Osteoprotective effects of flavonoids: Evidence from *in vivo* and *in vitro* studies (Review)

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Abstract. Osteoporosis is a systemic bone disease characterized by decreased bone mass and quality and bone micro-architecture degradation. Its primary cause is disorder of bone metabolism: Over-formation of osteoclasts, resulting in increased bone resorption and insufficient osteogenesis. Traditional herbal flavonoids can be used as alternative drugs to prevent and treat osteoporosis due to their wide range of sources, structural diversity and less adverse effects. The present paper reviewed six flavonoids, including quercetin, icariin, hesperitin, naringin, chrysin and pueraria, that promote bone formation and have been widely studied in the literature over the past five years, with the aim of providing novel ideas for the development of drugs for bone-associated disease.

Contents

1. Introduction

Osteoporosis is a systemic bone disease characterised by low bone mass and degeneration of bone tissue micro-architecture, leading to increased bone fragility and susceptibility to fractures (1). Strategies to prevent and treat osteoporosis include preventive (such as increased vitamin D and protein intake, exercise, smoking cessation, avoidance of excessive alcohol intake and prevention of falls) and therapeutic measures (2). Clinically used drugs include bisphosphonates (such as alendronate, risedronate and ibandronate), antibodies against nuclear factor-κB ligand receptor activator (RANKL; for example, denosumab), selective oestrogen receptor modulators (SERMs; for example, raloxifene), parathyroid hormone (PTH), PTH-related peptides (such as teriparatide and abaloparatide) and calcitonin (such as salmon calcitonin). However, clinical use of most anti-bone resorption drugs is limited by side effects of long-term inhibition of bone resorption, such as upper gastrointestinal bleeding, acute-phase reaction, hypocalcaemia, secondary hyperparathyroidism and most drugs on the market are expensive (3,4). With the ageing world population, the incidence of osteoporosis is increasing annually, with an increase of 70.1% in 2019 compared with 1990, endangering quality of life of middle-aged and older adults and imposing a burden on society and families (5).

Flavonoids are compounds formed when two benzene rings (A and B rings) are joined by a pyran heterocyclic ring (C ring) consisting of a central three-carbon chain (6). The compounds are found in the roots, stems, leaves, fruit and seeds of plants such as strawberries, onions and cucumbers (7). Flavonoids are diverse in structure and most are similar to oestrogen and exert anti-inflammatory, antibacterial, anti-cancer, antioxidant, osteogenic, osteoclast-inhibitory and oestrogen-like effects (8).

Multiple studies have confirmed that flavonoids have a promoting effect on osteogenesis and the underlying mechanism has received attention and become a hotspot in developing novel osteoporosis drugs (9,10). The present review focused on the role of flavonoids in osteoporosis.

2. Definition of osteoporosis, diagnostic criteria and epidemiological investigation

According to the World Health Organization (WHO), osteoporosis is defined as a bone disease characterized by a decrease in bone strength that puts a person at increased risk of fracture. Bone strength primarily reflects a combination of bone density and bone mass. The majority of the patient population with this

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disease is postmenopausal women (11). Postmenopausal osteoporosis is defined as bone density that is ≤2.5 standard deviations the mean for women aged 25-50 years. (T-score ≤2.5) (12,13). Therefore, clinical diagnosis and assessment of osteoporosis are based on measuring bone mineral density (BMD) (14). The results of an epidemiological survey on osteoporosis conducted by the National Health Council of China in 2019 demonstrated that the prevalence of osteoporosis in China is 3.2 for those aged 40-49, 19.2 for those aged 50-64 years and 32.0% for those aged ≥65 years (15). In the United States, ~10 million people aged >50 years have osteoporosis (16). In the United Kingdom, 50% of women and 20% of men aged >50 years suffer from osteoporotic fractures (17,18). Fragility fractures caused by osteoporosis lead to increased morbidity and mortality, resulting in a social burden (19). Thus, research on the prevention and treatment of osteoporosis has become an urgent priority (20).

3. Pharmacological studies of flavonoids

Flavonoids are compounds that are widely found in nature, and so far, >9,000 flavonoids have been reported (21,22) Flavonoids comprise subclasses, including flavonoids (such as lignans, rutin, bryophyllin and baicalin), flavanones (such as naringenin and hesperidin), isoflavones (such as soy flavonoids and genistein), pro-anthocyanidins, flavanols (such as catechins and epicatechin) and flavonols (such as kaempferol, yohimbine and quercetin), which all have a basic flavan structure (2-phenylchroman) (23). The present review focuses on six well-studied flavonoids (Fig. 1).

Quercetin. Quercetin is derived from the genus *Quercus* and has been used as a dietary supplement since 1857 (24,25). It has been isolated from >20 plant materials in the United States, Europe, Asia and South Africa (26-28). Moreover, it is present in fruits and vegetables, such as onions, tomatoes, peppers, lettuce, radish leaves, papaya, grapes, oranges and strawberries, as well as many seeds, nuts (including almonds and pistachios), flowers, bark and leaves (26,29-37). Quercetin is a key flavonol, accounting for the highest percentage of total dietary flavonoid intake (38). Quercetin is 3,3,4,5,7-penthydroxy flavonoid and most other flavonoids exhibit this basic structure (39,40). Due to the presence of phenolic hydroxyl groups and double bonds, quercetin exhibits antioxidant activity (41). It exerts pharmacological activity, such as anti-cancer, anti-inflammatory and antimicrobial activity, as well as anti-ulcer, anti-allergy, antitumor, anti-viral, anti-diabetic, anti-hypertensive, anti-infective, stomach-protective and immunomodulatory effects and protects against bone loss (42,43).

Icariin (ICA). With the development of modern separation techniques and ethnopharmacology, increasing evidence has shown that ICA is one of the primary biologically active monomers extracted from *Epimedium* (44,45). *Epimedium*, also known as Ninebark, was recorded 400 years ago in the traditional Chinese medical text Shennong Ben Cao Jing and is used in various herbal formulations (46). The herb is considered a complementary and alternative medicine and has been demonstrated to possess therapeutic effects in fractures, joint disease and gonadal dysfunction (36). There are >40 species of the genus worldwide, primarily found in southwest and central China, with a total of 27 species and four varieties, accounting for ~70% of the total global number (47). More than 260 components have been extracted from *Epimedium*, including 141 flavonoids and 31 lignans. Among them, flavonoid glycosides have been identified as key pharmacologically active ingredients (48,49). ICA is the primary active ingredient of *Epimedium* and was selected as a chemical marker for quality control of *Epimedium* in the Chinese Pharmacopoeia (44). It is a light-yellow powder with a molecular formula of $C_{33}H_{40}O_{15}$ and a molecular weight of 676.67 g/mol. ICA has a variety of pharmacological activities, including anti-inflammatory, anti-oxidant, anti-cancer, anti-osteoporosis, anti-hepatotoxic, anti-depressant and neuroprotective effects and protects against cardiac ischaemia and atherosclerosis (50,51).

Hesperitin. Hesperitin (3',5,-trihydroxy-4-methyl-7-xanthone) is a key component of citrus plants in the *Rutaceae* family. It has the molecular formula $C_{16}H_{14}O_6$ and is a member of the flavonoid subclass flavones, primarily found in citrus fruits (52). Like most flavonoids, hesperetin naturally occurs in the form of glycosides, known as hesperidin, first isolated from citrus peel by the French chemist Le Breton (53). Citrus bioflavonoids (including hesperidin) appear safe and do not cause side effects even during pregnancy. Dietary hesperidin is deglycosylated to hesperetin by intestinal bacteria before absorption (54,55). Its structure consists of ketone carbonyl, ether, methoxy and phenolic hydroxyl groups, allowing for a wide range of pharmacological effects, such as antioxidant, anti-allergic, and anti-inflammatory effects (56-59).

Naringin. Naringin is a flavonoid and key secondary metabolite. Bioactive naringin compounds are found in plant-based foods, such as vegetables, fruit, tea and wine (60). Naringin-derived drugs are used in traditional medicine because of their non-addictive and non-toxic properties (61). Studies have demonstrated that naringin has antioxidant, anti-microbial, anti-mutagenic, anti-cancer, bone-protective, anti-inflammatory and cholesterol-lowering effects (62-65).

Chrysin. Chrysin, also known as poplar flavonoid, is 5,7-dihydroxyflavone. It is found in propolis, honey, passion fruit, mushrooms and other plant sources. Due to its multiple pharmacological (anticancer, antitumor, antidiabetic, antioxidant stress, antiinflammatory, anti-obesity, antiallergic, hepatoprotective, reproductive organ-protective, neuroprotective and cardioprotective) effects and low toxicity. Therefore, chrysin has potential medicinal value (66-76).

Pueraria. Puerarin is an isoflavone derived from dried root of the legume *Pueraria lobata*, known as wild kudzu or 'Asian ginseng' (77). It was first reported in Shennong Ben Cao Jing and has a long history of treating disease. Kudzu root contains abundant isoflavones, primarily soybean base and isoflavones and puerarin (78). Puerarin, has a structure similar to estradiol and has the molecular formula $C_{21}H_{20}O_9$ and a relative molecular weight of 416; it was separated from *Pueraria lobata* in the late 1950s. Since then, its pharmacological properties have been extensively researched (68). It has been used to treat vascular disease, diabetes and its complications, cancer, bone-associated, Parkinson's and Alzheimer's

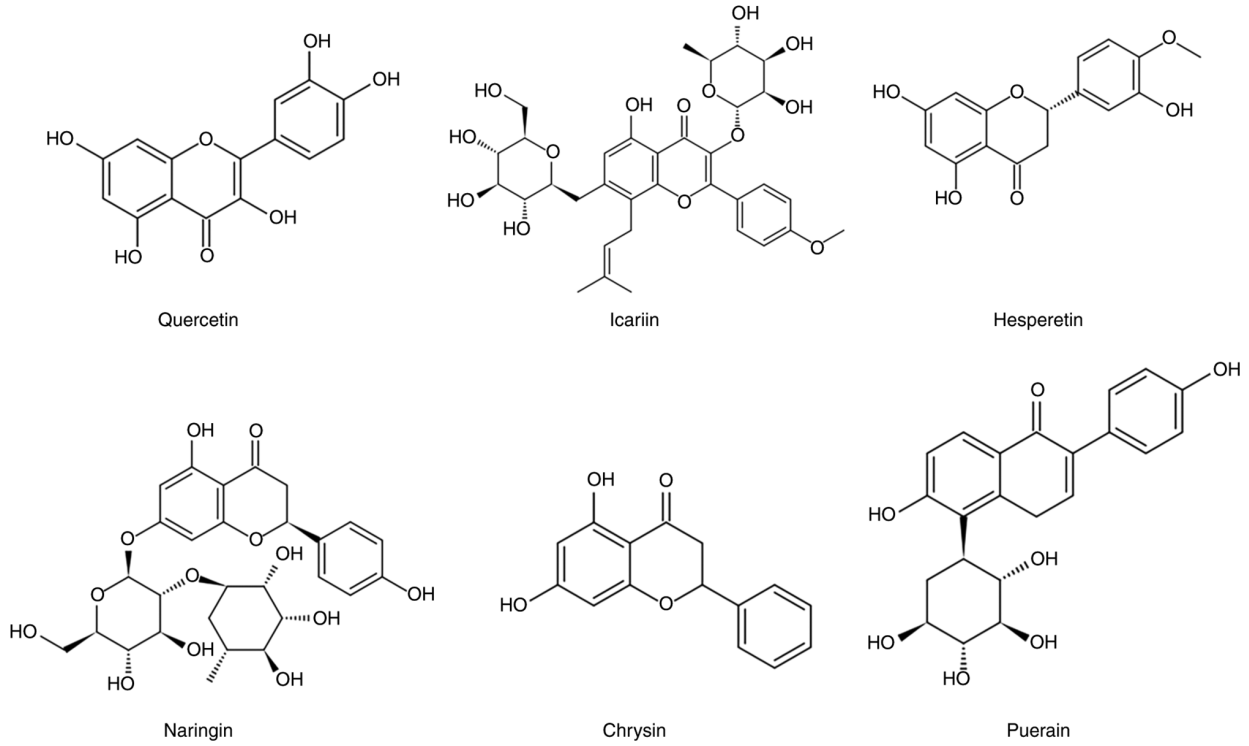

Figure 1. Chemical structure of six flavonoids.

disease, inflammation and alcohol-induced disease and exhibits antioxidant activity (79-88).

4. Use of flavinoids to prevent and treat osteoporosis at the cellular level and its signaling pathway

Physiological bone remodelling process. Bone is in a constant state of remodeling, which is key for maintaining structure and function. Imbalances can lead to disease, such as osteoporosis. Numerous types of cell and cytokine, hormones and signaling pathway are involved in bone remodeling. Osteoblasts and osteoclasts are responsible for new bone formation and resorption, respectively (89-91).

Osteogenic effects of flavonoids on osteoblasts and associated signaling pathways

Bone morphogenetic protein (BMP)/Smads signalling pathway. BMPs are members of the transforming growth factor β superfamily and were first identified in 1960 (92). However, they were not isolated and purified until the late 1980s (93,94). To date, ~20 BMPs have been identified. BMP signaling is associated with bone formation (95,96). BMP is the initial inducer of osteoblastogenesis during bone development. It binds to type II BMP receptors to phosphorylate them; activated type II BMP receptors phosphorylate type I BMP receptors and bind to form complexes (97). The activated complex further activates the downstream BMP signaling protein receptor (R-Smad). Phosphorylated R-Smad binds to Smad4 and migrates to the nucleus, where it serves as a transcriptional enhancer and interacts with the transcription factors Runx2 and Osterix to affect the transcription of osteogenic-associated genes (98). Differentiation of bone precursor cells and initiation of osteoblast-specific factors (such as alkaline phosphatase) promote bone formation (99-101).

Quercetin has been shown to possess positive pharmacological effects on bone metabolism, such as preventing bone loss (43,102). Zhou *et al* (103) showed that 10 and 50 μM quercetin stimulates gene expression of the osteoblast markers, bone morphogenetic protein 2 (BMP-2), Runx2, osteopontin (OPN), osteocalcin (OCN), collagen type 1 (COL-1) and osterix in mouse adipose stem cells (mASCs) *in vitro* but does not affect proliferation. The osteogenic effect of quercetin at certain concentrations has not yet been determined (104). By contrast to Zhou, another study showed that quercetin enhances proliferation of bone marrow mesenchymal stem cells (BMSCs) on days one, four and seven after dosing in a dose-dependent manner with the greatest effect at a concentration of 2 μM. In the aforementioned study, quercetin enhanced alkaline phosphatase (ALP) activity and especially the middle and late markers (bone sialoprotein (BSP), BMP-2, OPN and OCN) in a dose-dependent manner, with the greatest stimulation occurring at 2 μM. Furthermore, quercetin not only promoted osteogenic differentiation of BMSCs, but also promoted secretion of angiogenic factors in a dose-dependent manner, with the most significant effect at a concentration of 2 μM (103). Liu *et al* (105) found that naringin addition had a bidirectional effect on the cell proliferation and ALP activity of human amniotic fluid-derived stem cells (hAFSCs). At a concentration of 200 μg/ml, naringin inhibited the growth and moderately increased the ALP activity of hAFSCs; while at lower concentrations (1-100 μg/ml), naringin significantly enhanced the proliferative capacity and ALP activity of hAFSCs in a dose-dependent manner. In addition, naringin promotes osteogenic differentiation of hAFSCs via BMP signalling pathways; this finding, however, has only been

assessed *in vitro*. Menon *et al* (106) showed that chrysin released from a chitosa/carboxymethyl cellulose/nanohydroxyapatite stent stimulates proliferation of mouse mesenchymal stem cells (mMSCs) and promotes osteoblast differentiation; this may be due to upregulation of Runx2 and downregulation of Runx2 co-repressors.

Wnt/β-catenin signalling pathway. The Wnt/β-catenin signalling pathway activates transcription of Wnt gene in the nucleus via β-catenin (107). When the extraneous osteoblast Wnt factor binds to the membrane receptor frizzled, a series of membrane and cytoplasmic protein interactions lead to dimer formation. This results in β-catenin accumulation in the cytoplasm and subsequent entry into the nucleus (108). T cell/lymph enhancement factors combine to form a complex, which activates transcription of downstream target genes and promotes differentiation and proliferation of osteoblasts (109).

Lin *et al* (110) demonstrated that naringin promotes osteoblast formation via activation of osteogenic genes such as forkhead box protein C2 (Foxc2), core binding factor α1 (Cbα1) and OCN in *in vitro* osteogenic differentiation of BMSCs while decreasing peroxisome proliferator-activated receptor γ2 (PPARγ2) and upregulating Foxc2 expression partly via the Indian hedgehog signaling pathway. This revealed the mechanism by which naringin promotes osteogenesis of bone marrow MSCs. However, no *in vivo* animal experiments were performed to verify this. Liu *et al* (105) revealed that naringin may promote osteogenic differentiation of hAFSCs via the Wnt/β-catenin signaling pathway.

MAPK signalling pathway. The MAPK signaling pathway is key for regulation of osteoblast proliferation and differentiation. MAPKs are a group of serine/threonine kinases that serve key signaling transducer roles in translating extracellular stimuli into cellular responses (111). Activation of the MAPK cascade occurs via sequential phosphorylation of three protein kinases. Upon stimulus, MAPKK kinases (MAPKKKs) is activated, phosphorylating MAPK kinases (MAPKKs), which then phosphorylates MAPK (112). Certain studies have suggested that there are three primary pathways involved in MAPK signaling: Extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 pathway; different pathways receive different stimuli and serve different roles (113,114).

Zhou *et al* (103) reported that quercetin activates the ERK and p38 signaling pathways but not the JNK signaling pathway. Wu *et al* (115) demonstrated that the optimal concentration of ICA to promote osteogenic differentiation of bone marrow MSCs is 20 μM and ERK, p38 and JNK MAPK signaling pathways are involved in this process. Liu *et al* (116) discovered that hesperetin relieves glucocorticoid-induced osteogenic differentiation of BMSCs via the ERK signaling pathway. Nonetheless, the aforementioned study has certain limitations. First, bone marrow MSCs and glucocorticoid-induced osteoporosis (GIOP) osteogenic differentiation involve multiple signaling pathways. This experiment only investigated the ERK signaling pathway and whether other signaling pathways are involved in this process remains unclear. To the best of our knowledge, there is only one *in vitro* study and the effect of hesperetin on GIOP *in vivo* is still ambiguous. Xue *et al* (117) revealed that hesperetin promotes osteogenic differentiation of hBMSCs *in vitro,* potentially via ERK and Smad signaling pathways. The aforementioned study has limitations. First, only a low concentration (1 μM) of hesperetin was used to investigate the effect of hBMSC osteogenic differentiation and its underlying mechanism. Therefore, the effect of different concentrations of hesperetin on osteogenic differentiation of hMSCs and its mechanism needs to be further studied. Secondly, both ERK and Smad1/5/8 signaling pathways are involved in hesperetin-induced MSC osteogenic differentiation and it is unknown whether there is crosstalk between the ERK and Smad1/5/8 pathways. Yang *et al* (118) suggested that activation of ERK1/2 and p38 MAPK signaling pathways is involved in Puerarin-mediated osteogenesis and that the ERK1/2 signaling pathway plays a more significant role than the p38 signaling pathway in the induction of osteogenic phenotypic differentiation of BMSCs by puerarin.

PI3K/AKT signaling pathway. The PI3K/AKT signaling pathway is widely present in cells (119). PI3K family members are proto-oncogenes and key kinases of inositol and phosphatidylinositol (120). AKT is a serine/threonine-protein kinase and downstream target kinase in the PI3K signal transduction pathway (121). The PI3K/AKT signaling pathway regulates proliferation, differentiation and apoptosis of osteoblasts and osteoclasts (122,123).

Zhai *et al* (124) revealed that ICA promotes osteogenesis of BMSCs via the PI3K/AKT/endothelial NO synthase (eNOS)/NO/cyclic guanosine monophosphate (cGMP)-protein kinase-G (PKG) signaling pathway. However, the mechanism underlying PI3K activation by ICA should be further investigated. Lv *et al* (125) demonstrated that Puerarin at concentrations <10 μM stimulates proliferation and osteogenic differentiation of bone marrow MSCs in a dose-dependent manner. The estrogen receptor mediates this effect via the NO/cGMP/PKG II signaling pathway. However, higher concentrations of Puerarin impairs proliferation and differentiation of osteoblasts. Zhang *et al* (126) showed that Puerarin significantly inhibits lipopolysaccharide (LPS)-induced osteoclast differentiation in osteoclast precursor RAW264.7 cells. LPS stimulates activation of AKT in osteoclast precursor RAW264.7 cells, while puerarin inhibits activation of AKT.

Inhibitory effect of flavonoids on osteoclasts and associated signaling pathways. Osteoprotegerin (*OPG)*/nuclear factor-κB (RANK)/RANK ligand (RANKL) signalling pathway. In 1997, the OPG/RANK/RANKL signaling pathway was discovered by Simonet *et al* (127) and investigated in areas such as orthopedic disease. RANK is the only known RANKL receptor activator that binds to the C-terminus of RANKL to initiate intracellular signaling events and promote activation and maturation of osteoclasts (128-130). OPG competitively binds to RANKL and inhibits the RANK/RANKL signaling pathway. This antagonizes RANKL to promote osteoclast differentiation and osteoblast function and or activates RANKL to inhibit osteoclast differentiation (131,132). RANKL/OPG ratio regulates RANKL-mediated signaling. A high RANKL/OPG ratio increases osteoclast

Table I. Use of flavonoids in the prevention and treatment of osteoporosis at the cellular level.

Flavonoid	First author, year	Cell type	Effect	(Refs.)
Quercetin	Sharan, 2014	hAFSCs	Increased BMP-2, Runx2, OPN, OCN, COL-1 and Osx expression	(104)
	Zhou and Lin, 2015	rBMSCs	Increased BSP, BMP-2, OPN and OCN expression	(103)
Icariin	Zhai, 2014	rBMSCs	PI3K-AKT-eNOS-NO-cGMP-PKG signalling pathway(+)	(124)
	Wu, 2015	rBMSCs	ERK, p38 and JNK MAPK signalling pathways(+)	(115)
	Huang, 2017	rBMSCs	Wnt signalling pathway(+)	(140)
Hesperetin	Liu, 2020	hBMSCs	ERK signaling pathway(+)	(116)
Naringin	Lin, 2016	rBMSCs	Increased Foxc2, Cbfα1 and OCN and decreased PPARγ2 expression; IHH signaling pathway(+)	(110)
	Liu, 2017	hAFSCs	BMP and Wnt/β-catenin(+)	(105)
Chrysin	Huo, 2021	DPSC	Increased ALP, COL-1, Runx2 and OCN expression	(138)
	Menon, 2018	mMSC	Increased Runx2 and decreased Runx2 co-repressor expression	(106)
Puerarin	Zhang, 2016	RAW264.7	Inhibited activation of Akt	(126)
	Lv, 2015	hBMSCs	NO-cGMP-PKG II signalling pathway(+)	(125)
	Yuan, 2016	MC3T3-E1; RAW 264.7	Inhibited osteoclast formation; increased osteoblast RANKL OPG expression	(135)
	Shan, 2018	MC3T3-E1	Targeting RANKL to upregulate miR-106b promotes osteogenic differentiation	(136)
	Liu, 2016	rBMSCs	Increased osteogenic differentiation; decreased fat formation	(139)
	Yang, 2018	MC3T3-E1	ERK1/2 and p38 MAPK signalling pathways(+)	(118)

Table II. Animal experiments on use of flavonoids to prevent osteoporosis.

Flavonoid	Author, year	Animal model	Effect	(Refs.)
Icariin	Huang, 2017	OVX mice	Wnt signalling pathway(+)	(140)
Hesperetin	Xue, 2017	Rat osteotomy	ERK and Smad1/5/8 signaling pathways(+)	(117)
Naringin	Wang, 2015	OVX mice	Wnt/β-Catenin signalling pathways(+)	(141)
Chrysin	Huo, 2021	Ectopic osteogenesis in nude mice; rat cranial defect	Increased ALP, COL-1, Runx2 and OCN expression	(138)
Puerarin	Zhang, 2016	Mouse osteolysis	Inhibited activation of Akt	(126)
Hesperetin	Yuan, 2016	OVX mice	Inhibited osteoclast formation and increased osteoblast RANKL OPG expression	(135)
Hesperetin	Liu, 2016	OVX rat	Promotion of osteogenic differentiation; decreased fat formation	(139)

hAFSCs, human Amniotic Fluid-derived Stem Cells; rBMSC, rat Bone Marrow Mesenchymal Stem Cells; DPSC, dental Pulp Stem Cell; mMSC, mouse Mesenchymal Stem Cell; OVX, ovariectomy; BMP, Bone morphogenetic protein; ALP, alkaline phosphatase; COL-1, collagen type 1; OCN, osteocalcin; RANKL, RANK ligand; OPG, osteoprotegerin; OPN, osteopontin; Osx, osterix; BSP, bone sialoprotein; eNOS, endothelial nitric oxide synthase; PKG, Protein kinase-G; Foxc2, Forkhead box protein C2; Cbfα1, Core binding factor α1; PPARγ2, Peroxisome proliferator-activated receptor γ2; IHH, Indian hedgehog; c, cyclic; miR-106b, microRNA-106b; +, promote.

differentiation (127,133). Low RANKL/OPG ratio negatively regulates osteoclast differentiation in mature osteoclasts. Therefore, OPG/RANKL/RANK is a key mechanism regulating the coupled balance of osteoclast formation and differentiation and bone remodeling (134).

Yuan *et al* (135) suggested that Puerarin has a time-dependent promoting effect on expression of OPG mRNA in MC3T3-E1 cells and inhibits expression of RANKL mRNA. Shan *et al* (136) demonstrated that Puerarin at 20 μM promotes proliferation of MC3T3-E1 cells. Moreover, Puerarin promotes MC3T3-E1 cell differentiation at certain concentrations and positively affect osteogenic differentiation by directly targeting RANKL-induced upregulation of microRNA-106b (Table I).

5. Animal studies on use of flavonoids to prevent osteoporosis

The primary types of osteoporosis are postmenopausal, disuse and GIOP (137). Huo *et al* (138) revealed that chrysin upregulates expression of osteogenic proteins (ALP, COL-1, Runx2 and OCN) both *in vivo* and *in vitro* and that induced osteogenic differentiation of dental pulp stem cells (DPSCs) relies on activation of the Smad3 pathway. Furthermore, mineralized bone tissue formation is induced in DPSCs *in vivo* in an ectopic osteogenesis model in nude mice and a rat cranial defect model. However, the mechanism by which chrysin increases Smad3 expression and activation requires further investigation. Liu *et al* (139) showed that the combined application of Puerarin and zinc promotes serum levels of OCN and ALP expression in ovariectomy (OVX) rats and inhibits serum levels adiponectin and adiposity in bone marrow. Co-administration of Puerarin (low dose) and zinc partially reverses OVX-induced bone loss in rats and inhibits osteoporosis, suggesting the potential use of Puerarin and zinc in treating osteoporosis. Huang *et al* (140) suggested that ICA promotes fracture healing in OVX mice *in vivo* and induces bone formation and inhibits adipogenesis in BMSCs; these bone-promoting and anti-adipogenic effects are mediated by the Wnt signaling pathway. Using OVX mice *in vivo* to simulate osteoporosis, Wang *et al* (141) found that naringin treatment improves bone strength by activating the Wnt/β-Catenin signaling pathway. Xue *et al* (117) used a rat osteotomy model to confirm that hBMSCs combined with hesperetin/gelatin sponge scaffolds accelerates fracture healing *in vivo*. Yuan *et al* (135) verified that Puerarin effectively prevents bone loss in OVX mice. The anti-osteoporotic activity of Puerarin may be associated with its effect on osteoclast formation and RANKL OPG expression in osteoblasts. Zhang *et al* (126) revealed that Puerarin attenuates LPS-induced bone loss in a mouse cranial osteolysis model (Table II).

6. Prospects

Osteoporosis is a global public health issue and is considered the second most common health problem after coronary heart disease by the WHO. Current approaches toward osteoporosis prevention and treatment focus on drug therapy including bisphosphonates, calcitonin and SERMs. However, because these drugs have side effects, such as increased risk of cardiovascular events, breast cancer and venous thromboembolism, researchers have turned to traditional Chinese medicine (142,143).

Traditional Chinese medicine has developed over thousands of years based on a different perspective from Western medical knowledge. The natural abundance flavonoids, low price, high osteogenesis rate and low immune rejection during clinical treatment have made the application of flavonoids in bone tissue engineering research more widespread. However, current investigations have certain limitations. First, most research on flavonoids remains at the cellular level and research at the animal level needs to be more extensive. Additionally, the pharmacological effect of flavonoids is a complex process that involves multiple systems. Although certain mechanisms have been elucidated, further research is needed. In addition to the aforementioned signalling pathways, there are other overlapping signalling pathways that interact with each other. Current research has limitations, such as focusing on only independent pathways and molecular targets and there are few clinical studies (50).

More in-depth research on flavonoids is needed to develop effective and inexpensive novel drugs for clinical applications.

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Availability of data and materials

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Authors' contributions

LC wrote the manuscript. FT, JW and YZ edited the manuscript. CW revised the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Christiansen C: Consensus development conference: Diagnosis, prophylaxis, and treatment of osteoporosis. Osteoporosis Int 295: 914-915, 1987.
2. Management of osteoporosis in postmenopausal women: 2010 position statement of The North American Menopause Society. Menopause 17: 25-56, 2010.
3. Langdahl BL and Harslof T: Medical treatment of osteoporotic vertebral fractures. Ther Adv Musculoskel 3: 17-29, 2011.
4. Leong KH: Medical treatment of osteoporosis-increasing options. Ann Acad Med Singap 31: 43-47, 2002.
5. Nahas NE, Samy AM and Omer MO: Global, regional, and national burden of bone fractures in 204 countries and territories, 1990-2019: A systematic analysis from the global burden of disease study 2019. Lancet Healthy Longev 2: e580-e592, 2021.
6. Harborne JB and Baxter H: The Handbook of Natural Flavonoids. Vol 2. pp1800, 1999.
7. Kimira M, Arai Y, Shimoi K and Watanabe S: Japanese intake of flavonoids and isoflavonoids from foods. J Epidemiol 8: 168-175, 1998.
8. Cushnie T and Lamb AJ: Antimicrobial activity of flavonoids. Int J Antimicrob Ag 26: 343-356, 2005.
9. Kim SY, Lee JY, Park YD, Kang KL, Lee JC and Heo JS: Hesperetin alleviates the inhibitory effects of high glucose on the osteoblastic differentiation of periodontal ligament stem cells. PLoS One 8: e67504, 2013.
10. Zhang P, Dai KR and Yan SG: Effects of naringin on the proliferation and osteogenic differentiation of human bone mesenchymal stem cell. Eur J Pharmacol 607: 1-5, 2009.

11. Black DM and Rosen CJ: Clinical practice. Postmenopausal osteoporosis. N Engl J Med 374: 254-262, 2016.
12. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser 843: 1-129, 1994.
13. Kanis JA, Melton LJ III, Christiansen C, Johnston CC and Khaltaev N: The diagnosis of osteoporosis. J Bone Miner Res 9: 1137-1141, 1994.
14. Johnell O, Kanis JA, Oden A, Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, Eisman JA, *et al*: Predictive value of BMD for hip and other fractures. J Bone Miner Res 20: 1185-1194, 2007.
15. Jianning Y: Osteoporosis prevalence and community-based diagnosis and management of osteoporosis-related chronic pain in China. Chin Gen Pract 23: 2223-2228, 2020.
16. Office of the Surgeon General (US): Bone Health and Osteoporosis: A report of the surgeon general. Rockville (MD), Office of the Surgeon General (US), 2004.
17. Clynes MA, Harvey NC, Curtis EM, Fuggle NR, Dennison EM and Cooper C: The epidemiology of osteoporosis. Br Med Bull 133: 105-117, 2020.
18. van Staa T, Dennison EM, Leufkens H and Cooper C: Epidemiology of fractures in England and Wales. Bone 29: 517-522, 2001.
19. Ensrud KE and Crandall CJ: Osteoporosis. Ann Intern Med 167: ITC17-ITC32, 2017.
20. Dewan N, Macdermid JC, Grewal R and Beattie K: Risk factors predicting subsequent falls and osteoporotic fractures at 4 years after distal radius fracture-a prospective cohort study. Arch Osteoporos 13: 32, 2018.
21. Balasuriya B and Rupasinghe H: Plant flavonoids as angiotensin converting enzyme inhibitors in regulation of hypertension. Funct Foods Health Dis 5: 172-188, 2010.
22. Wang TY, Li Q and Bi KS: Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian J Pharm Sci 13: 12-23, 2018.
23. Alvarez-Arellano L, Salazar-García M and Corona JC: Neuroprotective effects of quercetin in pediatric neurological diseases. Molecules 25: 5597, 2020.
24. Manca ML, Castangia I, Caddeo C, Pando D, Escribano E, Valenti D, Lampis S, Zaru M, Fadda AM and Manconi M: Improvement of quercetin protective effect against oxidative stress skin damages by incorporation in nanovesicles. Colloids Surf B Biointerfaces 123: 566-574, 2014.
25. Andres S, Pevny S, Ziegenhagen R, Bakhiya N, Schäfer B, Hirsch-Ernst KI and Lampen A: Safety aspects of the use of quercetin as a dietary supplement. Mol Nutr Food Res: 62, 2018 doi: 10.1002/mnfr.201700447.
26. David A, Arulmoli R and Parasuraman S: Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacogn Rev 10: 84-89, 2016.
27. Mehrbod P, Abdalla MA, Fotouhi F, Heidarzadeh M, Aro AO, Eloff JN, McGaw LJ and Fasina FO: Immunomodulatory properties of quercetin-3-O-α-L-rhamnopyranoside from Rapanea melanophloeos against influenza a virus. BMC Complement Altern Med 18: 184, 2018.
28. Flores IR, Vásquez-Murrieta MS, Franco-Hernández MO, Márquez-Herrera CE, Ponce-Mendoza A and Del Socorro López-Cortéz M: Bioactive compounds in tomato (Solanum lycopersicum) variety saladette and their relationship with soil mineral content. Food Chem 344: 128608, 2021.
29. Torres N, Martínez-Lüscher J, Porte E, Yu R and Kurtural SK: Impacts of leaf removal and shoot thinning on cumulative daily light intensity and thermal time and their cascading effects of grapevine (*Vitis vinifera* L.) berry and wine chemistry in warm climates. Food Chem 343: 128447, 2020.
30. Maria P, Vivian O, Tatiana P and Sandra P: Physicochemical stability, antioxidant activity, and acceptance of beet and orange mixed juice during refrigerated storage. Beverages 3: 36, 2017.
31. Santiago B, Calvo AA, Gullón B, Feijoo G, Moreira MT and González-García S: Production of flavonol quercetin and fructooligosaccharides from onion (*Allium cepa* L.) waste: An environmental life cycle approach. Chem Eng J 392: 123772, 2020.
32. Ribes-Moya AM, Adalid AM, Raigón MD, Hellín P, Fita A and Rodríguez-Burruezo A: Variation in flavonoids in a collection of peppers (*Capsicum* sp.) under organic and conventional cultivation: Effect of the genotype, ripening stage, and growing system. J Sci Food Agr 100: 2208-2223, 2020.
33. Sun J, Janisiewicz WJ, Takeda F, Evans B, Wayne JM Mengliang Z, Liangli Y and Chen P: Effect of nighttime UV-C irradiation of strawberry plants on phenolic content of fruit: Targeted and non-targeted metabolomic analysis. J Berry Res 10: 365-380, 2020.
34. Zhou W, Liang X, Dai P, Chen Y, Zhang Y, Zhang M, Lu L, Jin C and Lin X: Alteration of phenolic composition in lettuce (*Lactuca sativa* L.) by reducing nitrogen supply enhances its anti-proliferative effects on colorectal cancer cells. Int J Mol Sci 20: 4205, 2019.
35. Formica JV and Regelson W: Review of the biology of Quercetin and related bioflavonoids. Food Chem Toxicol 33: 1061-1080, 1995.
36. Nugroho A, Hesty H, Choi JS and Park HJ: Identification and quantification of flavonoids in Carica papaya leaf and peroxynitrite-scavenging activity. Asian Pac J Trop Biomed 7: 208-213, 2017.
37. Bolling BW, Mckay DL and Blumberg JB: The phytochemical composition and antioxidant actions of tree nuts. Asia Pac J Clin Nutr 19: 117-123, 2010.
38. Stavric B: Quercetin in our diet: From potent mutagen to probable anticarcinogen. Clin Biochem 27: 245-248, 1994.
39. Batiha ES, Beshbishy AM, Ikram M, Mulla ZS, El-Hack MEA, Taha AE, Algammal AM and Elewa YHA: The pharmacological activity, biochemical properties, and pharmacokinetics of the major natural polyphenolic flavonoid: Quercetin. Foods 9: 374, 2020.
40. Beecher GR, Warden BA and Merken H: Analysis of tea polyphenols. Proc Soc Exp Biol Med 220: 267-270, 1999.
41. Hirpara KV, Aggarwal P, Mukherjee AJ, Joshi N and Burman AC: Quercetin and its derivatives: Synthesis, pharmacological uses with special emphasis on anti-tumor properties and prodrug with enhanced bio-availability. Anticancer Agents Med Chem 9: 138-161, 2009.
42. Boots AW, Haenen GR and Bast A: Health effects of quercetin: From mechanism to nutraceutical. Eur J Pharmacol 585: 325-337, 2006.
43. Yamaguchi M and Weitzmann MN: Quercetin, a potent suppressor of NF-κB and Smad activation in osteoblasts. Int J Mol Med 28: 521-525, 2011.
44. Chen M, Wu J, Luo Q, Mo S, Lyu Y, Wei Y and Dong J: The Anticancer properties of Herba Epimedii and its main bioactive Componentsicariin and Icariside II. Nutrients 8: 563, 2016.
45. Huang W, Zeng S, Xiao G, Wei G, Liao S, Chen J, Sun W, Lv H and Wang Y: Elucidating the biosynthetic and regulatory mechanisms of flavonoid-derived bioactive components in Epimedium sagittatum. Front Plant Sci 6: 689, 2015.
46. Huang KC: The pharmacology of Chinese herbs. Pharmacol Chin Herbs, 1993.
47. Makarova MN, Pozharitskaya ON, Shikov AN, Tesakova SV, Makarov VG and Tikhonov VP: Effect of lipid-based suspension of Epimedium koreanum Nakai extract on sexual behavior in rats. J Ethnopharmacol 114: 412-416, 2007.
48. Ma H, He X, Yang Y, Li M, Hao D and Jia Z: The genus Epimedium: An ethnopharmacological and phytochemical review. J Ethnopharmacol 134: 519-541, 2011.
49. Xie X, Pei F, Wang H, Tan Z, Yang Z and Kang P: Icariin: A promising osteoinductive compound for repairing bone defect and osteonecrosis. J Biomater Appl 30: 290-299, 2015.
50. Wang Z, Wang D, Yang D, Zhen W, Zhang J and Peng S: The effect of icariin on bone metabolism and its potential clinical application. Osteoporos Int 29: 535-544, 2018.
51. He C, Wang Z and Shi J: Pharmacological effects of icariin. Adv Pharmacol 87: 179-203, 2020.
52. Gary AB and McIntosh C: Radioimmunoassay for the quantitative determination of hesperidin and analysis of its distribution in Citrus sinensis. Phytochemistry 27: 249-254, 1988.
53. Rady H: Pharmacographia: A History of the principal Drugs of Vegetable Origin met with in Great Britain and British India. Nature 11: 42-44, 1874.
54. Garg A, Garg S, Zaneveld LJ and Singla AK: Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. Phytother Res 15: 655-669, 2001.
55. Parhiz H, Roohbakhsh A, Soltani F, Rezaee R and Iranshahi M: Antioxidant and anti-inflammatory properties of the citrus flavonoids hesperidin and hesperetin: An updated review of their molecular mechanisms and experimental models. Phytother Res 29: 323-331, 2015.
56. Li R, Cai L, Xie XF, Peng L, Wu TN and Li J: 7,3'-dimethoxy hesperetin inhibits inflammation by inducing synovial apoptosis in rats with adjuvant-induced arthritis. Immunopharmacol Immunotoxicol 35: 139-146, 2013.
57. Yang Z, Liu Z, Wang J and Zhu H: Antioxidative effects of hesperetin against lead acetate-induced oxidative stress in rats. Indian J Pharmacol 45: 395-398, 2013.
58. Itoh K, Masuda M, Naruto S, Murata K and Matsuda H: Antiallergic activity of unripe Citrus hassaku fruits extract and its flavanone glycosides on chemical substance-induced dermatitis in mice. J Nat Med 63: 443-450, 2009.

59. Trzeciakiewicz A, Habauzit V, Mercier S, Lebecque P, Davicco MJ, Coxam V, Demigne C and Horcajada MN: Hesperetin stimulates differentiation of primary rat osteoblasts involving the BMP signalling pathway. J Nutr Biochem 21: 424-431, 2010.
60. Ashraful AM, Nusrat S, Mahbubur RM, Uddin SJ, Reza HM and Sarker SD: Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr 5: 404-417, 2014.
61. E. P. O. Additives and Products or Substances used in Animal Feed: Scientific Opinion on the safety and efficacy of naringin when used as a sensory additive for all animal species. EfSA J 9: 2416, 2011.
62. Joshi R, Kulkarni YA and Wairkar S: Pharmacokinetic, pharmacodynamic and formulations aspects of Naringenin: An update. Life Sci 215: 43-56, 2018.
63. Wang Y, Yin L, Li Y, Liu P and Cui Q: Preventive effects of puerarin on alcohol-induced osteonecrosis. Clin Orthop Relat Res 466: 1059-1067, 2008.
64. Akintunde JK, Akintola TE, Hammed MO, Amoo CO, Adegoke AM and Ajisafe LO: Naringin protects against Bisphenol-A induced oculopathy as implication of cataract in hypertensive rat model. Biomed Pharmacother 126: 110043, 2020.
65. Zhang YF, Meng NN, Li HZ, Wen YJ, Liu JT, Zhang CL, Yuan XH and Jin XD: Effect of naringin on oxidative stress and endoplasmic reticulum stress in diabetic cardiomyopathy. Zhongguo Zhong Yao Za Zhi 43: 596-602, 2018 (In Chinese).
66. Choi JH and Yun JW: Chrysin induces brown fat-like phenotype and enhances lipid metabolism in 3T3-L1 adipocytes. Nutrition 32: 1002-1010, 2016.
67. Mohammadian F, Abhari A, Dariushnejad H, Nikanfar A, Pilehvar-Soltanahmadi Y and Zarghami N: Effects of Chrysin-PLGA-PEG nanoparticles on proliferation and gene expression of miRNAs in gastric cancer cell line. Iran J Cancer Prev 9: e4190, 2016.
68. Kang MK, Park SH, Kim YH, Lee EJ, Antika LD, Kim DY, Choi YJ and Kang YH: Chrysin ameliorates podocyte injury and slit diaphragm protein loss via inhibition of the PERK-eIF2α-ATF-CHOP pathway in diabetic mice. Acta Pharmacol Sin 38: 1129-1140, 2017.
69. Shoieb SM, Esmat A, Khalifa AE and Abdel-Naim AB: Chrysin attenuates testosterone-induced benign prostate hyperplasia in rats. Food Chem Toxicol 111: 650-659, 2018.
70. Zeinali M, Rezaee SA and Hosseinzadeh H: An overview on immunoregulatory and anti-inflammatory properties of chrysin and flavonoids substances. Biomed Pharmacother 92: 998-1009, 2017.
71. Vedagiri A and Thangarajan S: Mitigating effect of chrysin loaded solid lipid nanoparticles against Amyloid β25-35 induced oxidative stress in rat hippocampal region: An efficient formulation approach for Alzheimer's disease. Neuropeptides 58: 111-125, 2016.
72. Khan R, Khan AQ, Qamar W, Lateef A, Ali F, Rehman MU, Tahir M, Sharma S and Sultana S: Chrysin abrogates cisplatin-induced oxidative stress, p53 expression, goblet cell disintegration and apoptotic responses in the jejunum of Wistar rats. Br J Nutr 108: 1574-1585, 2012.
73. Khan MS, Devaraj H and Devaraj N: Chrysin abrogates early hepatocarcinogenesis and induces apoptosis in N-nitrosodiethylamine-induced preneoplastic nodules in rats. Toxicol Appl Pharmacol 251: 85-94, 2011.
74. Shen Y, Tian P, Li D, Wu Y, Wan C, Yang T, Chen L, Wang T and Wen F: Chrysin suppresses cigarette smoke-induced airway inflammation in mice. Int J Clin Exp Med 8: 2001-2008, 2015.
75. Rehman MU, Ali N, Rashid S, Jain T, Nafees S, Tahir M, Khan AQ, Lateef A, Khan R, Hamiza OO, *et al*: Alleviation of hepatic injury by chrysin in cisplatin administered rats: Probable role of oxidative and inflammatory markers. Pharmacol Rep 66: 1050-1059, 2014.
76. Ravishankar D, Salamah M, Attina A, Pothi R, Vallance TM, Javed M, Williams HF, Alzahrani EMS, Kabova E, Vaiyapuri R, *et al*: Ruthenium-conjugated chrysin analogues modulate platelet activity, thrombus formation and haemostasis with enhanced efficacy. Sci Rep 7: 5738, 2017.
77. Shibata S, Murakami T, Nishikawa Y and Harada M: The constituents of pueraria root. Chem Pharm Bull 7: 134-136, 1959.
78. Keung WM and Vallee BL: Kudzu root: An ancient Chinese source of modern antidipsotropic agents. Phytochemistry 47: 499-506, 1998.
79. Brenner R, Perez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT and Aldrich RW: Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. Nature 407: 870-876, 2000.
80. Hsu FL, Liu IM, Kuo DH, Chen WC, Su HC and Cheng JT: Antihyperglycemic effect of puerarin in streptozotocin-induced diabetic rats. J Nat Prod 66: 788-792, 2003.
81. Hao LN, Zhang YQ, Shen YH, Wang ZY and Wang YH: Inducible nitric oxide synthase and Fas/FasL with C3 expression of mouse retinal pigment epithelial cells in response to stimulation by peroxynitrite and antagonism of puerarin. Chin Med J 124: 2522-2529, 2011.
82. Shao HM, Tang YH, Jiang PJ, Zhu HQ, Zhang YC, Ji JM, Ji O and Shen Q: Inhibitory effect of flavonoids of puerarin on proliferation of different human acute myeloid leukemia cell lines in vitro. Zhongguo Shi Yan Xue Ye Xue Za Zhi 18: 296-299, 2010 (In Chinese).
83. Cheng Y, Zhu G, Guan Y, Liu Y, Hu Y and Li Q: Protective effects of puerarin against 1-methyl-4-phenylpyridinium-induced mitochondrial apoptotic death in differentiated SH-SY5Y cells. Zhongguo Zhong Yao Za Zhi 36: 1222-1226, 2011 (In Chinese).
84. Zou Y, Hong B, Fan L, Zhou L, Liu Y, Wu Q, Zhang X and Dong M: Protective effect of puerarin against beta-amyloid-induced oxidative stress in neuronal cultures from rat hippocampus: Involvement of the GSK-3β/Nrf2 signaling pathway. Free Radical Res 47: 55-63, 2013.
85. Song JL, Baek HJ, Chang HL and Kim HP: Antiinflammatory activity of isoflavonoids from *Pueraria* radix and biochanin A derivatives. Arch Pharm Res 17: 31-35, 1994.
86. Overstreet DH, Kralic JE, Morrow AL, Ma ZZ, Zhong ZM and Lee D: NPI-031G (puerarin) reduces anxiogenic effects of alcohol withdrawal or benzodiazepine inverse or 5-HT2C agonists. Pharmacol Biochem Behav 75: 619-625, 2003.
87. Yong PH and Jeong HG: Mechanism of phytoestrogen puerarin-mediated cytoprotection following oxidative injury: Estrogen receptor-dependent up-regulation of PI3K/Akt and HO-1. Toxicol Appl Pharmacol 233: 371-381, 2008.
88. Cho HJ, Jun HJ, Ji HL, Jia Y, Hoang MH, Shim JH, Park KH and Lee SJ: Acute effect of high-dose isoflavones from Pueraria lobata (Willd.) Ohwi on lipid and bone metabolism in ovariectomized mice. Phytother Res 26: 1864-1871, 2012.
89. Chen X, Wang Z, Duan N, Zhu G, Schwarz EM and Xie C: Osteoblast-osteoclast interactions. Connect Tissue Res 59: 99-107, 2018.
90. Compston JE, McClung MR and Leslie WD: Osteoporosis. Lancet 393: 364-376, 2019.
91. Lerner UH, Kindstedt E and Lundberg P: The critical interplay between bone resorbing and bone forming cells. J Clin Periodontol 46 (Suppl 21): S33-S51, 2019.
92. Urist MR: Bone: Formation by autoinduction. Science 150: 893-899, 1965.
93. Luyten FP, Cunningham NS, Ma S, Muthukumaran N, Hammonds RG, Nevins WB, Woods WI and Reddi AH: Purification and partial amino acid sequence of osteogenin, a protein initiating bone differentiation. J Biol Chem 264: 13377-13380, 1989.
94. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM and Wang EA: Novel regulators of bone formation: Molecular clones and activities. Science 242: 1528-1534, 1988.
95. Xu C and Di C: The BMP signaling and in vivo bone formation. Gene 357: 1-8, 2005.
96. Hinck AP, Mueller TD and Springer TA: Structural biology and evolution of the TGF-β family. Cold Spring Harb Perspect Biol 8: a22103, 2016.
97. von Bubnoff A and Cho KW: Intracellular BMP signaling regulation in vertebrates: Pathway or network? Dev Biol 239: 1-14, 2001.
98. Nohe A, Keating E, Knaus P and Petersen NO: Signal transduction of bone morphogenetic protein receptors. Cell Signal 16: 291-299, 2004.
99. Javed A, Afzal F, Bae JS, Gutierrez S, Zaidi K, Pratap J, van Wijnen AJ, Stein JL, Stein GS and Lian JB: Specific residues of RUNX2 are obligatory for formation of BMP2-induced RUNX2-SMAD complex to promote osteoblast differentiation. Cells Tissues Organs 189: 133-137, 2009.
100. Phimphilai M, Zhao Z, Boules H, Roca H and Franceschi RT: BMP signaling is required for RUNX2-dependent induction of the osteoblast phenotype. J Bone Mineral Res 21: 637-646, 2006.

101. Tan X, Weng T, Zhang J, Wang J, Li W, Wan H, Lan Y, Cheng X, Hou N, Liu H, *et al*: Smad4 is required for maintaining normal murine postnatal bone homeostasis. J Cell Sci 120: 2162-2170, 2007.
102. Liang W, Luo Z, Ge S, Li M, Du J, Yang M, Yan M, Ye Z and Luo Z: Oral administration of quercetin inhibits bone loss in rat model of diabetic osteopenia. Eur J Pharmacol 670: 317-324, 2011.
103. Zhou C and Lin Y: Osteogenic differentiation of adipose-derived stem cells promoted by quercetin. Cell Prolif 47: 124-132, 2014.
104. Sharan K, Mishra JS, Swarnkar G, Siddiqui JA, Khan K, Kumari R, Rawat P, Maurya R, Sanyal S and Chattopadhyay N: A novel quercetin analogue from a medicinal plant promotes peak bone mass achievement and bone healing after injury and exerts an anabolic effect on osteoporotic bone: The role of aryl hydrocarbon receptor as a mediator of osteogenic action. J Bone Miner Res 26: 2096-2111, 2011.
105. Liu M, Li Y and Yang ST: Effects of naringin on the proliferation and osteogenic differentiation of human amniotic fluid-derived stem cells. J Tissue Eng Regen Med 11: 276-284, 2017.
106. Menon AH, Soundarya SP, Sanjay V, Chandran SV, Balagangadharan K and Selvamurugan N: Sustained release of chrysin from chitosan-based scaffolds promotes mesenchymal stem cell proliferation and osteoblast differentiation. Carbohyd Polym 195: 356-367, 2018.
107. Willert K and Nusse R: Beta-catenin: A key mediator of Wnt signaling. Curr Opin Genet Dev 8: 95-102, 1998.
108. Clevers H and Nusse R: Wnt/β-catenin signaling and disease. Cell 149: 1192-1205, 2012.
109. Kestler HA and Kühl M: From individual Wnt pathways towards a Wnt signalling network. Philos Trans R Soc Lond B Biol Sci 363: 1333-1347, 2008.
110. Lin FX, Du SX, Liu DZ, Hu QX, Yu GY, Wu CC, Zheng GZ, Xie D, Li XD and Chang B: Naringin promotes osteogenic differentiation of bone marrow stromal cells by up-regulating Foxc2 expression via the IHH signaling pathway. Am J Transl Res 8: 5098-5107, 2016.
111. Johnson GL and Lapadat R: Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 298: 1911-1912, 2002.
112. Mercedes SS, Diniz FF, Gomes GN and Diana B: The Mitogen-activated protein kinase (MAPK) pathway: Role in immune evasion by trypanosomatids. Front Microbiol 7: 183, 2016.
113. Arthur JS and Ley SC: Mitogen-activated protein kinases in innate immunity. Nat Rev Immunol 13: 679-692, 2013.
114. Ronkina N and Gaestel M: MAPK-activated protein kinases: Servant or partner? Annu Rev Biochem: Feb 18, 2022 (Epub ahead of print). doi: 10.1146/annurev-biochem-081720-114505.
115. Wu Y, Xia L, Zhou Y, Xu Y and Jiang X: Icariin induces osteogenic differentiation of bone mesenchymal stem cells in a MAPK-dependent manner. Cell Prolif 48: 375-384, 2015.
116. Liu L, Zheng J, Yang Y, Ni L, Chen H and Yu D: Hesperetin alleviated glucocorticoid-induced inhibition of osteogenic differentiation of BMSCs through regulating the ERK signaling pathway. Med Mol Morphol 54: 1-7, 2020.
117. Xue D, Chen E, Zhang W, Gao X, Wang S, Zheng Q, Pan Z, Li H and Liu L: The role of hesperetin on osteogenesis of human mesenchymal stem cells and its function in bone regeneration. Oncotarget 8: 21031-21043, 2017.
118. Yang X, Yang Y, Zhou S, Gong X, Dai Q, Zhang P and Jiang L: Puerarin Stimulates osteogenic differentiation and bone formation through the ERK1/2 and p38-MAPK signaling pathways. Curr Mol Med 17: 488-496, 2018.
119. Franke TF, Hornik CP, Segev L, Shostak GA and Sugimoto C: PI3K/Akt and apoptosis: Size matters. Oncogene 22: 8983-8998, 2004.
120. Lien EC, Dibble CC and Toker A: PI3K signaling in cancer: Beyond AKT. Curr Opin Cell Biol 45: 62-71, 2017.
121. Kashii Y, Uchida M, Kirito K, Tanaka M, Nishijima K, Toshima M, Ando T, Koizumi K, Endoh T, Sawada K, *et al*: A member of Forkhead family transcription factor, FKHRL1, is one of the downstream molecules of phosphatidylinositol 3-kinase-Akt activation pathway in erythropoietin signal transduction. Blood 96: 941-949, 2000.
122. Gu YX, Du J, Si MS, Mo JJ, Qiao SC and Lai HC: The roles of PI3K/Akt signaling pathway in regulating MC3T3-E1 preosteoblast proliferation and differentiation on SLA and SLActive titanium surfaces. J Biomed Mater Res A 101: 748-754, 2013.
123. Xi JC, Zang HY, Guo LX, Xue HB, Liu XD, Bai YB and Ma YZ: The PI3K/AKT cell signaling pathway is involved in regulation of osteoporosis. J Recept Signal Transduct Res 35: 640-645, 2015.
124. Zhai YK, Guo XY, Ge BF, Zhen P, Ma XN, Zhou J, Ma HP, Xian CJ and Chen KM: Icariin stimulates the osteogenic differentiation of rat bone marrow stromal cells via activating the PI3K-AKT-eNOS-NO-cGMP-PKG. Bone 66: 189-198, 2014.
125. Lv H, Che T, Tang X, Liu L and Cheng J: Puerarin enhances proliferation and osteoblastic differentiation of human bone marrow stromal cells via a nitric oxide/cyclic guanosine monophosphate signaling pathway. Mol Med Rep 12: 2283-2290, 2015.
126. Zhang Y, Yan M, Yu QF, Yang PF, Zhang HD, Sun YH, Zhang ZF and Gao YF: Puerarin prevents LPS-induced osteoclast formation and bone loss via inhibition of Akt activation. Biol Pharm Bull 39: 2028-2035, 2016.
127. Simonet WS, Lacey DL, Dunstan CR, Kelley MC and Boyle WJ: Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell 89: 309-319, 1997.
128. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, *et al*: Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93: 165-176, 1998.
129. Xu J, Tan JW, Huang L, Gao XH, Laird R, Liu D, Wysocki S and Zheng MH: Cloning, sequencing, and functional characterization of the rat homologue of receptor activator of NF-kappaB ligand. Bone 15: 2178-2186, 2000.
130. Burgess TL, Qian Y, Kaufman S, Ring BD, Van G, Capparelli C, Kelley M, Hsu H, Boyle WJ, Dunstan CR, *et al*: The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. J Cell Biol 145: 527-538, 1999.
131. Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, Sato Y, Nakagawa N, Yasuda H, Mochizuki S, *et al*: Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. Biochem Biophys Res Commun 247: 610-615, 1998.
132. Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuriyama M, *et al*: Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): A mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology 139: 1329-1337, 1998.
133. Kostenuik P and Shalhoub V: Osteoprotegerin: A physiological and pharmacological inhibitor of bone resorption. Curr Pharm Design 7: 613-635, 2001.
134. Hofbauer LC, Kühne CA and Viereck V: The OPG/RANKL/RANK system in metabolic bone diseases. J Musculoskelet Neuronal Interact 4: 268-275, 2004.
135. Yuan SY, Sheng T, Qi L, Zhang YL, Liu XM, Ma T, Zheng H, Yan Y, Ishimi Y and Wang XX: Puerarin prevents bone loss in ovariectomized mice and inhibits osteoclast formation in vitro. Chin J Nat Med 14: 265-269, 2016.
136. Shan Z, Cheng N, Huang R, Zhao B and Zhou Y: Puerarin promotes the proliferation and differentiation of MC3T3-E1 cells via microRNA106b by targeting receptor activator of nuclear factor-κB ligand. Exp Ther Med 15: 55-60, 2018.
137. Turner RT, Maran A, Lotinun S, Hefferan T and Sibonga JD: Animal models for osteoporosis. Rev Endocr Metab Disord 2: 117-127, 2001.
138. Huo JF, Zhang ML, Wang XX and Zou DH: Chrysin induces osteogenic differentiation of human dental pulp stem cells. Exp Cell Res 400: 112466, 2021.
139. Liu H, Li W, Ge X, Jia S and Li B: Coadministration of puerarin (low dose) and zinc attenuates bone loss and suppresses bone marrow adiposity in ovariectomized rats. Life Sci 166: 20-26, 2016.
140. Huang J, Bao Y, Xiang W, Jing XZ, Guo JC, Yao XD, Wang R and Guo FJ: Icariin regulates the bidirectional differentiation of bone marrow mesenchymal stem cells through canonical Wnt signaling pathway. Evid Based Complement Alternat Med 2017: 8085325, 2017.
141. Wang D, Ma W, Wang F, Dong J, Wang D, Sun B and Wang B: Stimulation of Wnt/β-catenin signaling to improve bone development by naringin via interacting with AMPK and Akt. Cell Physiol Biochem 36: 1563-1576, 2015.
142. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, *et al*: Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results From the Women's Health Initiative randomized controlled trial. JAMA 288: 321-333, 2002.
143. Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J, *et al*: Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: Results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. JAMA 282: 637-645, 1999.