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Original Article

Effects of Dietary from Safflower Bud on the Osteoporosis in Ovariectomized Rats

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It has been reported that safflower seeds have preventive effects against osteoporosis. Recently, safflower buds (SB) were found to have more useful functional ingredients than safflower seeds. In the current study, we evaluated the anti-osteoporosis effects of SB diet in ovariectomized (OVX) rats. The rats were divided into five groups; sham operated group, OVX alone group, OVX plus 17β-estradiol (E_2 10 μg/kg, *i.p.*) and OVX plus SB diet feeding group (0.3% or 1%). Feeding of SB diet (0.3% or 1%) to OVX rats markedly increased bone mineral density (BMD) of femurs, compared to the OVX group. The OVX rats exhibited a marked increase in trabecular separation (Tb.Sp) and this change was inhibited by the feeding of SB diet, similar to that seen with OVX+E2 group. Moreover, feeding of SB diet to OVX rats decreased the markers of bone turnover, including osteocalcin and alkaline phosphatase (ALP). These results suggest that SB extract has a bone sparing effect in OVX-induced trabecular bone loss and prevents deterioration of bone microarchitecture by suppressing the rate of bone turnover. Therefore, SB may be useful for preserving bone mass and structure in estrogen deficient women with a potential role in reducing postmenopausal osteoporosis

Key Words: Ovariectomized rats, Safflower bud, Bone biomechanics, Bone turnover

INTRODUCTION

Osteoporosis is a bone disease which is characterized by the decrease of mineral density in bone (Cauley et al., 2000; Cummings and Melton, 2002). In particular, in the case of women, when women reach menopause, due to the rapid decrease of the estrogen hormone in calcium homeostasis, holes in bone occurs (Raisz et al., 2005). Ovariectomized (OVX)-estrogen hormone deficiency is a major risk factor for osteoporosis (Brennan et al., 2012). OVX rats were used as a model of postmenopausal model (Lelovas et al., 2008).

The Safflower (*Carthamus tinctorius* L.) seed has been used as a traditional herbal medicine in Korea and other Asian countries (Bae et al., 2002). In addition, fracture healing was repaired more rapidly in rats supplemented with safflower seed powder and its fractions than in control rats (Seo et al., 2000). Safflower seeds have been reported to have bone protecting effect in OVX rats (Kim et al., 2002; Alam et al, 2006). On the other hand, the safflower bud (SB) has increased contents of useful functional ingredients such as flavonoids and polyphenol contents, compared to

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safflower leaf, stem and root (Hiramatsu et al., 2009). Thus, in this study, we investigated the preventive effects of SB in osteoporosis in OVX rat models.

MATERIALS AND METHODS

Animals and treatments

The 8-week-old female Sprague-Dawley strain rats were purchased from Sam tacos (Oh San, Korea) and were housed for a week to obtain the adaptation period. The rats underwent either a sham-operation or were OVX (n = 8~10 for each group). Sham rats were fed a AIN-76 diet, whereas OVX rats were divided into 4 groups that were fed a AIN-76 diet and AIN-76 diet containing SB (0.3% and 1%) for 16 weeks. Rats were housed in clean environmental conditions with a temperature 23 ± 2℃, with a relative humidity of 55 ± 5%, and with a 12-h light/dark cycle during the 16-week intervention period. All rats had free access to distilled water and diet throughout the study. The food intake and body weight were measured at intervals of a week. Safflower buds (SB) used in this experiment is produced in Jangheung and was purchased from a market in Jangheung. SB was washed with water and air-dried SB was used. Safflower germinated sprouts are pulverized using a grinder to prepare powder of safflower germination. The diet for Ovx+ SB rats contained 0.3% or 1% SB mixed with the standard rat chow. The diet was prepared by mixing 0.3 or 1% SB into 100 g standard rat chow. 17β-estradiol (E_2) was purchased from Sigma-Aldrich Chemical Company (MO, USA). Rats were subcutaneously injected 5 times per week with 100 μL of vehicle in the sham and OVX groups, and with 17β-estradiol (10 μg/kg/day). For the experiments, rats were fed with 0.3% and 1% SB diets, as described above. The experimental protocol was approved by the Animal Care and Use Committee of Chonnam National University.

Body weights and uterus tissue weights

Body weights were measured on a weekly basis to monitor health and measure weight gain. Uterus tissue weights were measured immediately after necropsy. At necropsy, uteri were removed from fat tissue. Uterus tissue weights were measured to confirm the ovariectomy procedure.

Bone mineral density (BMD)

The femur was dissected from the femoral region and fixed with 4% paraformaldehyde fixed storage system and a high-resolution micro-computerized tomography (Three-dimensional micro focus computed tomography; micro-CT, Sky-Scan 1172TM, Skyscan, Kontich, Belgium) using a micro video image was obtained. Fine image was obtained from Nercon Ver 1.3 (Skyscan) and was as the gray scale level and a two-dimensional image reconstruction of the CTAn (SkyScan) software was used to reconstruct a three-dimensional model.

Serum analysis

After 16 weeks of experiment time, the experimental rats were fasted for 24 h and anesthetized. Blood samples were collected from the heart by cardiac puncture. Blood were centrifuged at 6,000 ×g for 10 min; then, serum samples were stored at -80℃ prior to biochemical analysis. The analysis of osteogenesis markers osteocalcin, estrogen, and ALP was measured using ELISA kits.

Statistical methods

Statistical analyses were performed using GraphPad Prism software version 4.0 (GraghPad Software, San Diego, CA, USA). The data for all of the measurements were analyzed using a one-way analysis of variance (ANOVA) with subsequent post hoc multiple comparison by Dunnett's test. Statistically significant values were defined as $P<0.05$.

RESULTS AND DISCUSSION

Effects of SB on food intakes and body weight in OVX rats

During the experimental period, food intakes and body weight were measured. Average daily dietary intake levels were similar in all groups. As a result, all groups including the sham group exhibited no differences in food intakes among OVX rats (Fig. 1A). On the other hand, body weights in OVX rats were increased compared with sham

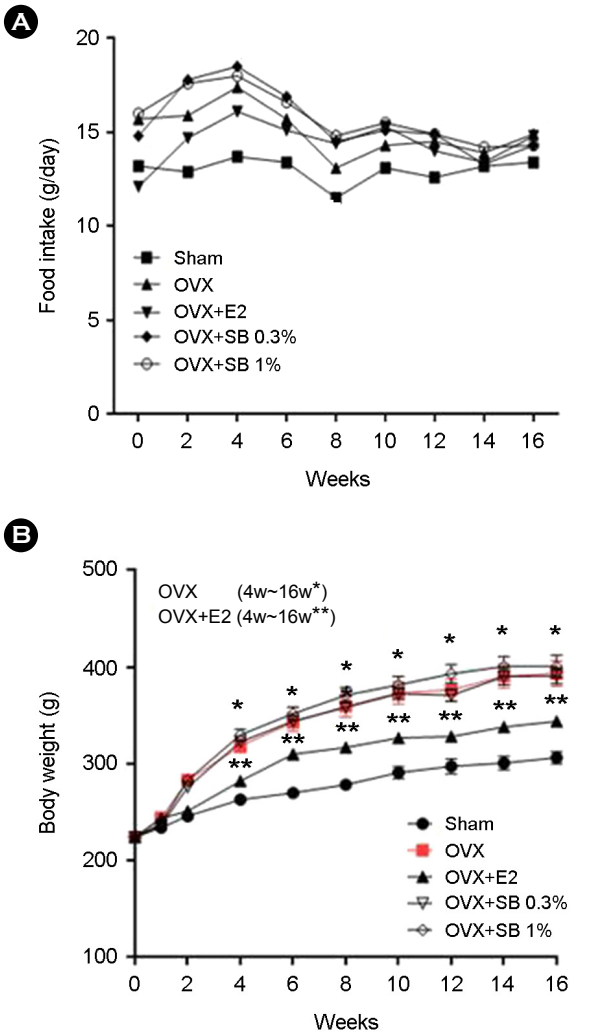

Fig. 1. Effects of safflower buds (SB) on food intake (A) and body weight (B) in ovariectomized (OVX) rats. OVX rats were treated with 17β-estradiol (E2, 10 μg/kg, i.p.) or fed with SB diet (0.3% or 1%). Each value is expressed as mean ± S.E., n = 8~10. *P<0.05 compared with the sham group and **P<0.05 compared with the OVX group.

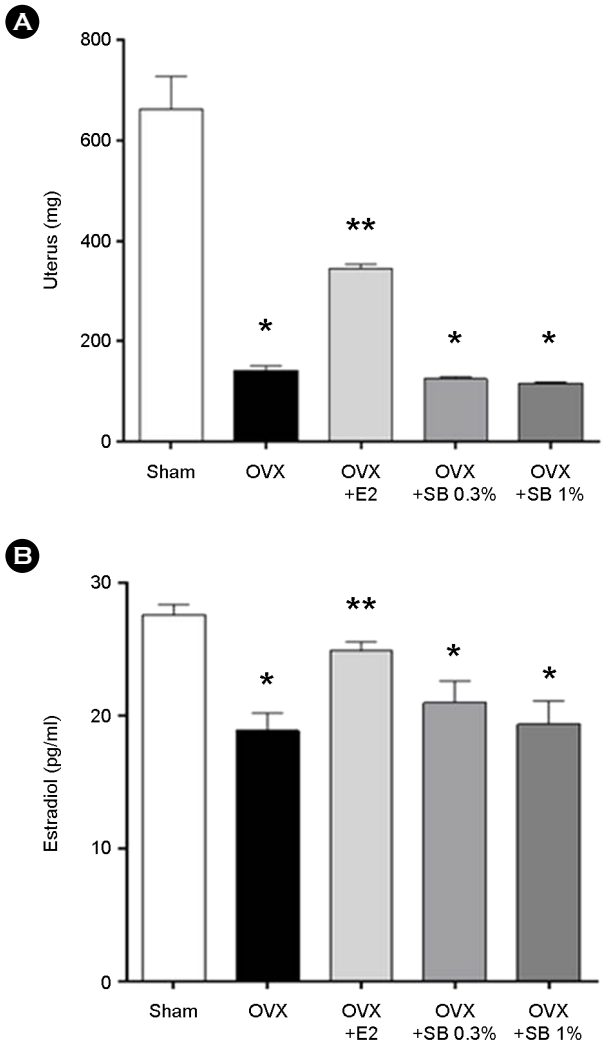

Fig. 2. Effects of safflower buds (SB) on uterine weight (A) and serum estradiol (B) in ovariectomized (OVX) rats. OVX rats were treated with 17β-estradiol (E2, 10 μg/kg, i.p.) or fed with SB diet (0.3% or 1%). Each value is expressed as mean ± S.E., n = 8~10. *P<0.05 compared with the sham group and **P<0.05 compared with the OVX group.

group and those in E_2-treated OVX rats were not increased compared to OVX rats. This weight gain is due to reduced secretion of ovarian hormones, which induce the obesity characterized by the accumulation of body fat in OVX animal models (Yoon and Lee, 1988). However, the feeding of SB diets in OVX rats did not prevent the increase of body weights (Fig. 1B). These results indicate that SB did not suppress body weight gain in OVX rats. Sarmiento et al. (1977) and Geinox et al. (1993) reported that fracture healing in rat femora was affected by functional weight bearing and nutritional differences. Our results indicate indirectly that food intake and weight gain induced by OVX were affected by feeding SB diets.

Effects of SB diets on uterine weights and serum estrogen level in OVX rats

Because estrogen is limited in clinical applications due to its tumor-promoting effects (Davis et al., 2006), we checked whether SB has estrogenicity by assessing the wet weights of the uterus. The uterine weights were also decreased in

OVX-induced rats compared to sham group. Uterine weights in OVX rats fed with SB diets (0.3% and 1%) were not different from OVX group (Fig. 2A). Also, serum estrogen levels were measured using ELISA Kit, and the results showed similar results to uterine weights. Serum estrogen levels in OVX rats alone and OVX rats fed with SB diets (0.3% or 1%) were not different (Fig. 2B). Our results are consistent with that safflower seed treatment in OVX rats did not affect serum estrogen levels (Kim et al., 2003). Since phytoestrogens are plant-derived compounds that have affinity to the estrogen receptor and are able to act as either estrogen agonists or antagonists (Lagari and Levis, 2012), our results suggest the possibility that SB may not be phytoestrogen and SB also do not affect estrogen levels.

Effects of SB diets on bone mineral density (BMD) in OVX rats

OVX rats are classically used as an animal model for

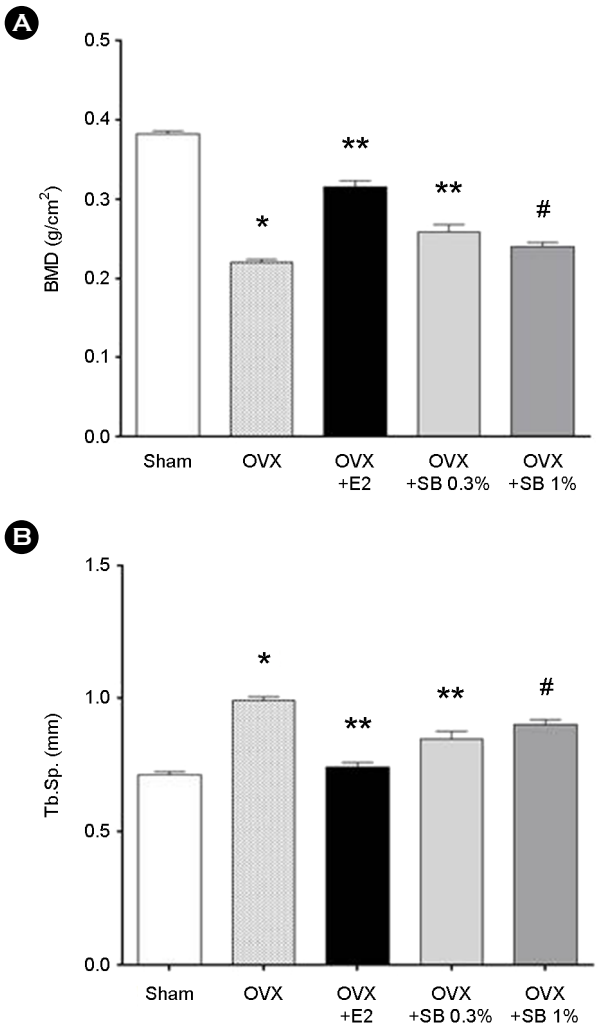

Fig. 3. Effects of safflower buds (SB) on bone mineral density (BMD) (A) and morphological characteristic Tb.Sp (B) in ovariectomized (OVX) rats. OVX rats were treated with 17β-estradiol (E2, 10 μg/kg, i.p.) or fed with SB diet (0.3% or 1%). Each value is expressed as mean ± S.E., n = 8~10. *P<0.05 compared with the sham group and **P<0.05 compared with the OVX group.

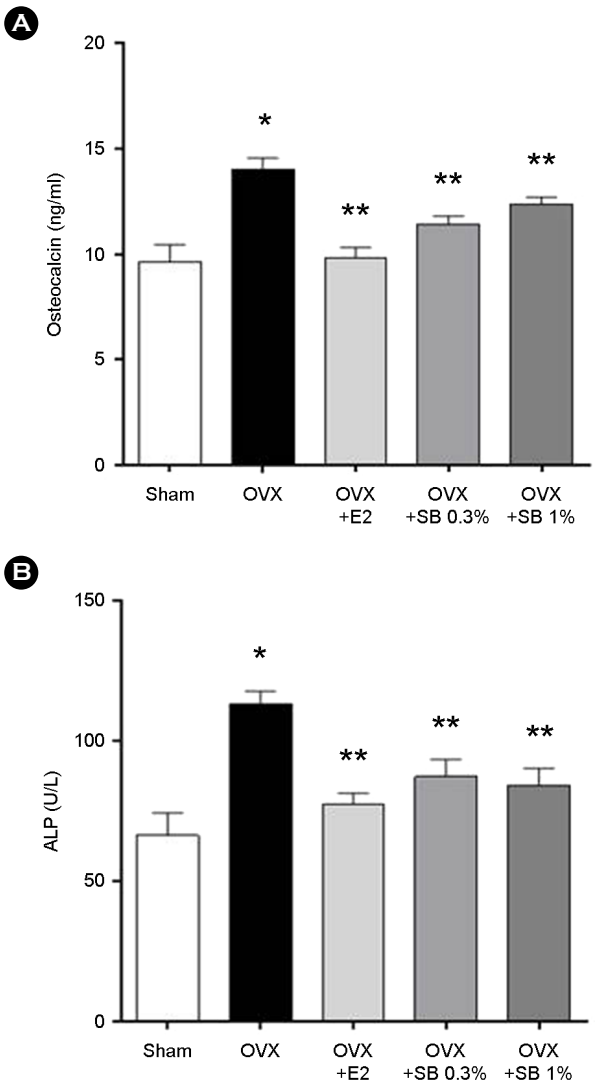

Fig. 4. Effects of safflower buds (SB) on serum osteocalcin concentrations (A) and serum alkaline phosphatase (ALP) concentrations (B) in ovariectomized (OVX) rats. OVX rats were treated with 17β-estradiol (E2, 10 μg/kg, i.p.) or fed with SB diet (0.3% or 1%). Each value is expressed as mean ± S.E., n = 8~10. *P< 0.05 compared with the sham group and **P<0.001 compared with the OVX group.

postmenopausal bone loss (Kalu, 1991). Currently, bone mineral density (BMD) measurements are commonly used to diagnose osteoporosis and to predict the risk of fractures (Cummings, 1995). So, we evaluated the effect of SB diets on BMD levels in OVX rats. According to several reports, the levels of osteoporosis in rats were shown to rise 2~6 weeks after removing the ovaries (O and Lee, 1993). In the present study, feeding of SB diets in OVX rats showed a significant protection effect on BMD analysis. However, dose dependent statistical significance between 0.3% and 1% SB diets feeding groups was not observed (Fig. 3A). Trabecular separation (Tb.Sp) is the average distance between the trabecular and is used as good micro-CT imaging biomarker to evaluate the protection effect in the ovariectomy mediated osteoporotic mouse model (Kang et al., 2014). The levels of Tb.Sp in OVX rats fed with SB diets were also significantly reduced compared with OVX alone group (Fig. 3B). These results suggest that feeding of SB diets improved the connectivity of trabecular bone in the OVX rats though to a lesser degree than treatment with E2.

Effects of SB treatment on serum bone turnover markers in OVX rats

Plasma osteocalcin levels in OVX group were higher than those in sham group. And the ovariectomy-induced osteocalcin levels were prevented by the feeding of SB diets (Fig. 4A). In the present study, we assessed the activity of serum bone-specific alkaline phosphatase (ALP) as biochemical marker of bone formation, which is synthesized in osteoblast and liver (Meller et al., 1984). Gomez et al. (1995) suggested that bone ALP (BAP) activity in serum can provide an index for the rate of bone formation. The changes of serum ALP activity are more dramatic than others in this study. As shown in Fig. 4B, the activities of serum ALP in OVX group significantly increased compared to sham group (i.e., 113 ± 12.3 U/L vs. 66 ± 21.3 U/L, $P<0.001$). And the OVX-induced ALP activity was prevented by the feeding of SB diets (0.3% or 1%). These results suggest that the feeding of SB diets improved bone mass by the decrease of OVX-induced APL activity. The most significant finding of this study is that SB diets can effectively prevent OVX-induced osteoporosis in rats. Feeding of SB diets can not only maintain bone mineral density but also prevent trabecular bone loss after OVX. Taken together, our results suggest that SB is a potential lead compound for the development of novel anti-osteoporosis agents, and may be an important source of new anti-osteoporosis components.

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