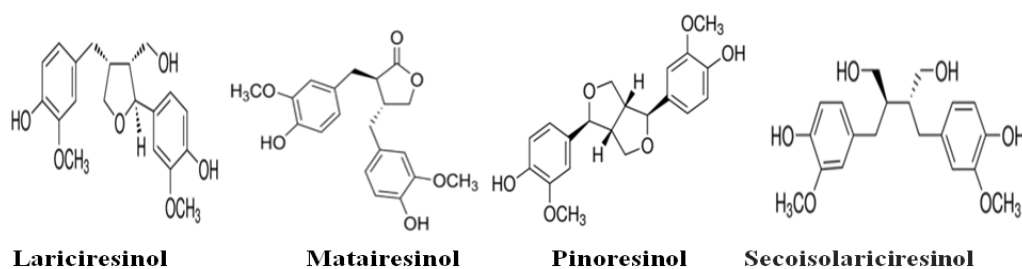


Fig. 5: Structures of Lariciresinol, Matairesinol, Pinoresinol and Secoisolariciresinol

Secoisolariciresinol and Matairesinol are among the first lignan precursors identified in the human diet. Lariciresinol and Pinoresinol contributed about 75% to the total lignan intake, while Secoisolariciresinol and Matairesinol contributed only about 25%. Lignan precursors are found in a wide variety of foods, including seeds (flax, pumpkin, sunflower, poppy, and sesame), whole grains (rye, oats, and barley), bran (wheat, oat, rye), beans, fruits (berries) and vegetables [54].

The enterolignans are formed by the action of intestinal bacteria on lignan precursors found in plants: Matairesinol is converted to Enterolactone and from Secoisolariciresinol is obtained Enterodiol (fig. 6) [16].

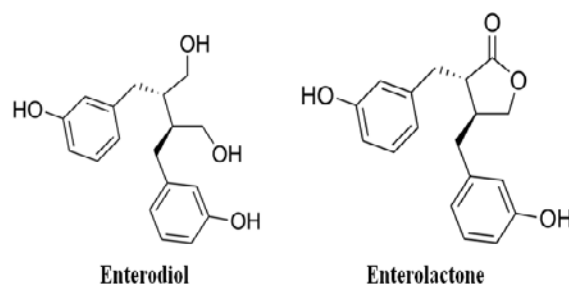


Fig. 6: Structures of Enterodiol and Enterolactone

Enterodiol can also be converted to Enterolactone by intestinal bacteria. Enterodiol and Enterolactone have weak estrogenic activity. Plant lignans have been shown to provide estrogenic support and antioxidant and anti-inflammatory activity [54]. Isotaxiresinol the main lignan isolated from *Taxus yunnanensis* L. Isotaxiresinol slightly increases bone formation and significantly inhibites bone resorption, which suggest that can be useful for treatment of postmenopausal osteoporosis, especially for prevention of bone fracture induced by estrogen deficiency [55].

Stilbene antioxidant Resveratrol (3, 5, 4'-trihydroxylstilbene) is a natural compound found in grapes, peanuts and wine. Resveratrol binds to estrogen receptors- $\alpha$  and  $\beta$  and exerts potential to attenuate bone loss due to multiple actions on both osteoblasts and osteoclasts [20].

Essential oils from thyme, sage, rosemary, juniper, pine, dwarf pine, turpentine, and eucalyptus) and their monoterpene components (thujone, eucalyptol, camphor, borneol, thymol, alpha-pinene, beta-pinene, bornylacetate, menthol) inhibit bone resorption. Pine oil protects from bone loss. The monoterpenes borneol, thymol and camphor are directly inhibitory in the osteoclast resorption. Verbenol, a metabolite of alpha-pinene inhibits osteoclast activity [56].

## CONCLUSION

Important strategy for the treatment of osteoporosis can be inhibition of the differentiation of marrow adipocytes and increasing of osteogenesis. Phytoestrogens like: isoflavonoids, flavonoids (flavones, flavanones, chalcones), lignans and stilbenes may act as potential replacement for estrogen deficiency and may be useful in prevention and treatment of postmenopausal osteoporosis.

## CONFLICT OF INTERESTS

Declared None

## REFERENCES

- Sanchez-Rodriguez MA, Ruiz-Ramos M, Correa-Munoz E, Mendoza-Nunez VM. Oxidative stress as a risk factor for osteoporosis in elderly Mexicans as characterized by antioxidant enzymes. *BMC Musculoskeletal Disord* 2007;8(124):1-7.
- Mao T, Kamakshi V. Bone grafts and bone substitutes. *Int J Pharm Pharm Sci* 2014;6(Suppl 2):88-91.
- Cummings-Vaughn L, Grammack J. Falls, osteoporosis and hip fractures. *Med Clin North Amer* 2011;95(3):495-506.
- Mazziotti G, Canalis E, Giustina A. Drug-induced osteoporosis: mechanisms and clinical implications. *Am J Med* 2010;123(10):877-84.
- Teucher B, Dainty JR, Spinks CA, Majsak-Newman G, Berry DJ, Hoogwerff JA, et al. Sodium and bone health: impact of moderately high and low salt intakes on calcium metabolism in postmenopausal women. *J Bone Miner Res* 2008;23(9):1477-85.
- Hallstrom H, Wolk A, Glynn A, Michaëlsson K. Coffee, tea and caffeine consumption in relation to osteoporotic fracture risk in a cohort of Swedish women. *Osteoporos Int* 2006;17(7):1055-64.
- Mahmood M, Saleh A, Al-Alawi F, Ahmed F. Health effects of soda drinking in adolescent girls in the United Arab Emirates. *J Crit Care* 2008;23(3):434-40.
- Khosla S, Bilezikian JP, Dempster DW, Lewiecki EM, Miller PD, Neer RM, et al. Benefits and risks of bisphosphonate therapy for osteoporosis. *J Clin Endocrinol Metab* 2012;97(7):2272-82.
- Green J, Czanner G, Reeves G, Watson J, Wise L, Beral V. Oral bisphosphonates and risk of cancer of oesophagus, stomach, and colorectum: nested case-control study. *BMJ* 2010;341(1):4444.
- Bowring CE, Francis RM. National osteoporosis society's position statement on hormone replacement therapy in the prevention and treatment of osteoporosis. *Menopause Int* 2011;17(2):63-5.
- Sacco SM, Horcajada MN, Offord E. Phytonutrients for bone health during ageing. *Br J Clin Pharmacol* 2013;75(3):697-707.
- Price CT, Langford JR, Liporace FA. Essential nutrients for bone health and a review of their availability in the average north american diet. *Open Orthop J* 2012;6(1):143-9.
- Weaver CM, Alekel DL, Ward WE, Ronis MJ. Flavonoid intake and bone health. *J Nutr Gerontol Geriatr* 2012;31(3):239-53.
- Putnam SE, Scutt AM, Bicknell K, Priestley CM, Williamson EM. Natural products as alternative treatments for metabolic bone disorders and for maintenance of bone health. *Phytother Res* 2007;21(2):99-112.
- Kalita JC, Milligan SR. *In vitro* estrogenic potency of phytoestrogen-glycosides and some plant flavanoids. *Ind J Sci Technol* 2010;3(12):1142-7.
- Cos P, De Bruyne T, Apers S, Berghe DV, Pieters L, Vlietinck AJ. Phytoestrogens: recent developments. *Planta Med* 2003;69(1):589-99.
- Marini H, Minutoli L, Polito F, Bitto A, Altavilla D, Atteritano M, et al. Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women. *Ann Intern Med* 2007;146(1):839-47.
- Nathiya S, Durga M, Devasena T. Quercetin, encapsulated Quercetin and its application-a review. *Int J Pharm Pharm Sci* 2014;6(10):20-6.
- Kyung TW, Lee JE, Shin HH, Choi HS. Rutin inhibits osteoclast formation by decreasing reactive oxygen species and TNF- $\alpha$  by inhibiting activation of NF- $\kappa$ B. *Exp Mol Med* 2008;40(1):52-8.
- Ornstrup MJ, Harslof T, Kjaer TN, Langdahl BL, Pedersen SB. Resveratrol increases bone mineral density and bone alkaline phosphatase in obese men: a randomized placebo-controlled trial. *J Clin Endocrinol Metab* 2014;99(12):4720-9.
- Habauzit V, Horcajada MN. Phenolic phytochemicals and bone. *Phytochem Rev* 2008;7(2):313-44.
- Riggs BL. The mechanisms of estrogen regulation of bone resorption. *J Clin Invest* 2000;106(10):1203-4.
- Krassas GE, Papadopoulou P. Oestrogen action on bone cells. *J Musculoskeletal Neuronal Interact* 2001;2(2):143-51.
- Cederroth CR, Nef S. Soy, phytoestrogens and metabolism: a review. *Mol Cell Endocrinol* 2009;304(1-2):30-42.
- Song L, Liang X, Zhou Y. Estrogen-mimicking isoflavone genistein prevents bone loss in a rat model of obstructive sleep apnea-hypopnea syndrome. *Int J Clin Exp Pathol* 2014;7(4):1687-94.
- Ward HA, Vogiatzoglou A, Luben RN, Mulligan A, Wareham NJ, Forouhi NG, et al. Association between dietary phytoestrogens and bone density in men and postmenopausal women. *Br J Nutr* 2011;106(7):1063-9.
- Bitto A, Polito F, Squadrito F, Marini H, D'Anna R, Irrera N, et al. Genistein aglycone: a dual mode of action anti-osteoporotic soy isoflavone rebalancing bone turnover towards bone formation. *Curr Med Chem* 2010;17(27):3007-18.
- Poulsen RC, Kruger MC. Soy phytoestrogens: impact on postmenopausal bone loss and mechanisms of action. *Nutr Rev* 2008;66(7):359-74.
- Occhiuto F, Pasquale RD, Guglielmo G, Palumbo DR, Zangla G, Samperi S, et al. Effects of phytoestrogenic isoflavones from red clover (*Trifolium pratense* L.) on experimental osteoporosis. *Phytother Res* 2007;21(2):130-4.
- Kenny AM, Mangano KM, Abourizk RH, Bruno RS, Anamani DE, Kleppinger A, et al. Soy proteins and isoflavones affect bone mineral density in older women: a randomized controlled trial. *Am J Clin Nutr* 2009;90(1):234-42.
- Ishimi Y. Soybean isoflavones in bone health. *Forum Nutr* 2009;61(1):104-16.
- Sehmisch S, Erren M, Kolios L, Tezval M, Seidlova-Wuttke D, Wuttke W, et al. Effects of isoflavones Equol and Genistein on bone quality in a rat osteopenia model. *Phytother Res* 2010;(24, Suppl 2):S168-S74.
- Ming LG, Chen KM, Xian CJ. Functions and action mechanisms of flavonoids Genistein and Icarin in regulating bone remodeling. *J Cell Physiol* 2013;228(3):513-21.
- Hsieh TP, Sheu SY, Sun JS, Chen MH, Liu MH. Icarin isolated from *Epimedium pubescens* regulates osteoblasts anabolism through BMP-2, SMAD4, and Cbfa1 expression. *Phytomed* 2010;17(6):414-23.
- Yang L, Yu Z, Qu H, Li M. Comparative effects of Hispidulin, Genistein, and Icarin with effects of Hispidulin, Genistein, and Icarin with estrogen on bone tissue in ovariectomized rats. *Cell Biochem Biophys* 2014;70(1):485-90.

36. Atkinson C, Compston JE, Day NE, Dowsett M, Bingham SA. The effects of phytoestrogen isoflavones on bone density in women: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr* 2004;79(2):326-33.
37. Su SJ, Yeh YT, Su SH, Chang KL, Shyu HW, Chen KM, *et al.* Biochanin A promotes osteogenic but inhibits adipogenic differentiation: evidence with primary adipose-derived stem cells. *Evidence-Based Complementary Altern Med* 2013;2013(1):1-12.
38. Kuhnle GGC, Dell'Aquila C, Runswick SA, Bingham SA. Variability of phytoestrogen content in foods from different sources. *Food Chem* 2009;113(4):1184-7.
39. Mu H, Bai YH, Wang ST, Zhu ZM, Zhang YW. Research on antioxidant effects and estrogenic effect of Formononetin from *Trifolium pratense* (red clover). *Phytomed* 2009;16(4):314-9.
40. Wu XT, Wang B, Wei JN. Coumestrol promotes proliferation and osteoblastic differentiation in rat bone marrow stromal cells. *J Biomed Mater Res B Appl Biomater* 2009;90(2):621-8.
41. Tousen Y, Ezaki J, Fujii Y, Ueno T, Nishimuta M, Ishimi Y. Natural S-Equol decreases bone resorption in postmenopausal, non-equol-producing Japanese women: a pilot randomized, placebo-controlled trial. *Menopause* 2011;18(5):563-74.
42. Shen CL, Chyu MC, Yeh JK, Zhang Y, Pence BC, Felton CK, *et al.* Effect of green tea and Tai Chi on bone health in postmenopausal osteopenic women: a 6-month randomized placebo-controlled trial. *Osteoporosis Int* 2012;23(5):1541-52.
43. Puel C, Mathey J, Agalias A, Kati-Coulibaly S, Mardon J, Obléd C, *et al.* Dose-response study of effect of Oleuropein, an olive oil polyphenol, in an ovariectomy/inflammation experimental model of bone loss in the rat. *Clin Nutr* 2006;25(5):859-68.
44. Hooshmand S, Chai SC, Saadat RL, Payton ME, Brummel-Smith K, Arjmandi BH. Comparative effects of dried plum and dried apple on bone in postmenopausal women. *Br J Nutr* 2011;106(6):923-30.
45. Possemiers S, Bolca S, Grootaert C, Heyerick A, Decroos K, Dhooze W, *et al.* The prenylflavonoid isoxanthohumol from hops (*Humulus lupulus* L.) is activated into the potent phytoestrogen 8-prenylnaringenin *in vitro* and in the human intestine. *J Nutr* 2006;136(7):1862-7.
46. Keiler AM, Zierau O, Kretzschmar G. Hop extracts and hop substances in treatment of menopausal complaints. *Planta Med* 2013;79(7):576-9.
47. Asthana S, Agarwa T, Khursheed A, Dutta D. Molecular modeling and QSAR analysis to explore therapeutic potentials of phytoestrogens in osteoporosis. *Int J Pharm Pharm Sci* 2014;6(3):239-43.
48. Hajirahimkhan A, Dietz BM, Bolton JL. Botanical modulation of menopausal symptoms: mechanisms of action? *Planta Med* 2013;79(7):538-53.
49. Luo D, Kang L, Ma Y, Chen H, Kuang H, Huang Q, *et al.* Effects and mechanisms of 8-Prenylnaringenin on osteoblast MC3T3-E1 and osteoclast-like cells RAW264.7. *Food Sci Nutr* 2014;2(4):341-50.
50. Lü X, Zhou Y, Chen KM, Zhao Z, Zhou J, Ma XN. Inhibitory effect of 8-Prenylnaringenin on osteoclastogenesis of bone marrow cells and bone resorption activity. *Yao Xue Xue Bao* 2013;48(3):347-51.
51. Lv X, Chen KM, Ge BF, Ma HP, Song P, Cheng K. Comparative study on effect of 8-Prenylnaringenin and Narigenin on activity of osteoclasts cultured *in vitro*. *Zhongguo Zhong Yao Za Zhi* 2013;38(12):1992-6.
52. Ming LG, Lv X, Ma XN, Ge BF, Zhen P, Song P, *et al.* The prenyl group contributes to activities of phytoestrogen 8-Prenylnaringenin in enhancing bone formation and inhibiting bone resorption *in vitro*. *Endocrinol* 2013;154(3):1202-14.
53. Peng S, Zhang G, Zhang BT, Guo B, He Y, Bakker AJ, *et al.* The beneficial effect of icaritin on osteoporotic bone is dependent on the treatment initiation timing in adult ovariectomized rats. *Bone* 2013 Jul; 55(1):230-40.
54. Cornish SM, Chilibeck PD, Paus-Jennsen L, Biem HJ, Khozani T, Senanayake V, *et al.* A randomized controlled trial of the effects of flaxseed lignan complex on metabolic syndrome composite score and bone mineral in older adults. *Appl Physiol Nutr Metabol* 2009;34(2):89-98.
55. Yin J, Tezuka Y, Subehan, Shi L, Nobukawa M, Nobukawa T, *et al.* *In vivo* anti-osteoporotic activity of Isotaxiresinol, a lignan from wood of *Taxus yunnanensis*. *Phytomed* 2006;13(1-2):37-42.
56. Mühlbauer RC, Lozano A, Palacio S, Reinli A, Felix R. Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. *Bone* 2003;32(4):372-80.