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Review article

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PHARMACOLOGICAL AGENTS AND NATURAL COMPOUNDS: AVAILABLE TREATMENTS FOR OSTEOPOROSIS

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Osteoporosis, a systemic skeletal disease characterized by a decrease in bone mass and deterioration of bone structure leading to an increased risk of fragility fractures, represents one of the major health problems worldwide. Currently, there are numerous pharmacological products used for the treatment of osteoporosis. Anti-resorptive drugs include bisphosphonates, hormone therapy, selective estrogen-receptor modulators, calcitonin, denosumab, calcium and vitamin D supplementation. Anabolic drugs such as teriparatide, strontium ranelate, romosozumab have recently become available based on advanced clinical trials. In recent years, combination therapy of anabolic and anti-resorptive agents is expected to be ideal anti-osteoporosis option. The adverse side effects caused by the long-term administration of pharmacological drugs have prompted researchers to study natural therapeutic compounds to find an alternative and effective way for osteoporosis treatment. Natural compounds including phytoestrogens with estrogenic effects (*e.g.* genistein, daidzein, icariin, dioscin, *Ginkgo biloba*), antioxidant and anti-inflammatory agents (*e.g.* acteoside, curcumin, resveratrol, *Camellia sinensis*), treatments that exert their effects by multiple actions (*e.g.* kinsenoside, berberine, *Olea europaea*, *Prunus domestica*, *Allium cepa*) could provide a safer alternative to primary pharmacological strategies. In this review, both pharmacological agents and natural compounds as available treatments for osteoporosis are characterized. In addition, possible mechanisms of action of all aforementioned treatments associated with bone remodelling, osteoclastogenesis, osteoblastogenesis, bone cell activity, death, and oxidative stress are presented. Nevertheless, more high-quality clinical studies with natural compounds are needed to provide greater evidence of the beneficial and safer antiosteoporotic application for the candidate.

Key words: *osteoporosis, treatment, pharmacological agents, natural compounds, anti-resorptive drugs, receptor activator of nuclear factor κB, phytoestrogens*

INTRODUCTION

Osteoporosis is a bone disease characterized by increasing osseous fragility and fracture due to the reduced bone mass and microstructural degradation (1). The most important feature of osteoporosis is the decrease in bone mineral density (BMD) (2). According to the World Health Organization (WHO) criteria, osteoporosis is diagnosed when the BMD is 2.5 or more below the young-adult mean standard deviations (3). It is a silent epidemic affecting more than 200 million people worldwide in recent years and has one of the highest incidence of all diseases in the elderly population (4). In Europe, osteoporotic fractures are the fourth leading cause of morbidity associated with chronic diseases annually, contributing to over 2.6 million disability-adjusted life years, that is more than hypertensive heart disease and rheumatoid arthritis (5). The main consequence of osteoporosis is the manifestation of fragility bone fractures due to reduced bone strength and is often seen in the vertebrae, wrists and hip. Such fractures are associated with significant morbidity and mortality, and despite several different treatments available for osteoporosis, there is still an observed increasing burden of osteoporotic fractures throughout the world (6). It has been estimated worldwide that, one in three women and one in five men aged over 50 experience osteoporotic fractures (7). Furthermore, the risk of additional fractures will rise exponentially with each incidence of fracture (8). Therefore, treatments for osteoporosis with various medicines have been receiving increasing attention globally.

There are certain risk factors which differ among individuals and are linked to the development of osteoporosis and contribute to the likelihood of developing the disease. These factors can be divided into two categories: The first being non-modifiable factors such as gender, age, body size, ethnicity, and family history, the other being modifiable factors are sex hormones, anorexia nervosa, calcium and vitamin D intake, lifestyle, cigarette smoking, and alcohol intake (4). Another risk factors involve other diseases consistent with long-term use of certain medications, the most commonly implicated being glucocorticoids, anticoagulants, anticonvulsants, aromatase inhibitors, cancer chemotherapeutic drugs, and gonadotropin-releasing hormone (9, 10). Physical exercise, dietary supplement, and pharmacotherapy are usually used for prevention and treatment of osteoporosis.

On the molecular level, osteoporosis is caused by an imbalance in bone remodelling - a highly complex process by which old bone is removed by osteoclasts (bone resorbing cells) and new bone tissue is formed by osteoblasts (bone forming cells). In general, osteoclasts differentiate from hematopoietic stem cells through monocyte/macrophage lineage upon stimulation of macrophage colony stimulating factor (M-CSF) by binding to its receptor c-Fms and activation of receptor activator of nuclear factor κB (RANK) by its ligand (RANKL) (11). M-CSF promotes the proliferation and survival of osteoclast precursors *via* the activation of several kinases, including Src and Akt. RANKL, expressed by osteoblasts, osteocytes, and stromal cells, binds to RANK on osteoclast precursor cells. The RANKL/RANK interaction subsequently activates nuclear factor κB (NF-κB), mitogen-activated protein kinases (MAPKs, *e.g*. JNK, p38) and promotes the expression of other osteoclastogenic factors such as nuclear factor of activated T-cells 1 (NFATc1) (12). On the other hand, osteoprotegerin (OPG), which is also produced by cells in the osteoblast lineage, is a soluble decoy receptor of RANKL that prevents binding of RANKL to RANK. Thus, the RANKL/RANK/OPG system is a key mediator of osteoclastogenesis (13).

Osteoblasts originate from mesenchymal stem cells (MSCs). Runt-related transcription factor 2 (Runx2) is a key transcription factor for osteoblast differentiation from MSCs and pre-osteoblasts which has many targets and downstream regulators including Osterix (Osx) and Sp7 (14). Runx2 also regulates the secretion of bone matrix proteins such as osteocalcin (OC), bone sialoprotein (BSP), and collagen type I (COL1A1) (15). Runx2 is regulated by multiple signals, such as bone morphogenetic proteins (BMPs) and Wnt proteins. BMPs were identified as members of the transforming growth factor-β (TGF-β) superfamily of multifunctional cytokines. Within the BMP signalling, signal transducer proteins SMAD1/5/8 are phosphorylated particularly by BMP2 and BMP4, and finally, activate Runx2 and other target genes (16). Regarding Wnt/β-catenin signalling, Wnt proteins (particularly Wnt3a and Wnt1) bind to Frizzled (FZD) receptors and lipoprotein receptor-related protein (LRP5/6) complexes, which results in the stabilization and translocation of β-catenin into the nucleus. β-catenin is an important transcriptional co-activator that regulates transcription of target genes including Runx2 (17). In addition to the mentioned canonical pathways, both BMP and Wnt also act in a non-canonical manner which does not require SMAD or β-catenin (18, 19).

This article deals with a detailed review of available treatments for osteoporosis, including both pharmacological agents and natural compounds. Recently, combination therapy of anabolic and anti-resorptive agents (either pharmacological therapy or simultaneous combination of pharmacological and natural therapy) is attracting attention because it is expected to be ideal anti-osteoporosis option. These combination treatments are also characterized. In general, some treatments have similar and/or identical mechanisms of action. Therefore, possible mechanisms of action of all pharmacological and natural agents described in this review, are illustrated in *Fig. 1*. Generally, these mechanisms are associated with bone remodelling, osteoclastogenesis, osteoblastogenesis, bone cell activity, death, and oxidative stress.

PHARMACOLOGICAL AGENTS FOR TREATMENT OF OSTEOPOROSIS

The pharmacotherapy for osteoporosis is usually focused on accommodating the estrogen level or bone remodel. The mechanisms involve many aspects, such as stimulating parathyroid hormone (PTH) synthesis, inducing the expression of OPG, decreasing interleukin (IL)-1, 4, 6, and M-CSF, increasing estrogens or like-estrogens, supplementing calcium, phosphate in bones, inhibit the proliferation of osteoclasts and induce osteoclast apoptosis, and to enhance the proliferation and differentiation of osteoblasts (20). Currently, there are numerous pharmacological products used for the treatment of osteoporosis in the clinic (21). The drugs used mainly include bisphosphonates (alendronate, risedronate, ibandronate, zoledronic acid), selective estrogen receptor modulators (SERMs; raloxifene), hormone therapy (HT; estrogen), calcitonin, denosumab, calcium and vitamin D supplementation, teriparatide (parathyroid hormone), strontium ranelate, romosozumab. Pharmacological treatments are divided into two broad categories: anti-resorptive and anabolic agents. Generally, anti-resorptive agents decrease bone resorption through inhibiting the osteoclasts activity. Administration of anabolic agents results in enhanced bone formation through stimulating the osteoblasts function (22). These therapies are helpful for the treatment of osteoporosis as evidenced by numerous clinical trials (5, 9). However, nearly all aforementioned therapies have adverse side effects because of the long-term drug administration for osteoporosis treatment (3).

Anti-resorptive drugs

Bisphosphonates (BPs) are considered first-line therapy for osteoporosis (23). They are analogues of pyrophosphate that bind at sites of active bone remodelling. BPs reduce bone resorption by inhibiting the action of osteoclasts and induction of osteoclast apoptosis (2). They could increase matrix mineralization and bone density up to a certain point, but they cannot restore lost structure or substantially improve bone microarchitecture because of their inability to stimulate osteoblast activity (24). In general, BPs are available in multiple formulations. Some of the potent inhibitors of bone resorption include alendronate, risedronate, ibandronate. Alendronate and risedronate are the most commonly used BPs worldwide (22). Zoledronic acid is the third-generation BPs and the most potent available BPs (2). Bisphosphonates, including alendronate, risedronate, and zoledronic acid reduce vertebral, non-vertebral and hip fractures compared with placebo in postmenopausal osteoporotic women. Ibandronate reduces the risk for radiographic vertebral fractures, although evidence is insufficient to determine the effect of ibandronate on hip fractures (25). Recently, concerns have been raised about the long-term safety of bisphosphonate therapy. Multiple case series have illustrated a link between a prolonged bisphosphonate use and a typical fractures, *e.g.* subtrochanteric femoral fractures (26), as characterised by clinical and radiographic features. The proposed pathophysiology is suppressed bone turnover resulting in accumulated micro-damage and a subsequent insufficiency fracture at the point of maximal stress (23). Other side effects include nausea, vomiting, diarrhoea, peptic ulceration, abdominal pain and constipation along with allergic skin reactions and possibility of osteomalacia (27). An additional less common adverse effect of bisphosphonates is osteonecrosis of the long bones and jaws (28, 29). It was also observed that high doses of BPs could reduce the proliferation of MG-63 osteoblast-like cells by arresting the cell cycle and inducing apoptosis/necrosis (30). On the contrary, low BPs doses act directly on bone-forming cells by increasing the expression of negative bone mediators and impairing extracellular matrix quality, suggesting an overall anti-anabolic effect on bone milieu (31).

Estrogen deficiency is a major cause of the early post-menopausal increase in bone resorption, bone loss and osteoporosis (32). The mechanisms focusing on how estrogen

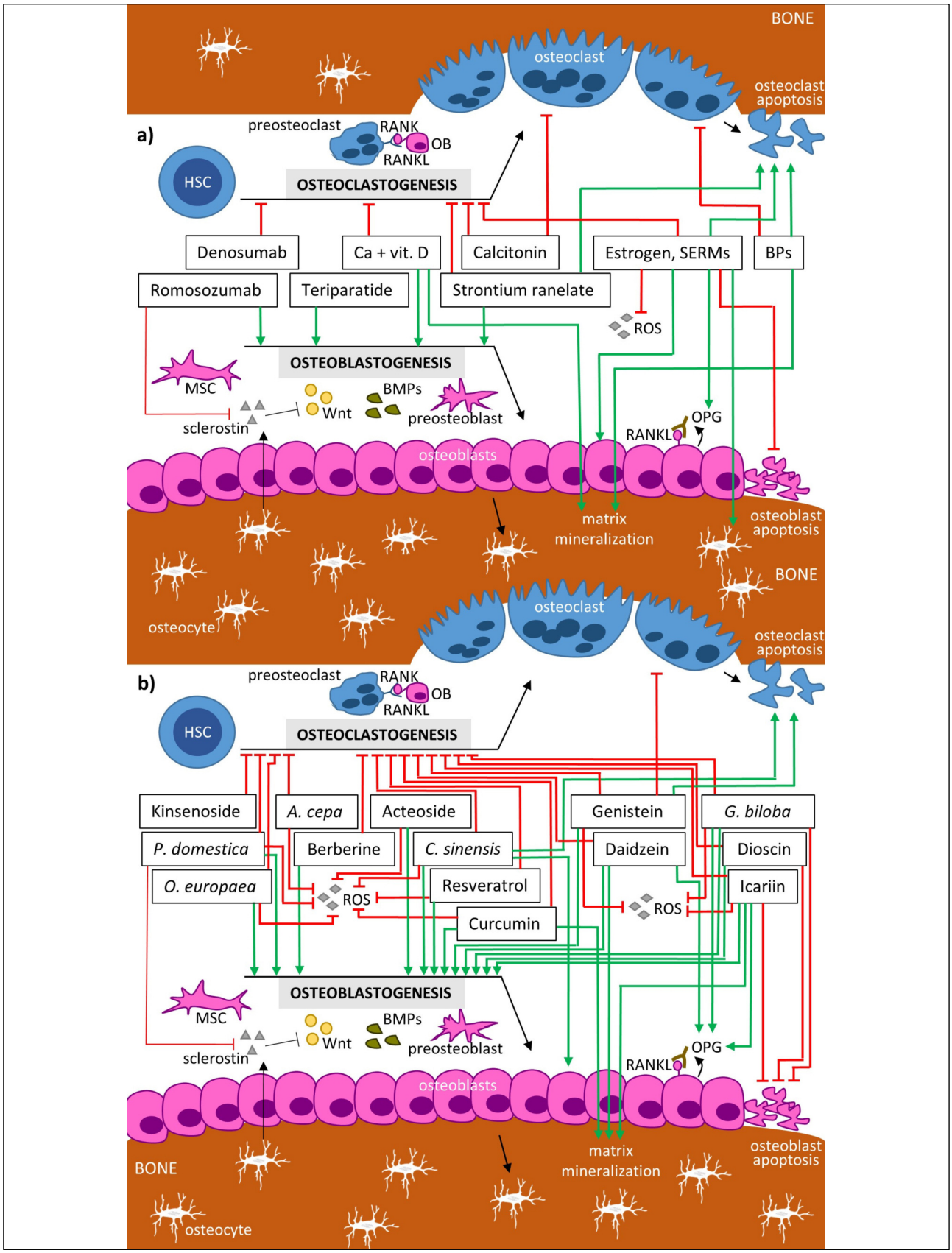

Fig. 1. Schematic representation of the effects of pharmacological agents (a) and natural compounds (b) on bone remodelling, osteoclastogenesis, osteoblastogenesis, bone cell activity and death, as well as their association with oxidative stress (ROS production). Negative and positive effects are depicted by red bookends and green arrowheads.

Abbreviations: BPs, bisphosphonates; SERMs, selective estrogen receptor modulators; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell; OB, osteoblast; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of nuclear factor κB ligand; OPG, osteoprotegerin; ROS, reactive oxygen species; BMPs, bone morphogenetic proteins; Wnt, Wnt glycoproteins.

deficiency negatively influences bone loss are complex. On the cellular level, estrogen blocks the new osteoclast formation by modulating RANK signalling in osteoclastic cells and induces apoptosis of osteoclasts (33). Although, the impact of estrogen on osteoblast lineage has not been fully elucidated, one of its most important downstream mediators is the OPG/RANKL system (34). In addition, it is likely that the anti-remodelling effects of estrogen are mediated *via* osteocytes. Therefore, estrogens influence the generation and lifespan of osteoclasts, osteoblasts, as well as the lifespan of osteocytes (35). In consequence, estrogen administration could be an appropriate therapy for osteoporosis. However, the long-term estrogen therapy is controversial because of increases in the risk of breast carcinoma, endometrial cancer, thromboembolic events, and cardiovascular disease (36). In post-menopausal women with osteoporosis and cardiovascular risk factors, combined estrogen and progestagen or individual estrogen therapy should be avoided in favor of alternative anti-resorptive agents (4). Due to these complications, estrogen is used for the prevention of post-menopausal symptoms only in the short term. In general, estrogen seems to positively control bone volume (3). The treatment efficiency of HT is also associated with the genetic background of individuals being treated (37).

Selective estrogen receptor modulators (SERMs) are chemically diverse set of compounds that do not have the steroid structure of estrogen, but have a tertiary structure that allows binding to the estrogen receptor to exert selective agonist or antagonist effects on different estrogen target tissues (33). The most studied SERM is raloxifene, which reduces vertebral fractures in osteoporotic women; however, it did not significantly decrease the risk for non-vertebral or hip fractures compared with placebo (25). Raloxifene can decrease the risk of breast cancer, but it also could enhance the rates of stroke, leg cramp, thromboembolism and post-menopausal vasomotor symptoms (38). Potential side effects include an increased risk of venous thrombosis similar to that with hormone therapy and exacerbation of hot flushes (33).

Calcitonin is a polypeptide hormone that strongly inhibits osteoclast function through a receptor-mediated process (23). It acts on a G protein-coupled calcitonin receptor, which primarily transduces signals *via* the cAMP and PLC/IP3 pathways. The calcitonin receptor is expressed at high levels in kidney and hypothalamus. In bone, the receptors are mainly located in the membranes of osteoclasts, thus reducing their motility and the bone resorption ability (39). It prevents osteoclast precursors from maturing (40). Additionally, it causes inhibition of carbonic anhydrase II, which disrupts the acidic environment that is optimal for osteoclast activity (41). Calcitonin therapy is usually given to patients who cannot take hormone therapy (2). It appears to reduce acute fracture-related pain, although long-term fracture prevention data are limited (3). The adverse effects include vomiting, nausea, allergic reactions, flushing, nasal adverse reactions, prostate cancer and hypocalcaemia (22).

Denosumab is a human monoclonal antibody against RANKL, an essential osteoclast cytokine. By binding this ligand, denosumab ultimately inhibits osteoclast-mediated bone resorption (23). It is an advancement in anti-resorptive therapy especially for enhancing adherence and persistence to treatment (42), as well as for a putative stimulation of osteoblastic activity in specific areas of cortical bone (43), as evidenced by animal studies, but not confirmed in humans yet (44). Treatment with denosumab reduces radiographic vertebral, non-vertebral, and hip fractures compared with placebo in post-menopausal osteoporotic women (25). The most commonly reported adverse effects include hypercholesterolemia, cystitis and musculoskeletal pain (26). In addition, long-term denosumab treatment is associated with gastrointestinal tract symptoms, dermatitis (25), and osteonecrosis of the jaw (45).

Calcium and vitamin D supplementation are essential nutrients for proper bone health. Since calcium together with phosphate makes up the mineral component of bone, vitamin D contributes to the maintenance of serum calcium levels. Direct effects of vitamin D are targeted to osteoblasts in order to enhance differentiation and mineralization (46). Active vitamin D has also been shown to inhibit human osteoclast formation and activity (47). For many years, a normal calcium balance together with normal vitamin D status have been considered crucial in the prevention and treatment of osteoporosis (48). However, in recent years, the usefulness of calcium supplementation (individual and simultaneous with vitamin D) has been questioned, since some studies reported only weak efficacy of these supplementations in reducing fragility fracture risk (49). According to the recent guidance by Kanis *et al*. (50), calcium and vitamin D supplementation is recommended for patients at high risk of calcium and vitamin D insufficiency, and in those who are receiving treatment for osteoporosis. Simultaneous supplementation with these nutrients may lead to a modest reduction in fracture risk, although population-level intervention has not been shown to be an effective public health strategy. Treatment with calcium alone does not reduce fracture risk. Side effects of calcium supplementation include renal stones and gastrointestinal symptoms. An increased cardiovascular risk consequent to calcium supplementation was not confirmed (50).

Anabolic drugs

Teriparatide is a recombinant form of human PTH, consisting of the first 34 N-terminal amino acids (51). It is the most commonly used bone anabolic drug that stimulates bone formation before it enhances bone resorption, generating a period when it is maximally anabolic (anabolic window), thus limiting further accrual of bone mass (52). Teriparatide exerts its positive effects on bone formation in two distinct fashions. The first is direct stimulation of bone formation that occurs within active remodelling sites (remodelling-based bone formation) and on surfaces of bone previously inactive (modelling-based bone formation). The second is an increase in the initiation of new remodelling sites (53). To maintain or increase BMD, teriparatide therapy is commonly followed by bisphosphonate therapy (23). Treatment with teriparatide reduces radiographic vertebral and non-vertebral fractures compared with placebo in post-menopausal osteoporotic women (25). However, teriparatide did not reduce hip fracture risk (51). Negative side effects include headache, nausea, hypercalcemia, and musculoskeletal pain. Studies in rats have demonstrated an increased incidence of osteosarcoma, so it is contraindicated for patients with enhanced risk for osteosarcoma or a history of radiotherapy (23).

Strontium ranelate is a unique anti-resorptive drug that may have anabolic properties. It inhibits osteoclast differentiation and promotes osteoclast apoptosis. For anabolic effects, there are several controversies, but it is known that it activates pre-osteoblasts and replaces calcium with strontium, which leads to an increase in BMD (54). It could be used as an alternative to bisphosphonate therapy in management of post-menopausal osteoporosis (2). The clinical trials in post-menopausal women revealed that it reduces the risk of fractures and was well-tolerated apart from a low rate of gastrointestinal side effects and increased risk of venous thrombosis (55).

Romosozumab is a monoclonal antibody directed against sclerostin and the only available therapeutic option targeting Wnt signalling, as both bone-forming and anti-resorptive intervention to treat osteoporosis and fragility fractures (56). Experimental studies on rats and ovariectomized primates treated with romosozumab had shown significant increases in

bone mass and strength (57). The efficacy of romosozumab in enhancing bone formation and preventing fragility fractures was assessed in several randomized controlled trials (58). Significantly, lower incidence of vertebral fractures in women previously treated with romosozumab versus placebo group was confirmed in the study by Cosman *et al.* (59). Saak *et al.* (60) reported a significantly lower risk of incident vertebral fractures and clinical fractures at 2-year follow-up in romosozumab groups versus placebo. Moreover, women receiving romosozumab had a significantly lower risk of hip fracture. The most commonly reported adverse effects include arthralgia, nasopharyngitis, back pain, hypersensitivity, osteoarthritis, osteonecrosis of the jaw, and cardiovascular events. An additional safety concern associated with romosozumab treatment is the potential tumorigenic effect (56).

Combination therapy

There is no pharmacological or physiological rationale for the simultaneous use of different anti-remodelling drugs (9). If one agent normalizes bone remodelling, there is little for an added agent to improve. The gains in BMD when estrogen was used in combination with either alendronate or risedronate were minimal, and much smaller than the increases observed with either estrogen or BPs alone (61). Generally, an ideal anti-osteoporosis agent should increase new bone formation and simultaneously inhibit bone resorption (62); however, there are currently no pharmacological anti-resorptive agents (such as BPs, denosumab, and SERMs) or no anabolic agents (such as PTH and its analogs) which meet both therapeutic goals. Therefore, combinations of anabolic pharmacological agents and anti-resorptive synthetic agents seems to be the most ideal anti-osteoporosis option. Lou *et al.* (63) evaluated the efficacies of combination therapies with PTH analogs and anti-resorptive agents including the BPs, denosumab, and estrogen-like drugs. This combination therapy exhibited superior efficacy over individual therapy with an additional 36% reduction in fracture risk. According to Finkelstein *et al*. (64), there was no evidence of synergy between BPs and PTH analogs in women with post-menopausal osteoporosis. However, a combination of teriparatide and intravenous zoledronic acid increased BMD more rapidly than individual drug administration in post-menopausal women (65). In the study by Leder *et al*. (66), simultaneous use of denosumab and teriparatide produces larger increases in lumbar spine, femoral neck, and total hip BMD than does monotherapy. It was suggested that the combination of teriparatide and denosumab may be useful to treat patients at a very high risk of fragility fracture (67). Combination therapy with raloxifene plus teriparatide in patients who had received only teriparatide before this simultaneous therapy, produced superior efficacy in lumbar spine BMD as compared to the continuation of teriparatide monotherapy (68). Conversely, Cosman *et al*. (69) revealed that there was no significant difference in lumbar spine BMD of patients with raloxifene and teriparatide combination therapy as compared to teriparatide monotherapy. Interestingly, most studies reported no significant increase in the frequency of serious adverse effects when combining anabolic agents with BPs, denosumab, or SERMs, in comparison with individual treatments (3).

NATURAL COMPOUNDS FOR TREATMENT OF OSTEOPOROSIS

Many medicinal plants have long been used to prevent and treat osteoporosis in many countries. These natural medicines derived from plants have fewer side effects and are more suitable for long-term use than synthesized drugs. The plant medicines containing numerous chemical constituents usually exert their therapeutic effects through multi-pathways and have multi-targets, this property is parallel with the multiple factors of osteoporosis pathogenesis (4). In general, three major groups of natural compounds or herbal remedies have been identified based on their mechanisms of action, including estrogen-like activity, antioxidant and anti-inflammatory properties, or by modulating the key signalling pathways in the pathogenesis of osteoporosis (70).

Plant-delivered phytoestrogens could act in a similar manner as mammalian estrogens but are believed to exert milder effects, particularly on sensitive tissues such as uterus and breast (71). Families of molecules that are classified as phytoestrogens include lignans, isoflavones and some other flavonoids. The two most commonly found soy isoflavones are genistein and daidzein (70). Epidemiological studies and clinical trials suggest that soy isoflavones have beneficial effects on BMD and mechanical strength in post-menopausal women (72). In addition, isoflavones preferentially bind to estrogen receptor β (ERβ); therefore, they mimic the effects of estrogens in some organs (including bone) and at the same time, block the estrogen effects in others (*e.g*. breast) and may prevent the aforementioned unwanted effects of estrogen therapy (73).

Generally, oxidative stress occurs as a result of an overproduction of reactive oxygen species (ROS) not balanced by adequate levels of antioxidants and it seems to be involved also in the pathogenesis of bone loss. ROS induce the apoptosis of osteoblasts, osteocytes and inhibit the mineralization and osteogenesis. Antioxidants either directly or by counteracting the action of oxidants contribute to activate the differentiation of osteoblasts, mineralization process and the reduction of osteoclast activity (74). Age-related chronic inflammation also plays an essential role in the pathogenesis of osteoporosis by influencing bone remodelling. In the presence of RANKL, the cytokines tumor necrosis factor alpha (TNF-α), IL-6, and IL-1 cause an excessive formation of osteoclasts while inhibiting osteoblast activities (75). Considering the involvement of oxidative stress and inflammation in the pathogenesis of osteoporosis, natural compounds with properties that could counteract these processes may be a suitable prophylactic agent to prevent bone loss (76).

In this review, selected natural compounds (genistein, daidzein, icariin, dioscin, acteoside, curcumin, resveratrol, kinsenoside, berberine) and also herbal remedies often used and/or consumed by humans (*Ginkgo biloba*, *Camellia sinensis*, *Olea europaea*, *Prunus domestica*, *Allium cepa*) as available treatments for osteoporosis are further characterized in each of the three major groups. Chemical structures of the natural therapeutic compounds are illustrated in *Fig. 2*.

Phytoestrogens

Genistein possesses numerous biological properties - anti-inflammatory, antiangiogenic, antiproliferative, antioxidant, immunomodulatory, analgesic and joint protection (77). In an ovariectomized (OVX) rat model, genistein has shown an antiosteoporotic activity. An extract of *Erythrina variegate* (containing genistein derivatives) was found to increase alkaline phosphatase (ALP) level, maintain serum calcium and phosphate levels, decrease urinary excretion of these elements, and had beneficial effects on cortical and trabecular bone structure (78). Compared with conventional drugs (alendronate, raloxifene and estradiol), genistein showed a greater tendency to increase BMD and bone mineral content (BMC) in OVX rats, accompanied by increased breaking strength and bone quality, elevated OPG and reduced C-terminal telopeptide of type I collagen (CTX) and

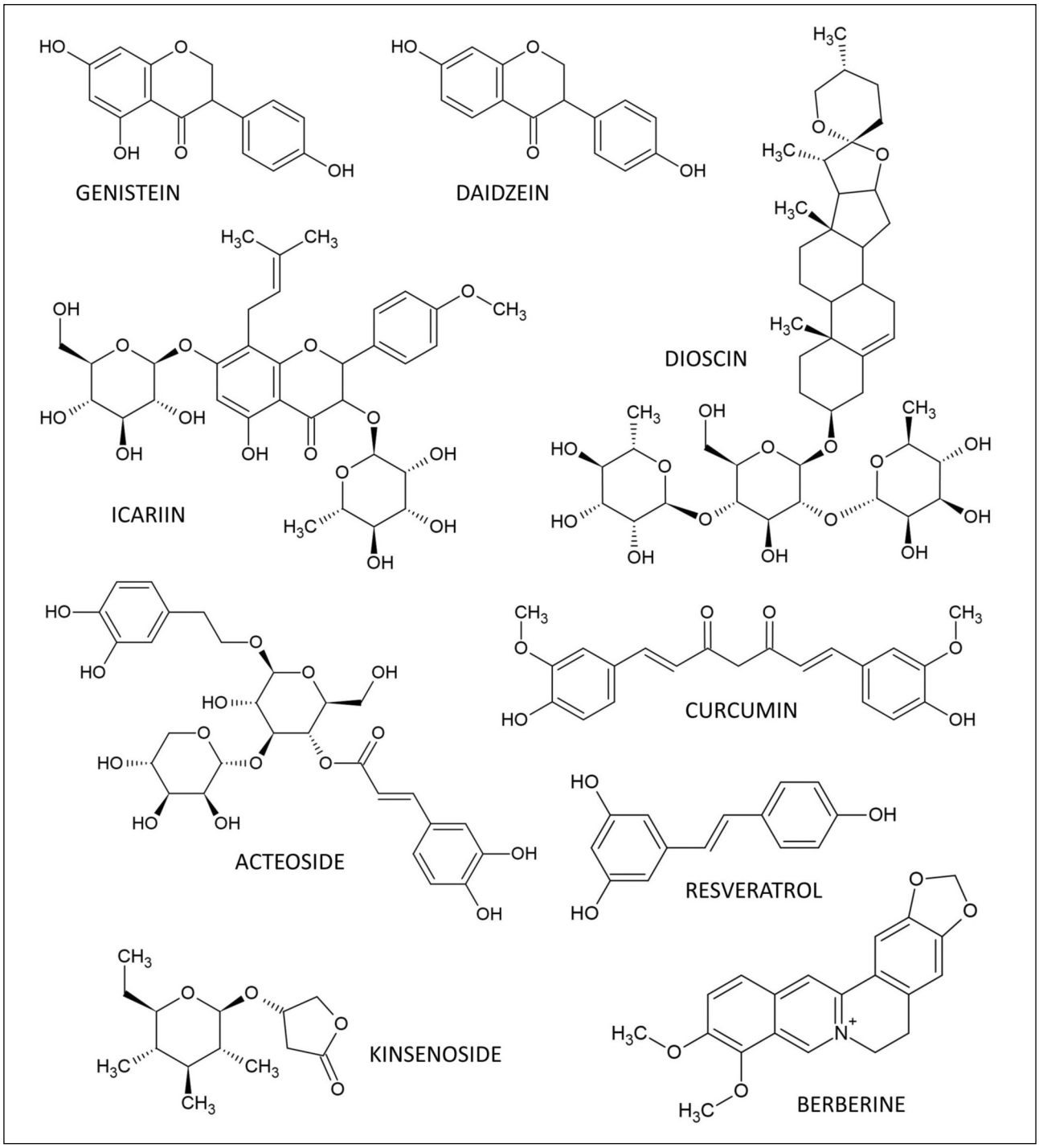

Fig. 2. Chemical structures of described natural therapeutic compounds.

RANKL (79). In osteoclasts, genistein suppresses the activation of protein phosphatase, induces apoptosis, and inhibits osteoclast cell formation through various pathways (2). It also enhances osteoblastic differentiation and maturation (80).

Daidzein has been shown to prevent the loss of BMD, and to increase trabecular bone area and thickness while decreasing trabecular separation in OVX rats (81). According to Picherit *et al*. (82), daidzein was more efficient in preventing OVX-induced bone loss when compared with genistein. Daidzein could inhibit the proliferation and differentiation of osteoclasts, this is possibly due to increasing apoptosis of osteoclast progenitors mediated by estrogen receptors (83). Other mechanisms may include activation of the protein tyrosine phosphatase, inhibition of cytokines, changes in intracellular calcium, and membrane depolarization (84). Low-dose daidzein induced production of OPG in osteoblasts which prevents maturation of pre-osteoclasts and thus, it decreased bone resorption. In addition, daidzein increased osteoblast differentiation and bone mineralization (85).

Icariin is the main active flavonoid glucoside isolated from *Epimedium* plant. It has a therapeutic effect on osteoporosis in OVX rat models and post-menopausal women. Icariin suppress the loss of bone mass and strengthen the distal femur and tibia of OVX rats through increasing the mRNA expression ratio of OPG/RANKL (86, 87) and, thus, inhibiting bone resorption

activity of osteoclasts. It is also capable of promoting osteoblast proliferation, differentiation, and mineralization, as well as inhibiting osteoblast apoptosis (88). Ma *et al*. (89) compared the impacts of icariin versus genistein on rat calvarial osteoblast *in vitro*. Their findings revealed that icariin yielded superior effects in comparison with genistein. The existence of a prenyl group on C-8 of icariin molecular structure has been suggested to be the reason why icariin is more potent than genistein in osteogenic activity (80).

Dioscin is a natural steroidal saponin isolated from some medicinal plants, most of which belong to the family of *Dioscoreaceae* (90). Generally, dioscin has anti-tumoral activity, anti-atherosclerotic properties, and inhibitory effects on hepatic fibrosis (91). In recent years, several studies have shown that dioscin alleviates the symptoms of osteoporosis (92). According to Wu *et al.* (91), it could improve BMD and strengthen the maximum bone stress in OVX rats. Furthermore, dioscin could promote bone tissue proliferation through PI3K/Akt and P38 signalling pathways, and inhibit bone tissue apoptosis *via* regulation of the Bcl-2/Bax signalling pathway in rat models. In addition, it could facilitate osteoblastic proliferation and differentiation through low-density LRP5 and ER pathways in mouse pre-osteoblast-like MC3T3-E1 cells and in human osteoblast-like MG-63 cells (92). Another beneficial effect of dioscin on osteoporosis is decreasing the expression of RANKL (93, 94). Therefore, it could also inhibit bone resorption by osteoclasts through down-regulating the Akt signalling cascades (93). The decrease in OPG mRNA level and OPG/RANKL ratio in femurs induced by ovariectomy could be rescued by dioscin in rodents, albeit, the effects on reversing osteoclastogenesis were in vain (94).

Components of *Ginkgo biloba* extract include phytoestrogens (*e.g.* kaempferol, quercetin), terpenoids and ginkgolic acid (70). The results by Lucinda *et al.* (95) showed that rats with glucocorticoid-induced osteoporosis and treated with *Ginkgo biloba* leaf extract had an increased percentage of alveolar and trabecular bone in the femur and jaw, comparable to those treated with alendronate. In a subsequent experiment using the same animal model (96), the investigators discovered that *Ginkgo biloba* also increased the expression of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) while decreased apoptosis regulator Bcl-2 associated X (Bax) expression in osteoblasts, suggesting its positive role in osteoblast cell survival. *Ginkgo biloba* extract also improved osteoblast proliferation and osteogenesis while it modulated BMP and Wnt/β-catenin signalling. In addition, the increased OPG expression and minimal expression of RANKL indicate that *Ginkgo biloba* inhibited osteoclastogenesis (97, 98).

Antioxidants and anti-inflammatory agents

Acteoside, also known as verbascoside, has been identified as the main bioactive compound of medicinal plants of genus *Verbascum* and *Cistanche*. It has a strong antioxidant and anti-inflammatory properties (99). In the *in vitro* study conducted by Lee *et al.* (100), acteoside was found to attenuate bone resorption by inhibiting osteoclast differentiation and formation. It was also revealed that acteoside decreased the inflammatory production of cytokines (*e.g.* TNF-α, IL-6), regulated the mitogen-activated protein kinase (MAPK)-mediated signalling which, together with ROS decrease, contribute to the suppression of osteoclastogenesis. In addition, acteoside increased the proliferation and differentiation of osteoblasts in diabetic rats (101). The study by Yang *et al.* (102) showed that acteoside exhibited potential activities for the prevention of bone loss in OVX mice, demonstrated by improved trabecular microarchitecture and bone biomechanical competence.

Curcumin, a major yellow natural polyphenolic compound, is extracted from the rhizomes of the turmeric (*Curcuma longa*) plant. The functional application and therapeutic potential of curcumin in the treatment of aging-associated diseases (including osteoporosis) is well documented (103). Mice receiving curcumin showed an increase in BMD and trabecular bone mass and reduction in the levels of TNF-α and IL-6. The protective effects of curcumin on OVX-induced bone loss have also been confirmed by Kim *et al*. (104). The authors found that curcumin inhibited bone loss by reducing osteoclastogenesis through increasing antioxidant activity and impairing RANKL signalling. This protective effect of curcumin on osteoclastogenesis seems to occur through the amelioration of the activation of Akt/NF-κB/NFATc1 (nuclear factor of activated T-cells 1) pathways (105). Large corpus of data presents curcumin as a robust downstream-acting inhibitor of Wnt signalling in tumor cells with low tissue specificity (106). It decreased the rate of migration and proliferation of Hep3B hepatocarcinoma cells through inhibition of the Wnt signalling pathway (107, 108), similarly to its effect in a medulloblastoma cell line (109), in glioblastoma (110), and in a non-small-cell lung cancer cell line A549 (111). However, curcumin was also reported to activate the Wnt pathway in some instances. It was concluded that curcumin stimulated Wnt signalling during adipogenesis (112). Curcumin restored the expression of Wnt pathway components, leading to the nuclear translocation of β-catenin, and the rescuing of dexamethasone-induced osteoporosis (113). In the cases of Wnt signalling stimulation, curcumin acts through modulating the expression of key pathway elements, rather than affecting the nuclear activity of the pathway. The clue to this riddle could lie in the fact that curcumin may interact with various microRNAs and thus interfere with gene expression. In addition, curcumin showed an immunomodulatory effect on macrophage polarization by down-regulating proinflammatory cytokines and up-regulating anti-inflammatory cytokines. Curcumin treatment also increased the expression of genes connected with osteoblast differentiation, and enhanced ALP activity and mineralization (114). According to Khanizadeh *et al*. (115), it could also be used in the combination therapy; the combination of curcumin and alendronate has beneficial effects on BMD and bone turnover markers in post-menopausal women with osteoporosis.

Resveratrol, a natural polyphenolic component extracted from red grapes, peanuts and other plants, is known to exert many beneficial pharmacological effects such as antitumor, scavenging free radicals, anti-inflammation, cardio-protection and vaso-protection activities (116). Clinical and experimental studies have shown that resveratrol prevented bone loss by attenuating the damage caused by oxidative stress. More specifically, resveratrol, due to its antioxidant effect, effectively decreased RANKL production and inhibited osteoclastogenesis. The preventive effects of resveratrol against oxidative damage and inhibition of osteoclastogenesis were associated with upregulated transcriptional activity of fork head box protein O1 (FoxO1), an important antioxidant defense factor (117). The mechanism of FoxO1 action is also related to levels of circulating serotonin (118). Moreover, resveratrol promoted the formation of osteoblasts by induction of BMP2 through Src kinase-dependent estrogen receptor activation (119). Treatment with resveratrol also activated the osteogenic factors Runx2 and Sirtuin 1 (SIRT1). *In vitro* studies have shown that resveratrol inhibited proliferation and promoted apoptosis in many types of tumor cells (120). This effect may be related to resveratrol-mediated inhibition of Wnt/β-catenin signalling (106). However, it could promote osteogenic differentiation in mouse and human mesenchymal stem cells by activating Wnt/β-catenin signalling (121). This effect of resveratrol is related to the concentration of

resveratrol and the type of cells involved. According to Zhao *et al.* (122), resveratrol also promoted osteoblastic differentiation of canine bone marrow stromal cells (BMSCs) by activating the Wnt/β-catenin; ERK/MAPK signalling pathways and was involved in osteogenic differentiation of canine BMSCs. In terms of other biological targets, resveratrol altered the expression of intracellular mediators which modulate variety of cellular processes such as cell cycle regulation, metabolism, post-translational modifications and inflammatory responses (123). Furthermore, resveratrol has also been shown to regulate modifications in DNA and histone proteins, thereby impacting gene expression and silencing of some important cellular processes including apoptosis, genomic imprinting, chromosome activation and stem cell pluripotency (124). The studies by Wang *et al.* (125, 126) confirmed that resveratrol significantly increased BMD, and inhibited the percentages of peak load and ultimate stiffness in osteoporotic rats. It also inhibited the serum levels of ALP and OC. Moreover, resveratrol exhibited no toxic effects, and therefore it could be safety used for the treatment and/or prevention of osteoporosis, even if used for a long time (127).

Camellia sinensis (Tea plant) belongs to the plant family *Theaceae*. Teas made from *Camellia sinensis* leaves are the second most consumed beverage in the world after water (128). Generally, teas could be classified depending on the degree of fermentation where green tea (unfermented tea) and black tea (fermented tea) belong to the most consumed (129). Tea drinking is closely associated with bone health and may provide protection against osteoporosis and osteoporotic fractures; these effects have been verified both *in vitro* and *in vivo* (130-132). Although there are obvious differences in the ingredients of black and green tea, tea drinkers of either kind showed similar positive results when correlated with BMD (27). (–)-Epigallocatechin-3-gallate (EGCG), one of the most abundant catechins and main active ingredient in green tea, showed a protective effect on bone microarchitecture in OVX rats (133). According to Vester *et al*. (134), green tea had a strong anti-oxidative stress effect in human primary osteoblasts. It increased the expression of OC and collagen in osteoblasts and improved the cell viability. EGCG had a regulative effect on osteogenic function, accompanied by increased ALP activity, the up-regulated expression of osteogenic genes and the formation of bone-like nodules. It is likely that EGCG affects osteogenetic differentiation through the modulation of BMP2 expression (135). EGCG has also been shown to induce apoptosis in mouse osteoclast-like cells (136) and inhibit the formation of osteoclasts (137). The antiosteoclastogenic effect of EGCG could be caused by inhibiting RANKL-induced activation of c-Jun N-terminal kinase (JNK/c-Jun) and NF-κB pathways, thereby suppressing the gene expression of c-Fos and NFATc1 in osteoclast precursors (138). Das *et al.* (139) have conducted an experiment to investigate the proposed antioxidant effect of black tea extract. Histochemical analysis revealed that OVX rats treated with black tea extract had improved bone remodelling and increased break strength of excised bone. In addition, serum levels of TNF-α, IL-6, and RANKL were reduced. It was also documented that theaflavin-3,3'-digallate, a black tea polyphenol, decreased the formation and differentiation of osteoclasts (132).

Treatments that exert their effects by multiple actions

Kinsenoside is a main active component isolated from plants of the genus *Anoectochilus* (family *Orchidaceae*), and exhibits many biological activities and pharmacological impacts, including hepatoprotective, anti-hyperglycemic, anti-hyperliposis, anti-inflammatory, vascular protective and anti-osteoporosis effects (140). *Anoectochilus* has been shown to elevate intestinal calcium absorption, increase trabecular bone volume and prevent bone loss associated with ovarian hormone deficiency in rats (141). Hsiao *et al.* (142) reported that kinsenoside could prevent bone loss in OVX mice *via* inhibiting RANKL-induced NF-κB and NFATc1 activities. It also suppresses early stage of osteoclast development *in vitro*, decreases plasma CTX and expression of tartrate-resistant acid phosphatase (TRAP) in the femur.

Berberine is an isoquinoline alkaloid present in several plants, including *Coptis sp*. and *Berberis sp*. (143). It prevents bone loss in SAMP6 senile osteoporosis model, in OVX rats (144), and in rats with glucocorticoid-induced osteoporosis by inhibiting bone resorption and improving bone formation (145). Berberine inhibited osteoclastogenesis - RANKL-mediated osteoclast formation and survival through suppressing NF-κB and Akt pathways (146). Inhibitory effect of berberine on osteoclast formation was confirmed by Xue *et al*. (147) who documented a reduced number of TRAP-positive multinucleated cells, suppressed activity of osteoclast differentiation markers TRAP and cathepsin K. In addition, simultaneous administration of berberine and icariin showed a synergistic inhibitory effects on these markers, suggesting the possibility to use these natural compounds in combination therapy. In osteoblastic cells, berberine enhanced the expression of osteogenic marker genes including osteopontin, osteocalcin and promoted osteoblast differentiation through activation of Runx2 by p38 MAPK (148). Moreover, berberine (and synergistically in combination with icariin) could regulate the OPG/RANKL system in osteoblasts, supporting the inhibition of osteoclast differentiation (147).

The consumption of olive oil (*Olea europaea*) especially extra virgin olive oil (EVOO) has been found to prevent bone loss in *in vivo* and *in vitro* studies. In patients who regularly consumed olive oil over a 1-year period, BMD at lumbar vertebrae L3, L4, and the femoral neck had improved (149). Also, dietary intake of olive oil has been positively associated with a better volumetric BMD in Spanish women (150). Phenolic compounds in EVOO, such as apigenin, luteolin, coumaric acid, ferulic acid and caffeic acid, have been found to increase the proliferative capacity and differentiation of osteoblasts (151, 152). Studies of markers related to bone resorption (*e.g*. OPG, RANKL) have demonstrated that phenolic compounds in EVOO inhibited osteoclastogenesis, and therefore bone tissue loss (153). According to Melguizo-Rodriguez *et al*. (154), EVOO phenolic compounds may have a beneficial effect on bone physiology, exerting a stimulatory effect on markers involved in osteoblast proliferation, differentiation and maturation. Generally, EVOO polyphenols are important at biological level not only for their antioxidant activity, but also for their implication in the modulation of several intracellular signals (155). In addition, results of Tagliaferri *et al*. (156) suggest the use of EVOO in combination therapy. EVOO fortified with vitamin D_3 was able to counteract the bone loss induced by estrogen deprivation. Such a bone sparing effect could be explained by an improvement of both inflammation status and oxidative stress.

Commercialized prunes (*Prunus domestica*, *European plum*), also commonly known as dried plums, are dehydrated version of the cultivar *Prunus domestica L*. cv d'Agen. Dried plum contains several bioactive compounds including dietary fiber, vitamins (*e.g*., vitamin K), minerals (*e.g*., boron, copper), and (poly) phenolic compounds such as chlorogenic acids (*i.e*. chlorogenic acid, neochlorogenic acid, and cryptochlorogenic acid) and proanthocyanidins (157). The exact nutrients and/or components contributing to the bone-protective effects of dried plum are unknown. However, many of these compounds are believed to exert bone-protective effects and therefore likely work additively and/or synergistically (158). A dietary supplement of dried plum in osteopenic post-menopausal women has been found to notably improve BMD in ulna and spine. This positive effect could be partly attributed to suppression of production of RANKL and sclerostin, and an increase in bone formation markers including serum ALP activity, OPG and insulin-like growth factor 1 (IGF-1)

(159). Animal studies have shown that dietary intake of dried plum prevented OVX-induced bone loss and preserved structural and biomechanical properties of bones (160). This beneficial effect might be partially attributed to antioxidant properties of dried plum, which causes an increase in glutathione peroxidase (GPX) activity (161) and modulates the immune response by restoring granulocyte and committed monocyte populations (162). In an OVX rat model, dried plum intake was shown to suppress bone turnover through upregulating of BMP4 and IGF-1 while down-regulating NFATc1, procollagen I N-terminal propeptide (P1NP) and deoxypyridinoline (DPD) (163). Dried plum polyphenols suppressed osteoclast differentiation and activity *in vitro* by down-regulating NFATc1 and pro-inflammatory products such as nitric oxide (NO) and TNF-α (164). They also down-regulated calcium and MAPK signalling, resulting in suppression of NFATc1 expression, which ultimately decreases osteoclast formation and activity (165). Additionally, dried plum polyphenols also enhanced osteoblast activity by upregulation of Runx2, Osx, and IGF-1 expressions (166). Therefore, dried plum may enhance osteoblast activity especially by upregulating BMP signalling (167). However, the components of dried plum, responsible for the increased osteogenesis, are not known yet.

Allium cepa (onion) is a bulbous vegetable ascribed to its strong flavour and various beneficial properties. Onion is one of the richest sources of flavonoids (*e.g.* quercetin, rutin, myricetin) and organosulfur compounds (*e.g.* S-methyl-L-cysteine sulphoxide, diallyl sulfide, alkyl sulfoxides, dipropyl trisulfide) that contribute to stronger antioxidant activities (168). In the study by Matheson *et al*. (169), post-menopausal women consuming onions once a day or more had an overall bone density that was 5% greater than in individuals who consumed onions once a month or less. Furthermore, women consuming onions decreased their risk of hip fracture by more than 20% versus those who never consumed onions. Animal studies conducted by Muhlbauer *et al*. (170) have also demonstrated a significant increase in BMD of rats fed with high onion diet. *In vitro* study revealed that onion extract effectively inhibited osteoclast differentiation and formation (osteoclastogenesis), due to the presence of flavonoids and phytoestrogens (168).

CONCLUSION

Osteoporosis and fragility fractures are relevant health issues because of their impact in terms of morbidity, mortality, and socioeconomic burden. Anti-resorptive pharmacological agents (*e.g.* bisphosphonates, hormone therapy, selective estrogen receptor modulators, calcitonin, denosumab, calcium and vitamin D supplementation) have been widely used for the treatment of osteoporosis. In addition, anabolic synthetic agents (*e.g.* teriparatide, strontium ranelate, romosozumab) have recently become available based on advanced clinical trials. To establish an ideal anti-osteoporotic therapy that increases new bone formation and simultaneously inhibits bone resorption, anabolic agents combined with anti-resorptive agents have been tested in several clinical trials. Recent advancements in the molecular understanding of bone metabolism and in bioengineering will open the door to future treatment paradigms for osteoporosis, including antibody agents, stem cells, and gene therapies. The adverse side effects caused by pharmacological agents have prompted researchers to study natural therapeutic compounds which might be effective and safe for the treatment of osteoporosis and had fewer negative effects. Some phytochemicals, which have estrogen-like and/or antioxidative activity, produce bone protective effects *via* estrogen receptor and/or improve antioxidative capacity. Some of them may directly regulate the proliferation and activity of osteoblasts and osteoclasts. Genistein, daidzein, icariin, dioscin, *Ginkgo biloba* have been reported to decrease bone loss through increasing osteoblast proliferation and activity *via* estrogen receptor. The phytochemicals with antioxidative capacity (*e.g.* acteoside, curcumin, resveratrol, *Camellia sinensis)* regulate bone metabolism through reducing the production of ROS and improving antioxidative and anti-inflammatory properties. Other compounds such as kinsenoside, berberine, *Olea europaea*, *Prunus domestica*, *Allium cepa* directly exert effects on osteoblasts and osteoclasts through modulating cytokines, and regulating important signalling pathways (*e.g.* MAPK, NF-κB, Wnt/β-catenin, and RANKL/RANK/OPG). Further research to isolate and characterize the bioactive anti-osteoporotic compounds from the classical and bone-specific drugs is necessary to extensively profile compound for pharmacological usage, especially their safety, efficacy, and potential chemical interactions with other drugs. The safety of herbal remedies should also be considered. Some herbal toxic effects have been revealed, out of which, the hepatotoxicity is the most frequently reported toxic effect. Generally, any plants produce toxic compounds as secondary metabolites, which may not easily be distinguishable from the active pharmacological constituents. Some of these herbs are produced in very unhygienic conditions (mainly in developing countries) using potentially toxic ingredients, subsequently exposing the consumers to multiple hepatotoxins (171). Also, there is a lack of data associated with long-term use of herbal supplements. Therefore, studies to determine the special and targeted cellular and molecular mechanisms of natural compounds are required to develop their potential application for the treatment of osteoporosis, as an effective, safe alternative to primary therapeutic strategies, or in combination with current primary pharmacological treatments. Nevertheless, more high-quality clinical researches with this natural medicines are needed to provide greater evidence for the candidate to beneficial and safer anti-osteoporotic application.

Abbreviations: ALP, alkaline phosphatase; Bax, Bcl-2-associated X; Bcl-2, B-cell lymphoma 2; BMC, bone mineral content; BMD, bone mineral density; BMP, bone morphogenetic protein; BMP2, bone morphogenetic protein 2; BMP4, bone morphogenetic protein 4; BPs, bisphosphonates; cAMP, cyclic adenosine monophosphate; c-Fms, macrophage colony-stimulating factor-1 receptor; COL1A1, collagen 1 alpha 1; CTX, C-terminal telopeptide of type I collagen; DNA, deoxyribonucleic acid; DPD, deoxypyridinoline; EGCG, epigallocatechin-3-gallate; ERK, extracellular signal-regulated kinases; FZD, Frizzled; JNK/c-Jun, c-Jun N-terminal kinase; HT, hormone therapy; IGF-1, insulin-like growth factor 1; IL, interleukin; IP3, inositol trisphosphate; FoxO1, forkhead box protein O1; JNK, c-Jun NH2-terminal kinase; LRP, low-density lipoprotein receptor related protein; MAPK, mitogen-activated protein kinase; M-CSF, macrophage colony stimulating factor; mRNA, messenger ribonucleic acid; NFATc1, nuclear factor of activated T cells 1; NF-κB, nuclear factor kappa B; NO, nitric oxide; OC, osteocalcin; OPG, osteoprotegerin; OVX, ovariectomized; P1NP, procollagen type I N-terminal propeptide; PI3K/Akt, phosphoinositide-3-kinase-protein kinase B/Akt; PLC, phospholipase C; PTH, parathyroid hormone; RANK, receptor activator of nuclear factor kappa B; RANKL, receptor activator of nuclear factor kappa B ligand; ROS, reactive oxygen species; Runx2, runt-related transcription factor 2; SERMs, selective estrogen receptor modulators; sp., species; SIRT1, sirtuin type 1; TGF-β, transforming growth factor beta; TNF, tumor necrosis factor; TNF-α, tumor necrosis factor alpha; TRAP, tartrate resistant acid phosphatase; WHO, World Health Organization.

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