Review

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Chinese single herbs and active ingredients for postmenopausal osteoporosis: From preclinical evidence to action mechanism

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Summary **Postmenopausal osteoporosis is a systemic metabolic skeletal disease generally ascribable to a dearth of estrogen. Whether traditional Chinese medicine is effective in management of postmenopausal osteoporosis remains unclear. This article reviews the experimental evidence of both *in vitro* and *in vivo* preclinical studies with the theme of the application of Chinese single herbs and active ingredients in postmenopausal osteoporosis. It includes three single herbs (Herba Epimedium, Rhizoma Drynariae, and Salvia miltiorrhiza) and eight active ingredients (saikosaponins, linarin, echinacoside, sweroside, psoralen, poncirin, vanillic acid, and osthole). The experimental studies indicated their potential use as treatment for postmenopausal osteoporosis and investigated the underlying mechanisms including osteoprotegerin/receptor activator of nuclear factor κB ligand (OPG/RANKL), extracellular-signal-regulated kinase/c-Jun N terminal kinase/mitogen-activated protein kinase (ERK/JNK/MAPK), estrogen receptor (ER), bone morphogenetic protein (BMP), transforming growth factor (TGF)-β, Wnt/β-catenin, and Notch signaling pathways. This review contributes to a better understanding of traditional Chinese medicine and provides useful information for the development of more effective anti-osteoporosis drugs.**

Keywords: Traditional Chinese medicine (TCM), single herbs, active ingredients, postmenopausal osteoporosis, bone morphogenetic protein (BMP), estrogen receptor (ER)

1. Introduction

Postmenopausal osteoporosis is a systemic metabolic skeletal disease characterized by structural deterioration and high fragility of bone tissue, generally ascribable to a dearth of estrogen. Osteoporosis affects 200 million women worldwide, and the probability of women over 50 affected by an osteoporotic fracture has been estimated to approach one third (*1*). With an aging population, the economic burden of osteoporosis increases exponentially. It is often associated with pain and fractures leading to reduced quality of life such as depression, morbidity, and increased mortality, which is considered to be an important public health issue.

Therapeutic agents currently used for osteoporosis include menopausal hormone therapy (MHT), bisphosphonates, calcitonin, selective estrogen receptor modulators (SERMs), parathyroid hormone (PTH) analogs, and so on. However, there have been long-term safety concerns about these drugs. Adverse events in clinical use include increased risk of cardiovascular disease, venous thromboembolism, breast and endometrial cancer, and stroke with hormone therapy; gastrointestinal intolerance, jaw osteonecrosis, atypical femoral fractures, and atrial fibrillation with bisphosphonates; a possible oncogenic association with

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salmon calcitonin; lower efficacy on fracture prevention and increased risk of venous thrombotic events with SERMs; nausea, headache, dizziness, hypercalcemia, hypercalciuria with PTH analogs (*2*). Therefore, safer and more effective alternatives for the management of osteoporosis are being explored.

In recent years, a growing interest has risen in the treatment of postmenopausal osteoporosis with traditional Chinese medicine (TCM). Compared with Western medicine, TCM has fewer adverse events with long-term use, for which extensive experience has been accumulated over thousands of years. Chinese medicinal herbs usually exert their therapeutic effects through a "multi-components, multi-pathways, and multi-targets" mode, which is in accordance with the multi-factorial and complicated nature of postmenopausal osteoporosis (*3*). TCM will be continuously adopted as a cost-effective alternative to chemically synthesized medicines as well as excellent and reliable sources for derivation of natural products for the development of new drugs. A meta-analysis in 2009, which included 14 randomized controlled trials involving 780 patients with postmenopausal osteoporosis, suggested that phytotherapy might possess a similar effect as hormone therapy on bone mineral density (BMD) values with a lower incidence of breast pain and uterine bleeding (*4*). It was later contradicted by new evidence in 2017 which included 10 randomized controlled trials involving 957 patients and concluded that Chinese herbal medicine alone did not significantly improve lumbar spine BMD (*5*). Further high-quality clinical trials are required in this field.

In TCM, there is no well-defined disease known as postmenopausal osteoporosis. However, symptom profiles in classical records such as low back pain, fracture, spine deformities, and limb atrophy could be the manifestations of osteoporosis. According to the TCM theoretical extraction of pathogenesis and symptoms, postmenopausal osteoporosis belongs to the TCM syndromes of "Gubi", "Gushi (bone loss)", "Guwei (bone atrophy)", "Guku (lack of bone marrow)", and "Guji (bone polarization)" (*6*). The role of "kidney" in governing the bone and generating marrow was the starting point to understand TCM, proposed in *Su Wen* (*Plain Questions*) as early as the Spring and Autumn period and the Warring States (more than 2000 years ago) (*7*). Therefore, "kidney deficiency" is regarded as the underlying cause of all skeletal pathologies, and Chinese herbal medicine accordingly follows the principle of "tonifying the kidney" to treat both the surface symptoms and internal balance in management of postmenopausal osteoporosis (*8*). The kidney's role in bone metabolism was later recognized by Western medicine, as it discovered the importance of the kidney in regulating calcium-phosphorus homeostasis, generating active metabolites of Vitamin D, and so on (*9*). It is noteworthy that the "kidney's role" in Chinese medicine cannot be equated directly with renal function in Western medicine and the kidney in TCM theory involves the neuro-endocrine and reproductive systems.

2. Single herbs commonly used in postmenopausal osteoporosis

2.1. *Herba Epimedium*

Epimedium brevicornum Maxim is a centuries-old traditional herb. Derived from the dried leaf of Epimedium brevicornum Maxim, Herba Epimedium (known as YinYangHuo in Chinese) is a popular Chinese traditional herb with a broad range of indications, especially for fatigue, sexual dysfunction, rheumatic diseases, and osteoporosis. To date, over 260 individual constituents have been derived from plants of the Epimedium genus, including icariin, icaritin, anhydroicaritin, epimedin, and so on (*10*). According to TCM theory, Herba Epimedium could tonify the kidney and expel dampness, which contributes to strengthening tendons and bones.

2.1.1. *Clinical trials*

To determine the therapeutic effect of Herba Epimedium and to provide clear evidence for clinical practice, Wang *et al*. identified 37 clinical trials using Herba Epimedium in co-prescription with other TCM herbs as anti-osteoporotic drugs to address postmenopausal and senile osteoporosis whose overall efficacy (with markedly and moderately symptom improvement) was between 73% and 100% (*11*). And Herba Epimedium contributed 4.1% to 21.7% of relative weight in these therapeutic formulas. However, these studies could be further criticized because few of them met the standards of randomized, double-blind, placebo-controlled or involved adequate sample size and treatment duration.

2.1.2. *In vivo findings*

In vivo studies found that Herba Epimedium extract and its bioactive components could prevent ovariectomized (OVX) induced bone loss in rats, as evidenced by the suppression of BMD descent and the improvement of biomechanical properties and trabecular microarchitecture.

In evaluation of bone turnover biomarkers, Herba Epimedium was found to decrease serum alkaline phosphatase (ALP) activity and urinary deoxypyridinoline levels compared to the OVX group (*12*). Total flavones of Epimedium (TFE) inhibited reduction of procollagen type I N-terminal propeptide (PINP), and increased serum osteocalcin and type I collagen in OVX rats (*13,14*). Icariin, one of the major components of Herba Epimedium, decreased activities of serum tartrate-resistant acid phosphatase (TRAP) and

bone alkaline phosphatase (BALP), decreased serum osteocalcin and ALP activity, decreased C-terminal telopeptide of type I collagen (CTX) levels compared with the OVX group (*15-17*). Flavonoid fraction of Epimedium (FE), ipriflavone, and anhydroicaritin inhibited serum ALP and TRAP in OVX rats (*18*).

For calcium and phosphate homeostasis, Herba Epimedium decreased urinary calcium excretion and corrected serum calcium (*12,19*). TFE decreased urinary calcium excretion, lowered the urinary calcium/creatinine and phosphate/creatinine ratio, suppressed PTH elevation, increased bone calcium and phosphorus content and serum calcium compared to OVX group (*14*). Icariin corrected the decreased serum calcium and phosphate (*15*). Anhydroicaritin decreased urinary calcium and D-pyruvate/creatinine ratio while increasing bone calcium and phosphate (*20*). FE prevented osteoporosis independent of intestinal calcium absorption (*21,22*).

For neuro-endocrine regulation, Herba Epimedium and icariin corrected estrogen decrease in OVX rats (*15*). TFE improved serum estrogen and increased estrogen receptor α (ERα) and ERβ mRNA expression of hypothalamus and hippocampus (*23*). Herba Epimedium and TFE inhibited the mRNA expression of interleukin (IL)-6 induced by OVX (*14*).

In gene profile, TFE enhanced osteoprotegerin (OPG) mRNA expression, increased OPG/receptor activator of nuclear factor κB ligand (RANKL) ratio, and recovered expression of runt-related transcription factor 2 (Runx2) compared to the OVX group (*13,14*). FE increased OPG protein expression and reduced the RANKL protein expression in OVX rats (*18*). Icariin increased the mRNA expression ratio of OPG/RANKL, up-regulated mRNA expression of low-density lipoprotein receptor-related protein 6 (Lrp6) receptor, while it down-regulated glycogen synthase kinase-3β and Runx2, following OVX (*24,25*). Icariin was also found to up-regulate expression of bone morphogenetic protein 2 (BMP2), BMP4, Runx2, osteocalcin, Wnt1, and Wnt3a in OPG knockout mice, and increase the expression of the direct target genes of β-catenin signaling such as AXIN2, dickkopf-related protein 1 (DKK1), T cell factor 1 (TCF1), and lymphoid enhancer-binding factor 1 (LEF1) (*26*). Icariin administration altered 23 proteins in bone and 8 metabolites in serum, involving bone remodeling, energy metabolism, cytoskeleton, lipid metabolism, mitogen-activated protein kinase (MAPK) signaling, and calcium signaling (*17*). Icaritin increased levels of osteoblast-related gene expression compared to pretreatment OVX levels and decreased adipocyte and osteoclast-related gene expression towards pretreatment sham levels (*27*).

2.1.3. *In vitro findings*

In vitro studies showed that Herba Epimedium and its bioactive components could stimulate the proliferation, differentiation and mineralization of osteoblasts (*12,28-40*), suppress the adipogenesis of bone marrow-derived mesenchymal stem cells (BMSCs) (*41-44*), inhibit the proliferation and differentiation of osteoclasts (*45-47*), and induce apoptosis and cell cycle arrest and suppress bone resorption of osteoclasts (*48*). In addition, icariin was found to significantly attenuate oxidative stress and apoptosis and preserve viability and osteogenic potential of osteoblasts exposed to hypoxia, which indicated that its anti-osteoporotic effect might be attributed to its anti-hypoxic activity (*49*).

Taken together, the following pathways were involved in the osteogenesis effect of Herba Epimedium and its constituents: *i*) activate extracellular-signal-regulated kinase (ERK), p38, c-Jun N terminal kinase (JNK)/MAPK pathways in rat BMSCs (*50*), *ii*) through ER-mediated ERK and JNK signal activation in MC3T3-E1 osteoblastic cell line (*51*), *iii*) *via* activating phosphatidylinositol-3-kinase (PI3K)-protein kinase B (AKT)-endothelial nitric oxide synthase (eNOS)-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) signal pathway in rat BMSCs (*36*), *iv*) *via* BMP or Wnt/β-catenin signaling pathway in human BMSCs (*28*), rat BMSCs (*26*), UMR-106 osteoblastic cells, and osteoblasts in neonatal rat calvaria cultures (*52*), *v*) *via* BMP2/SMAD4 signal pathway in hFOB 1.19 human osteoblastic cell line (*34*), *vi*) involve the ERα-Wnt/β-catenin signaling pathway in rat BMSCs (*38*), *vii*) through up-regulation of transforming growth factor (TGF)-β1, BMP2 expression in rabbit BMSCs (*30*), through up-regulation of BMP2 and Runx2 mRNA expression in calvarial osteoblasts from pups (*13*), *viii*) *via* Notch signaling pathway in rat BMSCs (*53*), *ix*) increase OPG and the OPG/RANKL ratio in UMR-106 osteoblastic cells (*12*), and *x*) stimulate ER-dependent osteoblastic functions and activate ER in a ligand-independent manner in UMR-106 osteoblastic cells (*24,39*).

At least three pathways were involved in the inhibition of adipogenesis in rat BMSCs: *i*) reduce peroxisome proliferator-activated receptor γ (PPARγ) mRNA expression (*41*), *ii*) down-regulate expression of DKK1 protein (*42*), and *iii*) activate Wnt/β-catenin signaling pathway (*43*).

The following pathways were involved in the anti-resorptive effect of Herba Epimedium and its constituents in co-culture of BMSCs and osteoblasts: *i*) suppress MAPKs/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) regulated hypoxia inducible factor (HIF)-1α and prostaglandin E2 (PGE2) synthesis (*45*), *ii*) inhibit p38 and JNK activation, *iii*) up-regulate expression of OPG while down-regulating RANKL, *iv*) repress the synthesis of cyclooxygenase-2 and PGE2, *v*) inhibit IL-6 and tumor necrosis factor (TNF)-α expression, and *vi*) interact with nuclear ERs *via* the mitochondrial pathway (*48*).

2.2. *Rhizoma Drynariae*

The traditional Chinese herb Rhizoma Drynariae (Gu-Sui-Bu) is commonly used to manage musculoskeletal traumatic disorders of orthopedics with satisfactory results, as it tonifies the kidney, activates blood circulation, and promotes tissue regeneration. Modern pharmacological studies have revealed more than 300 different constituents in this herb, including favonoids, triterpenoids, phenylpropanoids, lignans, phenolic acids, and so on (*54*).

2.2.1. *Clinical findings*

A meta-analysis performed in 2017, which included 6 randomized controlled trials involving 846 patients, showed that both the flavonoids from Rhizoma Drynariae and the combined therapy alone were better than conventional treatments in improving BMD value with no severe adverse drug reactions (*55*). This conclusion needs future research to confirm.

2.2.2. *In vivo preclinical data*

Rhizoma Drynariae had a similar effect compared to estrogen in maintaining normal trabecular structure and connections by inhibiting the increased bone turnover of postmenopausal osteoporosis (*56*). Drynariae total flavonoids could decrease cathepsin K mRNA and increase bending load compared to OVX group (*57*). Drynariae flavonoid fraction exerted dose-dependent effects in improving BMD, bone strength at the femur, tibia and lumbar spine in OVX mice (*58*). Naringin reversed OVX-induced bone loss *via* increasing BMD, bone volume, trabecular thickness, and mechanical strength (*59,60*). Naringin up-regulated vascular endothelial growth factor (VEGF) mRNA expression and vascular endothelial growth factor receptor (VEGFR)-2 mRNA and protein expression, which increased the number of vessels, vessel volume, and vessel thickness around the osteoporotic fracture sites (*61*).

2.2.3. *In vitro preclinical data*

Rhizoma Drynariae and its bioactive constituents were able to promote the osteoblastic proliferation, differentiation, and maturation (5*6,58,59,62-70*), inhibit osteoblastic apoptosis (*71*), suppress osteoclastogenesis (*72-74*), promote the apoptosis of osteoclasts (*60*), stimulate both cellular and humoral immunity (*75*), and inhibit cathepsins K processing (*76,77*).

Mechanisms involved in the osteogenesis and osteolysis effect of Rhizoma Drynariae and its components included: *i*) promote proliferation, differentiation, and maturation of rat calvarial osteoblasts (*64*) and UMR-106 osteoblastic cells (*58,68*) *via* ER pathway, *ii*) promote osteoblastogenesis from rat BMSCs *via* the Notch signaling pathway (*70*), *iii*) inhibit osteoclastogenesis of human amniotic fluid-derived stem cells (hAFSCs) *via* elevating OPG/RANKL ratio and induce the osteogenesis of hAFSCs *via* the BMP and Wnt/β-catenin pathways (*72*), and *iv*) promote the apoptosis of osteoclasts by regulating the mitochondrial apoptosis pathway using RAW 264.7 cells (*60*).

2.3. *Salvia miltiorrhiza*

Salvia miltiorrhiza, known as Danshen in Chinese, is one of the best-known Chinese traditional herbs whose root has been clinically exploited in treating postmenopausal syndrome. There are more than 100 compounds isolated from Salvia miltiorrhiza, such as tanshinone, salvianic acid, and flavonoids (*78*). As is recorded in *Qian Jin Fang*, the application of Salvia miltiorrhiza for treating blood stasis and injuries dated back over hundreds of years. In TCM, Salvia miltiorrhiza has been described to remove blood stasis, promote menstrual blood flow, and reduce pain. It is commonly used in patients with menstrual disorders, blood stasis, and rheumatism.

2.3.1. *Clinical data*

Guo *et al*. reported in 2014 that 25 clinical trials were conducted in which primary osteoporosis was treated with Salvia miltiorrhiza plus other herbs with an overall efficacy of 85% to 96% in markedly and moderately symptom improvement (*78*). Due to the huge variation in trial protocols, the exact therapeutic effects of Salvia miltiorrhiza could not be assessed from the available data.

2.3.2. *In vivo preclinical data*

Chae *et al*. demonstrated that the aqueous extracts of Salvia miltiorrhiza could enhance bone mechanical strength and prevent trabecular bone resorption in OVX Sprague-Dawley rats (*79*). Cui *et al*. found that Salvia miltiorrhiza prevented OVX-induced bone loss probably due to its anti-oxidative stress and partly *via* modulation of osteoclast maturation and number, because it decreased the osteoclast activation marker TRAP-5b and oxidative stress parameters malondialdehyde (MDA) and NO induced by OVX (*80*). Individual compound tanshinone also prevented a decrease in trabecular bone volume and trabecular number and an increase in osteoclast surface in vertebra, and partially prevented a decrease in trabecular bone volume and trabecular number in the tibia (*81*).

2.3.3. *In vitro findings*

Salvia miltiorrhiza increased osteoblast number and inhibited osteoclastogenesis (*82*). Isolated from Salvia

miltiorrhiza Bunge, tanshinone IIA, tanshinone I, cryptotanshinone, 15,16-dihydrotanshinone I, and ferruginol were found to reduce the formation of TRAP positive multinuclear osteoclasts (*83*). Tanshinol was able to ameliorate the accumulation of reactive oxygen species, decrease in cell viability, cell cycle arrest and apoptosis, and inhibition of osteoblastic differentiation induced by hydrogen peroxide (*84*). Salvianic acid A increased ALP activity, type I collagen mRNA and OPG mRNA expression, and stimulated nodule mineralization of rat osteoblasts. It stimulated osteogenesis and repressed adipogenesis from BMSCs (*85*).

Mechanisms involved in the osteogenesis and osteolysis effect of Salvia miltiorrhiza and its constituents included: *i*) first down-regulate and then up-regulate OPG/RANKL in MC3T3-E1 osteoblastic cell line (*86*), *ii*) promote osteogenesis through the ERK signaling pathway in human mesenchymal stem cells (*87*), *iii*) attenuate oxidative stress *via* down-regulation of forkhead box O3 (FoxO3a) signaling, and rescue the decrease of osteoblastic differentiation through up-regulation of Wnt signal under oxidative stress in pluripotent mesenchymal precursor C2C12 cells and preosteoblastic MC3T3-E1 cells (*84*), *iv*) inhibit osteoclast formation by inhibiting the expression of c-Fos and nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) induced by RANKL in a rat BMSCs/calvarial osteoblast co-culture system (*88*), *v*) prevent osteoclast differentiation by inhibiting RANKL expression and NF-κB induction in co-culture of monocyte-macrophage cell line RAW 264.7 and osteoblast cell line type CRL 12257 (*89*), and *vi*) reduce the number and activity of osteoclasts *via* suppression of RANK activated AKT, NF-κB, and MAPKs signal transduction in rat BMSCs/calvarial osteoblasts co-culture system (*90*).

3. Active ingredients commonly used in postmenopausal osteoporosis

As forms of Chinese herbal medicine, active ingredients of Chinese medicine are isolated from single herbs or traditional herbal formulas and prepared using modern advanced pharmaceutical technology, such as icariin from Herba Epimedium, naringin from Rhizoma Drynariae, and tanshinone from Salvia miltiorrhiza. Compared to traditional decoctions, active ingredients have various dosage forms including injections, tablets, pills, capsules, and liquids. They are safer, more effective, and easier to use. Thus, active ingredients of Chinese medicine become increasingly popular in China and attract worldwide attention.

3.1. *Saikosaponins*

Radix Bupleuri, made from dried roots of Bupleurum scorzonerifolium Willd, is commonly used in the prescriptions of traditional Chinese medicine. It has been utilized to treat various discomforts, including influenza, malaria, chronic hepatitis, and menstrual disorders. Saikosaponins, major bioactive compounds isolated from Radix Bupleuri, have exhibited anti-inflammatory, antimycotic, and immuno-regulatory pharmacological properties.

Saikosaponin A and saikosaponin D significantly repressed inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 expression, reduced TNF-α and IL-6 production, and inhibited NF-κB translocation in lipopolysaccharide (LPS)-induced murine macrophage cell line RAW264.7 cells (*91*). *In vitro* study revealed that saikosaponin A suppressed osteoclastogenesis in C57/BL6 mice bone marrow monocytes and mediated osteoclast differentiation through inhibiting RANKL-induced p38, ERK, JNK, and NF-κB activation in murine RAW264.7 cell line (*92*). Saikosaponins showed a potent anti-inflammatory and anti-osteoporotic effect as safe and effective agents for management of postmenopausal osteoporosis.

3.2. *Linarin*

Flos Chrysanthemi Indici, one of the most important drugs in traditional Chinese medicine, possesses biological properties such as antioxidative, antibacterial, antiviral, and antimycotic effects. Linarin, a natural flavonoid compound in Flos Chrysanthemi Indici, has been shown to preserve the trabecular bone microarchitecture of OVX C57/BL6 mice. Linarin enhanced osteoblast differentiation and mineralization in MC3T3 E1 cells, mediated by activating the BMP2/Runx2 pathway *via* protein kinase A (PKA) signaling pathway (*93*).

3.3. *Echinacoside*

Echinacoside is one of the major constituents of Herba Cistanches, a famous traditional Chinese medicine. As a natural polyphenolic compound, echinacoside possesses effective antiinflammatory, antioxidative, neuroprotective, hepatoprotective, and vasodilative properties. Administration of echinacoside could effectively and safely prevent bone loss in OVX-induced Sprague-Dawley rats through increasing OPG/RANKL ratio, which revealed its potential of developing into a novel agent for treatment in postmenopausal osteoporotic women (*94*).

3.4. *Sweroside*

Fructus Corni has wide application in the clinic with a long history, of which Sweroside is an important constituent. Modern pharmacology shows that sweroside has a variety of pharmacological functions including vasorelaxation, antihepatitis, antiinflammatory, and

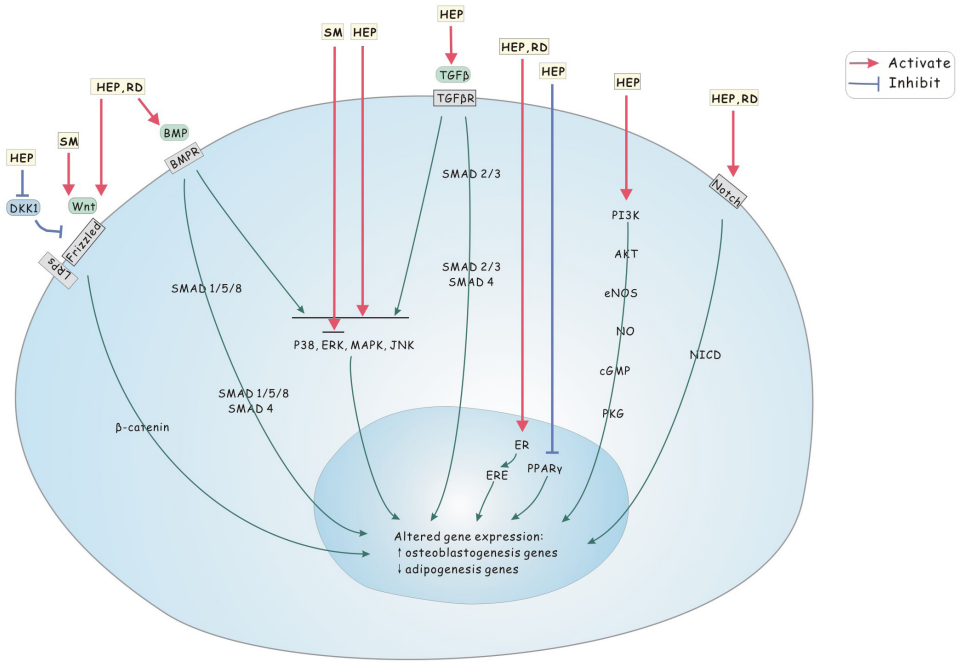

Figure 1. The mechanism of action of Chinese single herbs on pre-osteoblasts. Chinese single herbs interact with at least eight pathways for the treatment of osteoporosis in pre-osteoblasts: *i*) *via* BMP signaling pathway, *ii*) *via* Wnt/β-catenin pathway, *iii*) activate ERK, p38, JNK, MAPK pathways, *iv*) *via* up-regulation of TGF-β1 expression, *v*) through ER signal activation, *vi*) activate PI3K-AKT-eNOS-NO-cGMP-PKG signal pathway, *vii*) *via* Notch signaling pathway, and *viii*) reduce PPARγ mRNA and DKK1 protein to inhibit adipogenesis. (*Abbreviations*: HEP, Herba Epimedium; SM, Salvia miltiorrhiza; RD, Rhizoma Drynariae; DKK1, dickkopf-related protein 1; LRPs, lipoprotein receptor-related proteins; BMP, bone morphogenetic protein; BMPR, BMP receptor; JNK, c-Jun N terminal kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular-signal-regulated kinase; TGFβ, transforming growth factor β; TGFβR, TGFβ receptor; ER, estrogen receptor; ERE, estrogen-response element; PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; NICD, Notch intracellular domain; PPARγ, peroxisome proliferator-activated receptor γ.)

antiallergic effects. Sweroside was found to effectively induce proliferation and inhibit apoptosis in human osteosarcoma cell line MG-63 and Wistar rat osteoblastic cells (*95*), which displayed bright prospects as a therapeutic natural product for osteoporosis.

3.5. *Psoralen*

Psoralen is extracted from Psoralea corylifolia, which is one of the most commonly prescribed herbs for the treatment of bone and joint diseases. Psoralen was found to promote *in vitro* osteoblast differentiation dose-dependently, evidenced by increased ALP activity and enhanced expression of osteoblast-specific marker genes such as type I collagen and osteocalcin. Psoralen might act through the BMP signaling pathway as it could increase the gene expression of BMP2 and BMP4 as well as the protein level of phospho-SMAD1/5/8 (*96*).

3.6. *Poncirin*

Poncirin is isolated from Poncirus trifoliata and possesses anti-bacterial and anti-inflammatory activities. Studies have showed that poncirin could inhibit adipogenesis and enhance osteoblast differentiation in BMSCs. In C3H10T1/2 mesenchymal stem cells, poncirin prevented adipocyte differentiation, demonstrated by decreased accumulation of cytoplasm lipid droplets and down-regulated mRNA expression of PPAR-γ and CCAAT-enhancer-binding protein-β (C/EBP-β). In murine BMSCs, poncirin enhanced expression of Runx2, ALP, and osteocalcin, and increased mineral nodule formation (*97*).

3.7. *Vanillic acid*

Vanillic acid is a phenolic acid isolated from the bioactive fraction of Sambucus williamsii Hance. It stimulated the proliferation and ALP activity in rat osteoblast-like UMR 106 cells, and also increased mRNA expression of genes involved in osteoblast functions and osteoclastogenesis such as Runx2, osteocalcin, and the OPG/RANKL ratio. Its bone protective effects might be mediated through ER and MAPK pathways (*98*).

3.8. *Osthole*

Osthole, extracted from Fructus Cnidii, was found to notably improve bone microarchitecture, histomorphometric parameters, and biomechanical properties of OVX rats. It could activate Wnt/β-catenin pathways, up-regulate BMP2 expression, and stimulate

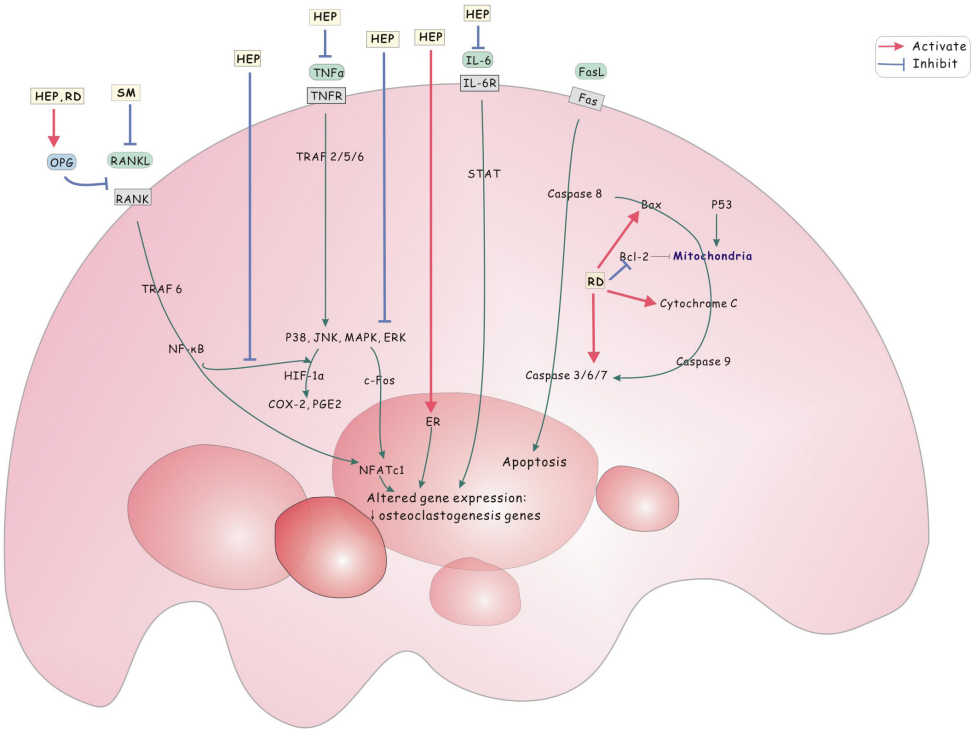

Figure 2. The mechanism of action of Chinese single herbs on pre-osteoclasts. Chinese single herbs interact with at least six pathways in pre-osteoclasts: *i*) up-regulate expression of OPG while down-regulate RANKL, *ii*) suppress MAPKs/NF-κB regulated HIF-1α and PGE2 synthesis, *iii*) inhibit p38, JNK, MAPK, ERK pathways, *iv*) inhibit IL-6 and TNF-α expression, *v*) interact with nuclear ERs, and *vi*) down-regulate the mRNA expression levels of bcl-2 and up-regulate Bax, caspase-3 and cytochrome c. (*Abbreviations*: HEP, Herba Epimedium; RD, Rhizoma Drynariae; SM, Salvia miltiorrhiza; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor κB; RANKL, RANK ligand; TNF-α, tumor necrosis factor α; TNFR, TNF receptor; TRAF, TNF receptor-associated factor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; JNK, c-Jun N terminal kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular-signal-regulated kinase; HIF-1α, hypoxia inducible factor 1α; COX-2, cyclooxygenase-2; PGE2, prostaglandin E2; NFATc1, nuclear factor of activated T-cells, cytoplasmic 1; ER, estrogen receptor; IL-6, interleukin 6; IL-6R, IL-6 receptor; STAT, signal transducer and activator of transcription; FasL, Fas ligand.)

osteoblast differentiation *in vitro* (*99*).

4. Conclusion

In conclusion, Chinese herbal medicine substantially influences postmenopausal osteoporosis as a safer and more effective alternative. However, current clinical studies are not well funded to prove their therapeutic efficacy because most of the studies contain a small sample size and short treatment duration, and their clinical parameters and biomarkers for analysis differ from each other. Both *in vivo* and *in vitro* studies reveal the anti-osteoporotic effect of single herbs targeting different pathways in bone metabolism (Figure 1 and Figure 2). Apart from restoring the balance between osteoblasts and osteoclasts, Chinese single herbs have also been shown to inhibit adipocyte differentiation and exert anti-inflammatory, immuno-regulatory, anti-oxidative, and estrogen-like functions. This review should contribute to a better understanding of Chinese single herbs and active ingredients as treatment for postmenopausal osteoporosis and provide useful information for the development of more effective anti-osteoporosis drugs.

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