Int J Clin Exp Med 2021;14(4):1741-1747
www.ijcem.com /ISSN:1940-5901/IJCEM0122969

Original Article

Study on the role of Psoralea corylifolia in osteoporotic rats

Zhi Deng, Zhoujin Li

Department of Orthopedics, The Second Affiliated Hospital of Hunan University of Chinese Medicine, Changsha, Hunan Province, China

Received September 23, 2020; Accepted November 6, 2020; Epub April 15, 2021; Published April 30, 2021

Abstract: Objective: To investigate the effect of Psoralea corylifolia on bone mineral density (BMD), bone biomechanics, and blood biochemistry indexes in osteoporotic rats and to explore the mechanism of Psoralea corylifolia in improving osteoporosis. Methods: Thirty-six female SD rats with osteoporosis were randomly divided into three groups: sham group, model group and Psoralea corylifolia group. Psoralea corylifolia was given by gastric perfusion for three months. BMD were detected by X-ray absorptiometry and biomechanical detector was used to examine the maximum load and stiffness of femoral bone. The serum levels of OCN, PICP, 25(OH)D_3 and 1,25$(OH)_2D_3$ were examined by ELISA methods. ALP and TRAP levels were detected by automatic biochemical analyzer. And OPG and RANKL mRNA expressions were detected by Real-time PCR. Results: Compared with those in model group, BMD, maximum load, stiffness, and the levels of OCN, PICP, 25(OH)D_3, 1,25$(OH)_2D_3$, OPG and RANKL were significantly increased while ALP and TRAP levels were obviously reduced, and there were statistically significant differences (all $P<0.05$). Conclusions: Psoralea corylifolia could significantly increase BMD and strength of bone, and promote the levels of bone metabolism in osteoporotic rats. It may improve osteoporosis through OPG/RANKL pathway.

Keywords: Psoralea corylifolia, osteoporosis, bone metabolism balance, bone mineral density, biomechanics

Introduction

It is well known that osteoporosis is a common systemic skeletal disorder which is associated with aging [1]. With the aging of the population becoming more and more serious, osteoporosis has become an important public health problem which faced by the whole world [2, 3]. The results of epidemiological investigation showed that osteoporosis had affected around 200 million people worldwide [4]. It was reported that osteoporosis could result in bone fragility and increase fracture risk, which usually leads to the great pain in patients and brings a heavy financial burden in families [5, 6]. Therefore, the strategies for management of osteoporosis and fracture prevention are of great importance.

Many studies have shown that traditional Chinese medicine had a certain curative effect on osteoporosis, with little side effects for long-term use [7, 8]. Psoralea corylifolia is widely used in clinical Chinese medicine [9]. The active components of Psoralea corylifolia included flax flavonoids, coumarin, and flavonoids. It was showed that Psoralea corylifolia had multiple effects such as antitumor, protection of cardiovascular disease, antibacterial, anti-cancer and anti-inflammation properties [10, 11]. Psoralea corylifolia has also been shown to promote osteogenic differentiation in the term of bone metabolism, which indicated that Psoralea corylifolia may be a target for intervention of osteoporosis [12]. However, the specific molecular mechanism how Psoralea corylifolia played an important role in osteoporosis remains unclear. In this context, the osteoporosis rat models were established as subjects to investigate the effect of Psoralea corylifolia on osteoporosis to get a better understanding of bone healing capacity in the osteoporosis population.

Materials and methods

Experimental animals

Thirty-six SPF healthy female SD rats, weighing about 220±20 g, were provided by the Center for Experimental Animals in our hospital. SD

rats were divided into three groups: Sham group, Model group and Psoralea corylifolia group. There were 12 SD rat in each group. SD rats from Psoralea corylifolia group were given 8 mg/kg Psoralea corylifolia (Sigma, USA) intragastrically once a day for three months. SD rats in model group were given the same volume of normal saline intragastrically. And Sham group did not make any processing. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Hunan University of Chinese Medicine.

Establishment of osteoporosis rat model

An osteoporosis rat model was established according to the method reported by Hoffmann et al [13]. The SD rats underwent anesthetization using 3% pentasorbital sodium solutions (40 mg/kg). Under aseptic conditions, the longitudinal incision was made along the abdominal white line. Tissues were separated layer by layer to expose the abdominal cavity. The ligation of fallopian tube and its surrounding blood vessels and adipose tissue was performed and bilateral ovaries were completely removed. After hemostasis, the tissues were stitched layer by layer. At 6 weeks following the operation, the SD rats underwent experimental treatment.

Detection of bone mineral density (BMD) and biomechanical test

The SD rats in each group were anesthetized, and then whole body bone mineral density, femoral bone mineral density and vertebral bone mineral density were examined using dual X-ray Absorptiometry (GE Company, USA). And the rats were killed and femur bone was separated for detection of maximum load and stiffness using biomechanics testing machine (858 type, MTS systems corporation, USA).

Detection of osteocalcin (OCN), procollagen I carboxyterminal (PICP) and 25(OH)D_3 and 1,25$(OH)_2D_3$ by ELISA

Heart blood was extracted from each group. The serum was separated by 5000 rpm ×15 min, and then placed in -80°C refrigerator for standby use. Enzyme-linked immunosorbent assay (ELISA) was used to detect the concentration of OCN, PICP, 25(OH)D_3 and 1,25$(OH)_2D_3$ (R&D Systems, USA). The sample, reference standard product, and HRP-labeled detection antibody were added in turn to microwells pre-coated with OCN, PICP, 25(OH)D_3 or 1,25$(OH)_2D_3$ primary antibody. According to the instructions, the OD value of each microwell was detected at 450 nm wavelength. The standard curve was used to calculate the concentration of each sample.

Detection of serum alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP)

According to the operating instructions, the serum levels of ALP and TRAP were detected by automatic biochemical analyzer (AU5800, Beckman Coulter, USA).

Detection of osteoprotegerin (OPG) and receptor activator of NF-κB ligand (RANKL) by Real-time PCR

The femoral bone was separated and squashed in each group. The total RNA was extracted using the Trizol reagent (Invitrogen, USA) and synthesized into cDNA via reverse transcription polymerase chain reaction. The primer and probe sequences of GAPDH, OPG and RANKL were as follows: GAPDH forward primer: 5'-AGTTCAACGGCACAGTCAAGG-3' and reverse primer: 5'-ACATACT CAGCACCAGCATCAC-3'; OPG forward primer: 5'-CACAGAGCAGCT CCGCATCTTG-3' and reverse primer: 5'-AAGTGCTTGAGTGCGT ACATCAGG-3'; RANKL forward primer: 5'-ATATCGTTGGATCACAGCA CATCAGAG-3' and reverse primer: 5'-TGTCGGTGGCATTAATAGTGAGAT GAG-3'; According to the instructions of PCR kits (Thermo Fisher Scientific, USA), Real-time PCR reactions was conducted in Applied Biosystems 7500 PCR System. The reaction system was as follows: cDNA 2.0 μL, forward and reverse primers of 1.0 μL each, SYBR Premix Ex TaqTM II (2×) 10 μL, ROX Reference Dye (50*) 0.6 μL, and dH_2O 5.4 μL. The reaction conditions were as follows: initial denaturation at 95°C for 20 sec, denaturation at 95°C for 12 sec, renaturation at 62°C for 45 sec, extension at 70°C for 1 min, with 45 cycles. The relative expression levels of OPG and RANKL in each group were calculated using the $2^{-\Delta\Delta Ct}$ method. The expression of GAPDH was selected as the internal reference.

Statistical analysis

Statistical analyses of all experimental data were conducted using SPSS statistical soft-

Table 1. Comparison of bone mineral density among three groups

Groups	Whole body bone BMD ($\times 10^2$ g/cm^2)	Femoral bone BMD ($\times 10^2$ g/cm^2)	Vertebral bone BMD ($\times 10^2$ g/cm^2)
Sham group	18.6±0.5	15.6±0.6	17.6±0.5
Model group	16.0±0.3***	13.8±0.4***	14.7±0.2***
Psoralea corylifolia group	17.1±0.4$^{***,\#\#\#}$	14.7±0.5$^{***,\#\#\#}$	15.8±0.3$^{***,\#\#\#}$
F value	122.600	37.870	203.100
P value	<0.001	<0.001	<0.001

Note: BMD: Bone mineral density. Compared with sham group, $^{***}P<0.001$; Compared with model group, $^{\#\#\#}P<0.001$.

Table 2. Comparison of maximum load and stiffness among different groups

Groups	Maximum load (N)	Stiffness (N/mm)
Sham group	129.8±5.1	228.7±8.4
Model group	79.6±4.8***	149.8±7.5***
Psoralea corylifolia group	113.7±4.3$^{***,\#\#\#}$	201.4±6.9$^{***,\#\#\#}$
F value	350.200	331.400
P value	<0.001	<0.001

Note: Compared with sham group, $^{***}P<0.001$; Compared with model group, $^{\#\#\#}P<0.001$.

ware, version 23.0. Measurement data were expressed as mean ± standard deviation, and comparisons among three groups were performed by One-way ANOVA test. Count data were presented as percentages, and the comparisons among different groups were conducted by chi-square test. P<0.05 was deemed to be statistically significant.

Results

Comparison of bone mineral density

As shown in **Table 1**, the whole body bone mineral density, femoral bone mineral density and vertebral bone mineral density in Psoralea corylifolia group and sham group were obviously higher than those in model group, and there were statistical differences (all P<0.001). Compared with those in sham group, the whole body bone mineral density, femoral bone mineral density and vertebral bone mineral density in Psoralea corylifolia group were obviously lower, and the statistical differences were found (all P<0.001).

Comparison of maximum load and stiffness

As seen in **Table 2**, compared with those in sham group, the maximum load and stiffness in model group and Psoralea corylifolia group were significantly lower, and there were significantly statistical differences (all P<0.001). The maximum load and stiffness in Psoralea corylifolia group were significantly higher than those in model group and the statistical differences were found (all P<0.001).

Comparison of OCN and PICP levels

As shown in **Figure 1**, the serum levels of OCN and PICP in Psoralea corylifolia group and sham group were remarkably higher than those in model group, and there were statistical differences (all P< 0.001). Compared with those in sham group, the serum levels of OCN and PICP in Psoralea corylifolia group were remarkably lower, and the statistical differences were found (all P<0.05).

Comparison of serum 25(OH)D_3 and 1,25$(OH)_2D_3$ levels

As shown in **Figure 2**, the serum levels of 25(OH)D_3 and 1,25$(OH)_2D_3$ in Psoralea corylifolia group were significantly higher than those in model group, and there were statistical differences (all P<0.01). Compared with those in sham group, the serum levels of 25(OH)D_3 and 1,25$(OH)_2D_3$ in model group were remarkably lower, and the statistical differences were found (all P<0.01). There were no statistical differences for serum levels of 25(OH)D_3 and 1,25$(OH)_2D_3$ between sham group and Psoralea corylifolia group.

Comparison of ALP and TRAP

As shown in **Table 3**, the serum levels of ALP and TRAP in Psoralea corylifolia group and model group were significantly higher than those in sham group, and there were statistical differences (all P<0.001). In contrast to those in model group, the serum levels of ALP and TRAP in Psoralea corylifolia group were remarkably lower, and the significantly statistical differences were found (all P<0.001).

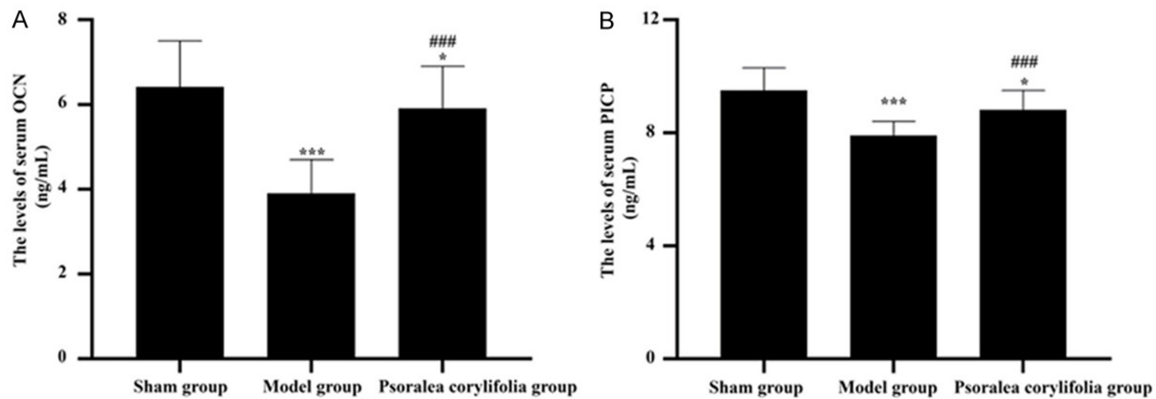

Figure 1. Comparison of serum OCN and PICP levels among three groups. Note: OCN: Osteocalcin; PICP: Procollagen I carboxyterminal. Compared with sham group, ***P<0.001 and *P<0.05; Compared with model group, ###P<0.001.

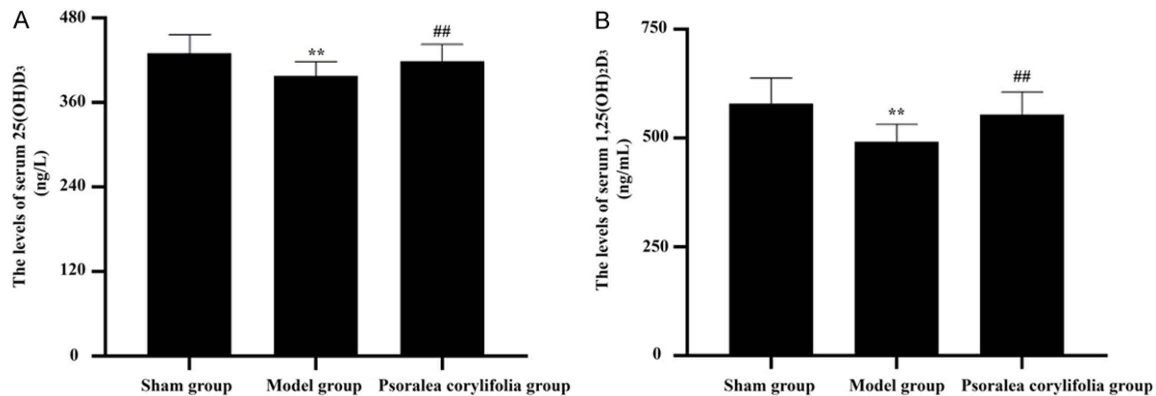

Figure 2. Comparison of serum $25(OH)D_3$ and $1,25(OH)_2D_3$ levels among three groups. Note: Compared with sham group, **P<0.01; Compared with model group, ##P<0.01.

Table 3. Comparison of ALP and TRAP among different groups

Groups	ALP (U/L)	TRAP (U/L)
Sham group	49.8±6.2	47.8±6.6
Model group	105.4±9.1***	100.2±8.3***
Psoralea corylifolia group	75.6±7.6***,###	66.5±7.5***,###
F value	125.850	117.390
P value	<0.001	<0.001

Note: Compared with sham group, ***P<0.001; Compared with model group, ###P<0.001.

Comparison of OPG and RANKL mRNA levels

As shown in **Figure 3**, compared with those in sham group, the mRNA levels of OPG in Psoralea corylifolia group and model group were significantly lower, while RANKL mRNA levels were obvious higher, and there were statistical differences (all P<0.001). Compared with those in mode group, OPG mRNA levels in Psoralea corylifolia group were remarkably higher while RANKL mRNA levels significantly lower, and the statistical differences were found (all P<0.001).

Discussion

Psoralea corylifolia was a common herb in traditional Chinese medicine prescriptions and widely used in clinical practice. Osteoporosis usually occurs during an imbalance between bone formation and bone resorption in the body [14]. At present, the strategy for osteoporosis treatment is to promote bone formation and/or reduce bone resorption, but many of the drugs for osteoporosis is to promote bone formation [15]. The specific mechanism via which Psoralea corylifolia improve osteoporosis was still unknown. In this study, the osteoporotic rats were successfully established and Psoralea corylifolia could significantly increase BMD and enhance biomechanics, and thus improve osteoporosis.

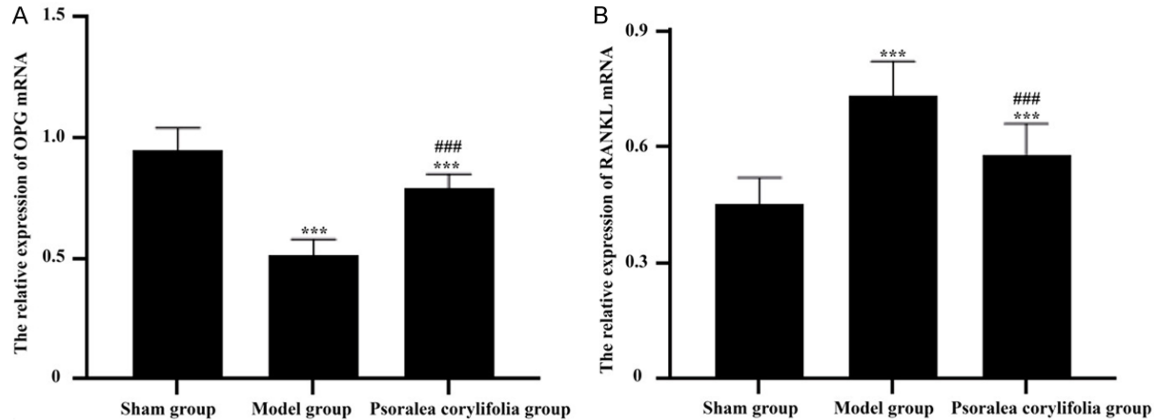

Figure 3. Comparison of OPG and RANKL mRNA levels among three groups. Note: OPG: Osteoprotegerin; RANKL: Receptor activator of NF-κB ligand. Compared with sham group, ***P<0.01; Compared with model group, ###P<0.01.

ALP was an extracellular enzyme for osteoblasts. The main function of ALP was to hydrolyze phospholipids during the process of bone formation and promote bone formation [16]. TRAP was mainly secreted from osteoclasts and has the resisting effect on the inhibition of tartaric acid. It was reported that TRAP could reflect the activity of osteoclasts and the conditions of bone resorption [17]. In this study, the levels of ALP and TRAP in Psoralea corylifolia group were significantly lower than those in mode group, which indicated that Psoralea corylifolia could obviously inhibit the activity of osteoclasts. These results were consistent with previous reports [18, 19].

Osteocalin (OCN) was secreted by osteoblasts and considered as one of the markers for evaluating activity of osteoblasts and physiological metabolism of bone [20]. It was reported that the serum levels of OCN increased when the balance between normal bone resorption and bone formation was disrupted [21]. Moreover, the production of OCN depended on the regulation of $1,25(OH)_2D_3$. And $1,25(OH)_2D_3$ was the strongest form of vitamin D in the body. The serum $25(OH)D_3$ level, as an index for reflecting nutritional status of vitamin D, was positively associated with BMD [22]. Down-regulation of serum $25(OH)D_3$ levels could induce increased osteoclastogenesis and then lead to osteoporosis [23]. In addition, serum PICP levels could reflect the activity of osteoblast, bone formation and synthesis of collagen I. It was reported that detection of PICP levels was helpful to investigate the change of bone metabolism [24]. In our study, we have shown that the serum levels of OCN, PICP, $25(OH)D_3$, $1,25(OH)_2D_3$ in model group were significantly lower than those in sham group, suggesting that the activity of osteoblasts was significantly reduced and bone metabolism was affected in osteoporotic rats. These results were consistent with previous report, in which, the activity of osteoblasts in Psoralea corylifolia group was obviously improved, bone resorption was significantly inhibited and bone formation was promoted [25]. It was also indicated that Psoralea corylifolia could improve the balance between bone resorption and bone formation, which was helpful for prevention and treatment of osteoporosis.

It is well known that OPG/RANKL signaling pathway is one of the important pathways involved in the interaction between osteoblasts and osteoclasts, which can effectively regulate the process of osteoclastogenesis and bone metabolism [26]. It was also reported that OPG/RANKL signaling pathway was closely associated with pathogenesis of osteoporosis [27]. OPG secreted by osteoblasts was one kind of RANKL decoy receptor, which could induce osteoclast apoptosis and inhibited bone resorption [28]. Many studies have reported that regulation of OPG and RANKL expression played a key role in the process of bone remodeling [29]. In this study, in contrast to sham group, the expression of OPG significantly decreased and the expression of RANKL obviously increased, indicating that increased bone resorption and reduced bone mass in osteoporotic rats may be related with OPG/RANKL signaling pathway. After treatment with Psoralea

corylifolia, OPG expression significantly increased and RANKL expression obviously decreased, which suggested that Psoralea corylifolia improved osteoporosis through OPG/RANKL signaling pathway. These results were similar to previous studies that have been reported by Ma et al [30].

In conclusions, in osteoporotic rats Psoralea corylifolia could increase BMD and biomechanics, up-regulate the serum levels of OCN, PICP, $25(OH)D_3$ and $1,25(OH)_2D_3$ and down-regulate the serum levels of ALP and TRAP. Moreover, the mechanisms for osteoporosis are very complicated, and there may be multiple signaling pathways or target sites. Psoralea corylifolia may improve osteoporosis via OPG/RANKL signaling pathway. However, additional studies are required to investigate and confirm the specific mechanisms of action for Psoralea corylifolia in the prevention and treatment of osteoporosis.

Disclosure of conflict of interest

None.

Address correspondence to: Zhoujin Li, Department of Orthopedics, The Second Affiliated Hospital of Hunan University of Chinese Medicine, No. 233 North Cai'e Road, Kaifu District, Changsha 410005, Hunan Province, China. Tel: +86-0731-84917810; Fax: +86-0731-84912978; E-mail: lizhoujin_tcm@163.com

References

[1] Sleeman A and Clements JN. Abaloparatide: a new pharmacological option for osteoporosis. Am J Health Syst Pharm 2019; 76: 130-135.
[2] Yavropoulou MP, Makras P and Anastasilakis AD. Bazedoxifene for the treatment of osteoporosis. Expert Opin Pharmacother 2019; 20: 1201-1210.
[3] Anthamatten A and Parish A. Clinical update on osteoporosis. J Midwifery Womens Health 2019; 64: 265-275.
[4] Cotts KG and Cifu AS. Treatment of osteoporosis. JAMA 2018; 319: 1040-1041.
[5] Johnston CB and Dagar M. Osteoporosis in older adults. Med Clin North Am 2020; 104: 873-884.
[6] Qaseem A, Forciea MA, McLean RM and Denberg TD; Clinical Guidelines Committee of the American College of Physicians. Treatment of low bone density or osteoporosis to prevent fractures in men and women: a clinical practice guideline update from the American College of Physicians. Ann Intern Med 2017; 166: 818-839.
[7] Jin YX, Wu P, Mao YF, Wang B, Zhang JF, Chen WL, Liu Z and Shi XL. Chinese herbal medicine for osteoporosis: a meta-analysis of randomized controlled trials. J Clin Densitom 2017; 20: 516-525.
[8] Wang YC, Chiang JH, Hsu HC and Tsai CH. Decreased fracture incidence with traditional Chinese medicine therapy in patients with osteoporosis: a nationwide population-based cohort study. BMC Complement Altern Med 2019; 19: 42.
[9] Alalaiwe A, Hung CF, Leu YL, Tahara K, Chen HH, Hu KY and Fang JY. The active compounds derived from Psoralea corylifolia for photochemotherapy against psoriasis-like lesions: the relationship between structure and percutaneous absorption. Eur J Pharm Sci 2018; 124: 114-126.
[10] Alam F, Khan GN and Asad M. Psoralea corylifolia l: ethnobotanical, biological, and chemical aspects: a review. Phytother Res 2018; 32: 597-615.
[11] Zhang X, Zhao W, Wang Y, Lu J and Chen X. The chemical constituents and bioactivities of psoralea corylifolia Linn.: a review. Am J Chin Med 2016; 44: 35-60.
[12] Wong RW and Rabie AB. Effect of Buguzhi (Psoralea corylifolia fruit) extract on bone formation. Phytother Res 2010; 24 Suppl 2: S155-160.
[13] Hoffmann DB, Komrakova M, Pflug S, von Oertzen M, Saul D, Weiser L, Walde TA, Wassmann M, Schilling AF, Lehmann W and Sehmisch S. Evaluation of ostarine as a selective androgen receptor modulator in a rat model of postmenopausal osteoporosis. J Bone Miner Metab 2019; 37: 243-255.
[14] Wang Y, Tao Y, Hyman ME, Li J and Chen Y. Osteoporosis in China. Osteoporos Int 2009; 20: 1651-1662.
[15] Liu GF, Wang ZQ, Liu L, Zhang BT, Miao YY and Yu SN. A network meta-analysis on the short-term efficacy and adverse events of different anti-osteoporosis drugs for the treatment of postmenopausal osteoporosis. J Cell Biochem 2018; 119: 4469-4481.
[16] Mukaiyama K, Kamimura M, Uchiyama S, Ikegami S, Nakamura Y and Kato H. Elevation of serum alkaline phosphatase (ALP) level in postmenopausal women is caused by high bone turnover. Aging Clin Exp Res 2015; 27: 413-418.
[17] Song ZH, Xie W, Zhu SY, Pan JJ, Zhou LY and He CQ. Effects of PEMFs on Osx, Ocn, TRAP, and CTSK gene expression in postmenopausal osteoporosis model mice. Int J Clin Exp Pathol 2018; 11: 1784-1790.

[18] Li X, Sui X, Yang Q, Li Y, Li N, Shi X, Han D, Li Y, Huang X, Yu P and Qu X. Oviductus Ranae protein hydrolyzate prevents menopausal osteoporosis by regulating TGFbeta/BMP2 signaling. Arch Gynecol Obstet 2019; 299: 873-882.
[19] Sun X, Wei B, Peng Z, Fu Q, Wang C, Zhen J and Sun J. Protective effects of Dipsacus asper polysaccharide on osteoporosis in vivo by regulating RANKL/RANK/OPG/VEGF and PI3K/Akt/eNOS pathway. Int J Biol Macromol 2019; 129: 579-587.
[20] Xu ZS, Dai F, Chen J, Lv M, Cheng JW, Zhang XM and Lin BW. Experimental research into the potential therapeutic effect of GYY4137 on Ovariectomy-induced osteoporosis. Cell Mol Biol Lett 2018; 23: 47.
[21] Zhang K, Li B, Chen Q, Zhang Z, Zhao X and Hou H. Functional calcium binding peptides from Pacific cod (Gadus macrocephalus) bone: calcium bioavailability enhancing activity and anti-osteoporosis effects in the ovariectomy-induced osteoporosis rat model. Nutrients 2018; 10: 1325.
[22] Liao EY, Zhang ZL, Xia WB, Lin H, Cheng Q, Wang L, Hao YQ, Chen DC, Tang H, Peng YD, You L, He L, Hu ZH, Song CL, Wei F, Wang J and Zhang L. Calcifediol (25-hydroxyvitamin D) improvement and calcium-phosphate metabolism of alendronate sodium/vitamin D_3 combination in Chinese women with postmenopausal osteoporosis: a post hoc efficacy analysis and safety reappraisal. BMC Musculoskelet Disord 2018; 19: 210.
[23] Hoeck HC, Li B and Qvist P. Changes in 25-Hydroxyvitamin D_3 to oral treatment with vitamin D_3 in postmenopausal females with osteoporosis. Osteoporos Int 2009; 20: 1329-1335.
[24] Chen P, Satterwhite JH, Licata AA, Lewiecki EM, Sipos AA, Misurski DM and Wagman RB. Early changes in biochemical markers of bone formation predict BMD response to teriparatide in postmenopausal women with osteoporosis. J Bone Miner Res 2005; 20: 962-970.
[25] Ostrowska Z, Kos-Kudla B, Swietochowska E, Marek B, Kajdaniuk D and Gorski J. Assessment of the relationship between dynamic pattern of nighttime levels of melatonin and chosen biochemical markers of bone metabolism in a rat model of postmenopausal osteoporosis. Neuro Endocrinol Lett 2001; 22: 129-136.
[26] El-Baz FK, Saleh DO, Abdel Jaleel GA, Hussein RA and Hassan A. Heamatococcus pluvialis ameliorates bone loss in experimentally-induced osteoporosis in rats via the regulation of OPG/RANKL pathway. Biomed Pharmacother 2019; 116: 109017.
[27] Ma L, Liu X and Sun G. Aspirin reduces the incidence of postmenopausal osteoporosis in rats through OPG-RANKL-RANK signaling pathway. Minerva Endocrinol 2019; 44: 408-410.
[28] Mo H, Zhang N, Li H, Li F and Pu R. Beneficial effects of Cuscuta chinensis extract on glucocorticoid-induced osteoporosis through modulation of RANKL/OPG signals. Braz J Med Biol Res 2019; 52: e8754.
[29] Li CW, Liang B, Shi XL and Wang H. Opg/Rankl mRNA dynamic expression in the bone tissue of ovariectomized rats with osteoporosis. Genet Mol Res 2015; 14: 9215-9224.
[30] Ma B, Zhang Q, Wu D, Wang YL, Hu YY, Cheng YP, Yang ZD, Zheng YY and Ying HJ. Strontium fructose 1,6-diphosphate prevents bone loss in a rat model of postmenopausal osteoporosis via the OPG/RANKL/RANK pathway. Acta Pharmacol Sin 2012; 33: 479-489.