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Original Article

Effects of *Pueraria Radix* Isoflavone Extract on Osteoporosis in Ovariectomized Rats

Rihyang Pak[1,*], Cholgyu Choe[2], Kyongjin Ma[3]

[1]Postgraduate School, Haeju University of Medicine, Guangsok Dong, Haeju, DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA.
[2]Department of Koryo Medicine, Pyongyang Medical College, Taedonggang District, Pyongyang, DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA.
[3]Department of Emergency, Pyongyang General Hospital, Taedonggang District, Pyongyang, DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA.

ABSTRACT

Background and Aim: Cessation of ovarian function is a major cause of postmenopausal osteoporosis. Ovariectomized rats and dogs have been used extensively in osteoporosis models. This study examined the effect of *Pueraria Radix* isoflavone extract not only on Bone Mineral Density (BMD) but also on bone strength using bone formation and resorption markers in ovariectomized rats as an osteoporosis model. **Methods:** The model of Ovariectomized (OVX) rats induced by ovariectomy as previously described and the effect of isoflavone extract was evaluated through the measurement of BMD, the strength of the distal femoral metaphysis and the levels of urinary deoxypyridinoline and serum osteocalcin. **Results:** In rat administrated with isoflavone extract (only 90 mg/kg) for 12 weeks, BMD was significantly increased, while the doses of 60 and 90 mg/kg decreased urinary deoxypyridinoline and serum osteocalcin to normal level and elevated the strength of the distal femoral metaphysis. **Conclusion:** These results suggested that *Pueraria Radix* isoflavone may be a useful traditional medicine for osteoporosis due to stimulated bone resorption.

Keywords: *Pueraria Radix* Isoflavone, Osteoporosis, Ovariectomized, Bone mineral density.

***Correspondence:**
Dr. Rihyang Pak
Postgraduate School, Haeju University of Medicine, Guangsok Dong, Haeju, DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA.
Email: shypinguo202123@163.com

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INTRODUCTION

Estrogen plays an important role not only in maintaining the reproductive function and the secondary sex characters of the female animals, but also in so-classical target tissues, such as the brain, bone, liver, kidney and the cardiovascular system.[1,2] A significant decrease in estrogen levels in women during the menopausal period also causes osteoporosis, a major public health concern. When the bone resorption of osteoclasts outpaces the bone formation of osteoblasts during menopause, bone density is decreased and osteoporosis becomes more severe. Epidemiological studies indicate that menopausa1 syndrome and osteoporosis caused by estrogen deficiency are the two major leading factors affecting the health and life quality of climacteric women.[3] Although Hormone Replacement Therapy (HRT) can help to prevent and treat the menopausal syndromes, the side effects of HRT, such an increased risk of developing breast and endometrial cancer, prevent the acceptance of HRT.[4,5] Recently, reliable evidence has indicated that phytoestrogens offer the best potential therapy for menopausal women because they are safe and have a high compliance rate. Phytoestrogens are naturally occurring plant chemicals that can produce an estrogen-like effect in the body, used as a natural alternative to hormone replacement therapy and to reduce menopausal symptoms. They are not chemically the same as the estrogens made in the body, but when digested and absorbed they can act somewhat as estrogen in the body.[6] Recent research has found leguminous plants rich in flavone have a certain kind of estrogen-like effect.[7,8] *Pueraria Radix*, consisting of the root of *Pueraria lobate Ohwi* (Leguminosae), has been clinically used as an antipyretic and spasmolytic agent in Traditional Korean medicine as well as Chinese. Many Koreas herbal formulas contain *Pueraria radix* as their major ingredient and these have been prescribed indicated for fever and chills with stiffness or rigidity of the neck and upper back. The compounds of *Pueraria radix* have been studied extensively with various isoflavonoids, such as puerarin, daidzin, daidzein, genistin, and genistein, having been identified.[9] Among them genistein has estrogenic activity[10] and a study demonstrated the positive effect of *Pueraria radix* flavones on liver lipid metabolism in OVX rat.[11] It might be associated to estrogenic activity of some flavones in chemical components of *Pueraria radix*. However, there has been no research to use *Pueraria radix* isoflavone extract as traditional medicine for the treatment of osteoporosis after menopause.

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MATERIALS AND METHODS

Reagents

Pueraria radix isoflavone extract was kindly provided by Traditional Medicine Centre of Pyongyang University of Medical Sciences. *Pueraria radix* isoflavone was extracted using microwave assisted extraction (MAE, microwave powers: 400 W, leaching time: 5min, and leaching temperature: 80°C). As described by manufacturer, the extract contained about 35.1% isoflavone and other unidentified compounds.

Animals

Mature female Wistar rats (12 weeks old) were purchased from the Laboratory Animal Centre of the Haeju University of Medicine. During the experimented period, rats had free access to tap water and commercially available standard solid food containing 1.18% calcium and 2.5 IU/g of Vitamin D_3. The temperature was maintained at 20±2°C and the humidity was 55%. After 7 days acclimatization, 50 rats were randomly divided into 5 groups (10 rats/group): Sham-group, OVX model group, OVX+ Extract-1, OVX+ Extract-2 and OVX+Extract-3. After anesthetized with 15g/L sodium pentobarbital intraperitoneally, the rats of OVX, OVX + extract (-1,2,3) groups were ovariectomized. Rats of sham group received only a squeeze in the connecting point between the ovary and the fallopian tube without having their ovary removed. From the second week after the operation, the rats of OVX and sham group were administrated with saline and the rats of extract groups were orally administrated with isoflavone extract (30mg/kg/d, 60 mg/kg/d and 90 mg/kg/d respectively) for 12 weeks. The study was approved by the Ethics Committee for Animal Experimentation, Pyongyang University of Medical Sciences.

Sample Preparation and Assay

After the final administration, urine (0–24 hr) was collected and then urinary deoxypyridinoline levels were measured using High Performance Liquid Chromatography (HPLA). Rats were anesthetized and sacrificed by withdrawing blood from the abdominal aorta using vacuum sampling tubes through a laparotomic incision. Serum samples were prepared by centrifuging (1000×g for 10 min) their blood, and serum osteocalcin levels were determined using a radioimmunoassay (RIA kits, BNIBT). Immediately after collecting urine and blood from rats, the second lumbar vertebrae and the femurs were removed. BMD of the second lumbar vertebrae were measured by dual energy X-ray absorptiometry using a bone densitometer (Hologic QDR 2000w). The distal epiphysis of each femur was removed and a 7-mm portion was cut from the edge of the bone. The strength of the distal femoral metaphysis under compression was measured by compressing the bone anterior-to-posterior in a bone strength tester (TK-252C).

Statistical Analysis of Data

Results were expressed as the mean and SEM. Data were analyzed by one-way Analysis of Variance (ANOVA) using SPSS 16.0 and the differences between the means assessed using Dunnet's multiple range test. A P value of < 0.01 was taken as the level of statistical significance.

RESULTS

Changes of Urinary Deoxypyridinoline Level

The urinary deoxypyridinoline levels in each group at the 12th week after ovariectomy were shown in Figure 1. At the 12th week after ovariectomy, urinary deoxypyridinoline level in OVX group was significantly elevated by 28.6 (pmol/μmol creatinine) compared to sham group (14.2 pmol/μmol creatinine). Oral administration of isoflavone extract showed some tendency to dose-dependently inhibit the elevation in urinary deoxypyridinoline level, and especially at doses of 60mg/kg and 90mg/kg urinary deoxypyridinoline levels were significantly decreased compared to OVX group.

Changes of BMD

After administration with different doses of Extract, the change of BMD in each group was measured using a bone densitometer. Figure 2 shows the effects of isoflavone extract on BMD. In Figure 2, BMD in only Extract group with 90 mg/kg increased significantly compared to OVX group (P <0.01). But extract at doses of 30 mg/kg and 60 mg/kg had no effect on BMD (P > 0.5).

Changes of Compressive Strength

The strength of femurs was measured by compressing the bone anterior-to-posterior. Table 1 shows some tendency to dose-dependently inhibition of isoflavone extract on compressive strength.

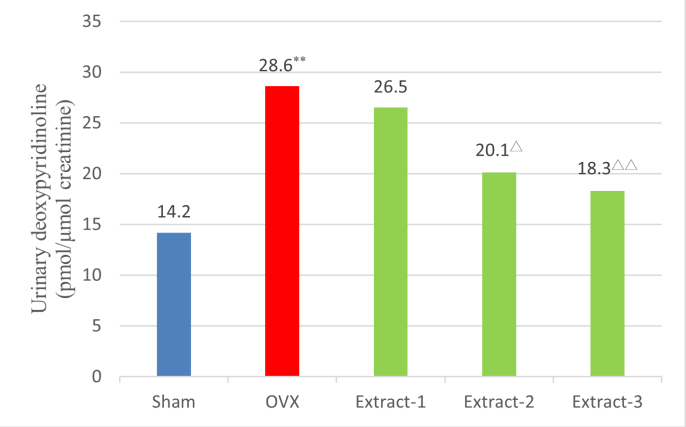

Figure 1: Effect of isoflavone extract on urinary deoxypyridinoline.

Extract-1: 30 mg/kg, Extract-2: 60 mg/kg, Extract-3: 90 mg/kg. Each value represents the mean ± SEM of 10 rats per group. **P <0.01 as compared with sham group. $^{\Delta}$ P <0.05 and $^{\Delta\Delta}$ P <0.01 as compared with OVX group

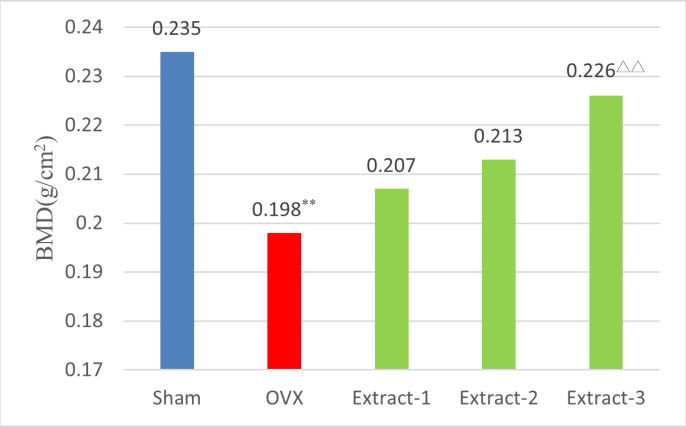

Figure 2: Effect of isoflavone extract on BMD.

Extract-1: 30 mg/kg, Extract-2: 60 mg/kg, Extract-3: 90 mg/kg. Each value represents the mean ± SEM of 10 rats per group. **P <0.01 as compared with sham group. ΔΔ P <0.01 as compared with OVX group.

Table 1: Effects on distal femoral metaphyseal compressive strength.

Group	Dose	Ultimate load (N)
Sham		175.2±16.5
OVX	-	62.7±8.3**
Extract-1	30 mg/kg	88.9±10.4
Extract-2	60 mg/kg	112.3±9.3ΔΔ
Extract-3	90 mg/kg	145.6±14.1ΔΔ

Each value represents the mean ± SEM of 10 rats per group. **P<0.01 as compared with sham group. ΔΔ P<0.01 as compared with OVX group.

Table 2: Effect on serum osteocalcin levels.

Group	Dose	Serum osteocalcin (ng/mL)
Sham		38.1±0.6
OVX	-	49.5 ± 1.3**
Extract-1	30 mg/kg	47.3± 0.9
Extract-2	60 mg/kg	41.3± 0.3ΔΔ
Extract-3	90 mg/kg	40.9±0.5ΔΔ

Each value represents the mean ± SEM of 10 rats per group. **P<0.01 as compared with sham group. ΔΔ P<0.01 as compared with OVX group.

After 12 weeks, the compressive strength of the distal femoral metaphysis declined by approximately 64% in OVX group compared to sham group. Isoflavone extract inhibited this decrease significantly at doses of 60 and 90 mg/kg.

Changes of Serum Osteocalcin Level

Table 2 shows the dose-dependently reduced serum osteocalcin levels after administration of isoflavone extract for 12 weeks. After 12 weeks, in OVX group serum osteocalcin level increased significantly compared to sham group, but isoflavone extract inhibited this increase significantly at doses of 60 and 90mg/kg.

DISCUSSION

With the decreases of estrogen in postmenopausal women, the incidences of fracture in osteoporosis, cardiovascular and neurodegenerative diseases often increase.[12,13] Estrogen replacement therapy is able to attenuate symptoms of menopausal syndrome and to reduce the incidence of above-mentioned diseases.[14,15] Many studies reported that the flavones in the leguminous plants have a certain kind of estrogen-like effect and, in particular, genistein in *Pueraria Radix* has estrogenic activity. As *Pueraria Radix* is the dried root of *Pueraria lobate* Ohwi *Pueraria thomsonii* Benth, it can strengthen the spleen and stomach, invigorate the spleen in Traditional Korean medicine as well as Chinese medicine. We thought that isoflavones including genistein in *Pueraria Radix* can improve osteoporosis in OVX, because its main component is flavone. The OVX rats can be used as models to reflect the pathologic changes in perimenopausal or postmenopausal women. In this study, the isoflavone extracts of *Pueraria Radix* at different doses were given to OVX rats and its estrogen-like effects were observed in this model. Metabolic markers, reflecting specific stages of bone catabolism and anabolism have been used to detect bone loss in early stages of morbidity.[16,17] These markers for bone formation include the protein osteocalcin and the enzyme alkaline phosphatase[18] produced by osteoblasts. To monitor bone resorption, urinary collagen deoxypyridinoline crosslinks, which are produced by the catabolism of collagen, have been assayed.[19] At the 12th week after ovariectomy, urinary deoxypyridinoline levels were measured by HPLC. Isoflavone extract dose-dependently inhibited the increase in urinary deoxypyridinoline levels after oral administration for 12 weeks and especially decreases at doses of 60 mg/kg and 90 mg/kg were significant compared to OVX group. OVX resulted in significantly decreased BMD of the second lumbar vertebrae in rats, with reductions of 15.7% at the 12th week after ovariectomy compared to non-OVX sham group. Oral administration of isoflavone extract at doses of 30, 60, and 90 mg/kg decreased in BMD. Only at dose of 90 mg/kg for 12 weeks this effect was statistically significant (Figure 2). We used serum osteocalcin levels as bone formation marker and urinary deoxypyridinoline levels as above-mentioned bone resorption marker to clarify the administrative effect of the *Pueraria Radix* isoflavone extract for 12 weeks on bone turnover in OVX rats. Isoflavone extract potently inhibited the increase in urinary deoxypyridinoline levels, a bone resorption marker and also serum osteocalcin levels, a bone formation marker, following oral administration for 12 weeks at doses of 60 and 90 mg/kg. These results suggest that during longer periods of isoflavone administration bone formation was reduced to original normal levels. The inhibition of bone formation by isoflavone might be attributed mainly to inhibition of bone resorption after restoration of the balance between bone resorption and bone formation. This maintenance of the balance between bone resorption and bone formation was demonstrated by the elevation of distal femoral metaphyseal compressive

strength (Table 2). Our results show that isoflavone extract in *Pueraria Radix* significantly may be useful for management and treatment of osteoporosis in postmenopausal women.

CONCLUSION

In conclusion, this study suggests that 90 mg/kg isoflavone extract in *Pueraria Radix* has the anti-osteoporosis potency in OVX rats. The results suggest that administration of *Pueraria Radix* isoflavone might pave the way for a therapeutic agent for the management of osteoporosis in postmenopausal women.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

HRT: Hormone Replacement Therapy; **OVX:** Ovariectomized; **BMD:** Bone Mineral Density.

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