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난소절제 흰쥐에서 엉겅퀴 추출물의 골다공증 보호 효과

김영옥*† · 김진성* · 이상원* · 조익현* · 나세원**
*농촌진흥청 국립원예특작과학원 인삼특작부, **전북대학교 수의과대학

Osteoprotective Effect of Extract from *Cirsium japonicum* var. *ussuriense* in Ovariectomized Rats

Young Ock Kim*†, Jin Seong Kim*, Sang Won Lee*, Ick Hyun Jo* and Sae Won Na**
**Department of Herbal Crop Research, NIHHS, RDA, Eumseong 369-873, Korea.*
***College of Veterinary Medicine, Chonbuk National University, Jeonju 561-756, Korea.*

ABSTRACT : This study was carried out to investigate the effects of the *Cirsium japonicum* var. *ussuriense* (*C. japonicum*) extract on serum level of hormones from induced osteoporosis by ovariectomized rats. Two month-old rats were ovariectomized (OVX), remained untreated for 8 weeks, and were subsequently administered *C. japonicum* (200 ㎎/㎏) every day for 8 weeks. We examined the effects of treated *C. japonicum* on ovariectomy-related changes in Insulin-like Growth Factors (IGF), Insulin-like Growth Factor binding protein-3 (IGBF-3), Estrogen, Calcium, and Phosporus. After 8 weeks, the serum levels of IGF-I, -II, and IGFBP-3 were higher presented as compared to the other two groups ($P < 0.05$), in the *C. japonicum* extract treatment on OVX rats. There were differences between OVX and *C. japonicum* extract treated OVX rats in serum levels of Ca^{2+}, but Ca^{2+} levels for the normal group was higher than for the other two groups. The *C. japonicum* extract increased both serum IGFs and IGFBP-3 levels on induced osteoporotic rat by ovariectomized. Thus, these results revealed that the *C. japonicum* extract is a possible role for improvement of osteoporosis induced-ovariectomized rats and has a great potential as an alternative tool for the treatment of osteoporosis.

Key Words **:** *Cirsium japonicum,* Osteoporosis, Ovariectomy, Serum

INTRODUCTION

Osteoporosis is a disease characterized by low bone mass and deterioration of bone tissue. This leads to increased bone fragility and risk of fracture. Osteoporosis is often known as “the silent thief” because bone loss occurs without symptoms (Christiansen, 1993). A decrease in in women at the time of menopause and a decrease in testosterone in men is another major cause of bone loss. After menopause, the normal balance between bone formation and resorption is disrupted : osteoclasts (giant multi-nucleated cells) become more active, decreasing bone mass and increasing the chance of fracture. It is now known that postmenopausal osteoporosis should be regarded as a product of inflammatory disease triggered by estrogen deficiency (Chung *et al*., 2006). Ovariectomy (OVX) in the rat results in bone loss and micro-architecture deterioration (Ammann *et al*., 1996) and is the most commonly used animal model of post-menopausal osteoporosis (Turner *et al*., 2001). Although hormone replacement therapy has been proven to be efficacious in preventing bone loss, yet, it is not desirable to many women due to its side effects (Epstein, 2006). Estrogen deficiency results in a marked increase in proinflammatory cytokines, interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-a (TNF-a), and macrophage colony-

†Corresponding author: (Phone) +82-43-871-5585 (E-mail) kyo9128@korea.kr
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stimulating factor (McLean, 2009). TNF-a, IL-1b, and IL-6 stimulate osteoclast differentiation independently or in synergistic fashion (Jiro *et al*., 2011). Because estrogen deficiency has been recognized as the key factor of osteoporosis, hormone replacement therapy (HRT) was used as an effective strategy for prevention and treatment of osteoporosis in postmenopausal women.

Recently, therapy for osteoporosis is aimed at the prevention of further bone loss, primarily by inhibiting bone resorption. Particularly, insulin-like growth factors (IGF) and their binding proteins (IGFBP), which could modulate IGF's actions, might play essential roles in bone formation (Nilsson *et al*., 1994; Rosen *et al*., 1994). In serum, most IGF-I (70-80%) forms a 150-kD complex by binding with IGFBP-3. The serum IGFBP-III level is considered to positively regulated by GH and/or IGF-I (Rosen and Pollak, 1999; Stewart and Rotwein, 1996). IGFBP-III acts as a regulator of growth-dependent IGF-I signaling through the enhancement of IGF-I stability in serum (Binoux and Hossenlopp, 1988). A major part of bound IGF-I is connected to IGFBP-III (Baxter, 1993). Low estrogen concentrations are known to increase the secretion of subsequent IGF-I synthesis during early puberty. Estrogens initiate the pubertal growth spurt and stimulate skeletal growth. Hence, sex steroid-related changes in GH and IGF-I secretion may impact on bone size and cross-sectional area (Juul, 2001). The popularity of herbal medicines is attributable to their easy take, therapeutic efficacy, relatively low cost, and assumed absence of toxic side effects (Park *et al*., 2013). The use of natural products as an alternative treatment has been on the rise in the last few decades *C. japonicum*, a wild perennial herb which belongs to *Asteraceae* family, has long been used as a natural medicine for treatment of hypertension, traumatic hemorrhage, and inflammation (Kim *et al*., 2010). Recent studies have found that the water extracts of *C. japonicum* induce the activation of estrogen receptors and has estrogenic effects (Park *et al*., 2008) and has been prescribed in the treatment of liver cancer (Lee *et al*., 2003), uterine cancer, and leukemia (Yin *et al*., 2008). But since now no studies have been carried out to evaluate whether *C. japonicum* has an antiosteoporosis activity in rats. The present research was conducted to investigate the effect of *C. japonicum* on postmenopausal osteoporosis in ovariectomized rats.

MATERIALS AND METHODS

1. Experimental animals

The Sprague-Dawley rats were purchased from the Jungang Lab animal co, South Korea. A total of 60 female rats, 2-month-old, weighing 180-200 g were randomly assigned into 6 groups (10 animals/group). (i) sham, (ii) OVX_control, (iii) 17β-estradiol, 17β-estradiol (E2) 30 mcg/㎏/24 hour, (Sigma, St. Lois, MO, USA) *C. japonicum*. Except 17β-estradiol (positive control), which was administered every three days for 8weeks, each treatment sample was administered once daily p.o. at a dose of (iv) 100, (v) 200 and (vi) 400 ㎎/㎏ for 8weeks. The animals were housed in stainless steel cages having wire mesh bottom (3 animals per cage) in a light-controlled (12 : 12 hours light-dark cycle) room with a constant temperature (25℃). All the rats had free access to food and water. The animals in group I were normal group. To induce menopause in rats, ovariectomies were performed in group I and group II. Ovariectomies were done under ketamine HCl (100 ㎎/㎏) and xylazine (3 ㎎/㎏) anesthesia. During 8 weeks, the animals of group I and II received solvent vehicle daily, whereas those of group III were administered *C. japonicum* orally (100, 200 and 400 ㎎/㎏) daily for eight weeks. Body weight was determined weekly.

2. Preparation of *C. japonicum* extraction

The aerial parts of *C. japonicum* were collected on July 20, 2013 from Imsil herbal farming the Act at Imsil in Chonnam, Republic of Korea, and then specimens were taxonomically identified by an oriental doctor, S. W. Lee at National Institute of Horticultural & Herbal Science, RDA. We used water extraction because most traditional oriental herbal materials are decocted with boiling water. The crushed plant materials (100 g for each) were kept in a breaker at room temperature (25 ± 1). And it extracted under reflux with distilled water three times. After the extraction, the sample residues were dissolved in a known quantity of water and were repeatedly extracted until the extracts became clear. The obtained extract was then filtered through Whatman No. 1 filter paper and the filtrate was concentrated in a rotary evaporator (Buchi Rotavapor R-215, Flawil, Switzerland) under controlled vacuum. The concentrated extract was then dried in a

freeze dryer (Labconco, Free Zone 6 Liter, Kansas City, MO, USA), and yields were 16% (wt/wt) for *C. japonicum* in the dried state. They kept at 4℃ in air tight container until further analysis.

3. Measurement of Femur/body weight

Body and femur weight was measured after *C. japonicum* seed treatment in osteoporosis induced-ovariectomized rats using chemical balance (Ohaus, Parsippany, NJ, USA).

4. Collection of blood and tissue samples

Serum was isolated from the blood samples by centrifugation at 3000 × g and 4℃ for 5 min and stored at −70℃ for biochemical measurements. Body and isolated femur weight was measured in osteoporosis induced-ovariectomized rats using chemical balance (Ohaus, Parsippany, NJ, USA)

5. Measurement of serum IGF-I, IGF-II and IGFBP-III levels

For the quantitative analysis of the serum IGF-I, IGF-II and IGFBP-III levels, free IGF-I and IGFBP-III enzyme-linked immunosorbent assay (ELISA) kits were used according to the manufacturer's protocols (R&D Systems, Minneapolis, MN, USA).

6. Estrogen, Ca^{2+} and P assay

IGFBP-III and estrogen were assayed using an immunoradiometric assay kit (Diagnostic Systems Laboratories, Webster, TX, USA). The Ca^{2+} and P assays used kits (Embiel Co., Gunpo, Korea).

7. Statistical analysis

All the data were presented as mean ± S.E.M. The effects of the different treatments were compared by Student's t test and were considered significant for values of $p < 0.05$ and $p < 0.01$. Post hoc analysis was carried out using Duncan's Multiple Range Test (DMRT).

RESULTS

1. Animal model

Body weight is shown in Fig.1. Ovariectomy increased the body weight (Normal: 266.60 ± 8.6; ovariectomized: 328.8 ± 7.4; *C. japonicum* (200 ㎎/㎏) treated: 267.5 ± 11.4 ($p < 0.001$): *C. japonicum* (400 ㎎/㎏) treated: 284.8 ± 16.5). There were no differences in body weight between normal, OVX and *C. japonicum*-treated until the end of the study, OVX group higher weights than the other groups. Body weight of OVX rats increased about 23.3%, after 8 week surgery. A relatively low dose of *C. japonicum*, 200 ㎎/㎏, more inhibited the increase in body weight than did a high dose, 400 ㎎/㎏.

2. Effect of ovariectomy and *C. japonicum* on serum IGF-I levels

Changes in serum level of IGF-I *C. japonicum* treatment in osteoporosis induced-OVX rat groups over time are shown in Fig. 2. On 8 weeks after ovariectomy, serum level of IGF-I was significantly higher in the 17-β estradiol and *C. japonicum* treated OVX group than in the OVX and sham groups ($p < 0.01$) whereas slight changes were between the normal and OVX groups.

3. Effect of ovariectomy and *C. japonicum* on serum IGF-II levels

Serum level of IGF-II after *C. japonicum* treatment in osteoporosis induced-OVX rat groups was shown in Fig. 3. On 8 weeks after ovariectomy, serum level of IGF-II on the 17β-estradiol treated group were significantly higher than that of the OVX group (174.4 ng/mL). Serum level of IGF-II after *C. japonicum* (400 ㎎/㎏) treatment was significantly higher than in the OVX groups ($p < 0.05$) whereas slight changes were between the 100 ㎎/㎏ and 400 ㎎/㎏ of *C. japonicum* treated groups.

4. Effect of ovariectomy and *C. japonicum* on serum IGFBP-III and Estrogen levels

IGFBP-III levels after *C. japonicum* treatment in osteoporosis induced OVX rat groups are shown in Fig 4. On 8 weeks, IGFBP-III levels in the normal group decreased and *C. japonicum* treatment group significant differences compared with the OVX group ($p < 0.05$). The serum levels of IGFBP-III in 17β-estradiol (600.1 ± 12.8 ng/mL) and the 200 ㎎/㎏ *C. japonicum* (579.7 ± 8.1 ng/mL) were higher than in OVX group (502.1 ± 30.1 ng/mL), but in *C. japonicum* (400 ㎎/㎏) the serum IGFBP-III decreased to 563.4 ± 9.7 ng/mL. The difference was significant ($p < 0.05$). Changes in estrogen levels of each group are shown in Fig. 5. On 8 weeks after ovariectomy, serum

estrogen levels of each group was difference as a consequence of OVX or the *C. japonicum* treated group, but there were no differences between *C. japonicum* treated group and OVX group.

5. Effect of ovariectomy and *C. japonicum* on serum Ca^{2+} and P levels

Changes in serum level of Ca^{++} and P after *C. japonicum* treated group are shown in Fig 6. 7. The serum levels of Ca^{++} and P in all groups were differences compared with the OVX group ($p < 0.05$). The serum levels of Ca^{++} in OVX + *C. japonicum* (100 ㎎/㎏) group (8.35 ± 0.2 ng/mL) was significantly lower than in OVX group (10.04 ± 0.39 ng/mL Fig. 6). On 8 weeks after ovariectomy, serum level of phosphorus on the 17β-estradiol and the 200 ㎎/㎏ *C. japonicum* treated group were significantly higher than that of the OVX group (6.96 ± 0.96 ng/mL), but in *C. japonicum* (400 ㎎/㎏) the serum phosphorus decreased to 8.29 ± 0.80 ng/mL.

6. Femur / body weight

In Fig. 8, the ratio of femur/body weight on the 17β-estradiol and the *C. japonicum* (200, 400 ㎎/㎏) treated group were significantly higher than that of the OVX group (0.38 ± 0.06 ng/mL).

DISCUSSION

This study showed that the *C. japonicum* enlargements IGF/IGFBP on osteoporosis induced-ovariectomize rats. OVX dramatically increases body weights, while *C. japonicum* treatment presents almost normal levels (Fig. 1). Circulating levels of IGF-I, -II is low in men and women with osteoporosis (Frystyk *et al*., 1994). The growth hormone/IGF-I system stimulates both the bone resorbing and bone-forming cells, but the superior effect is on bone formation, thus resulting in an increase in bone mass. Circulating level of IGF-I and IGFBP-III were also low in males and females with osteoporosis due to decreasing of bone formation or increasing of bone resorption (Nasu *et al*., 1997). and increasing serum IGF-I levels of ovariectomized rats have been decreased (Rosen *et al*., 1994). In serum IGF-II levels in *C. japonicum* treated OVX rats increased in 8 weeks just as osteoblast differentiation and activity aid. IGF-II appears to be due to stimulatory effect of *C. japonicum* on osteoblast and osteoclast bone formation in this study (Fig. 2, 3).

In this study we investigated IGFBP-III levels to determine the role of IGFBP-III in bone growth. The IGFBP-III levels were high in the *C. japonicum* group but not significantly different from those of the OVX group. The serum IGFBP-III levels did not exactly reflect the IGF-I level. However, the results indicate that the IGF-1 and IGFBP-III levels had a directly proportional relationship. Most rats with high IGF-1 levels had high IGFBP-III levels as well (Fig. 4). These findings suggest

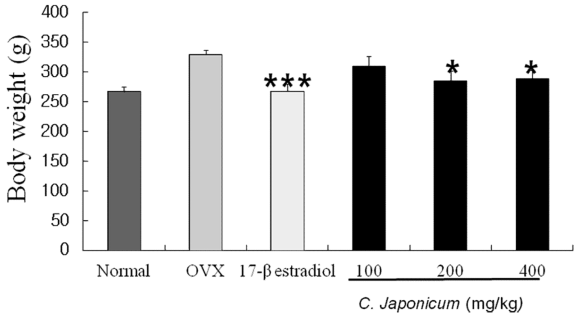

Fig. 1. Changes of body weight on OVX and *C. japonicum* treatment. Values are mean ± S.E.M. The number of animals is 10/group. *P*-values denote significant difference from the OVX group and 17-β estradiol treated group. ***$p < 0.001$ vs. the OVX group, *$p < 0.05$ vs. the OVX group. Normal; Normal and saline treated group, OVX; ovariectomized and saline treated group, *C. japonicum*; ovariectomized and *C. japonicum* treated group. 17-β estradiol; ovariectomized and 17-β estradiol (30 ㎍/㎏) treated group.

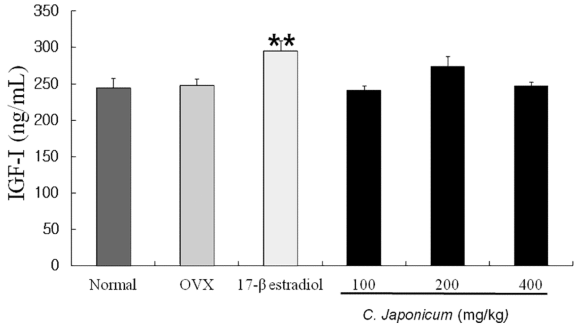

Fig. 2. Effects of OVX and *C. japonicum* on serum IGF-I levels determined. Values are mean ± S.E.M. The number of animals is 10/group. *P*-values denote significant difference from the OVX group and *C. japonicum* treated group. Normal; Normal and saline treated group, OVX; ovariectomized and saline treated group, *C. japonicum*; ovariectomized and *C. japonicum* treated group, 17-β estradiol; ovariectomized and 17-β estradiol (30 ㎍/㎏) treated group. **$p < 0.01$ vs. the OVX group.

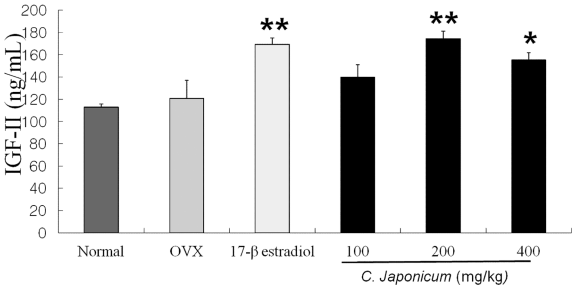

Fig. 3. Effects of OVX and *C. japonicum* on serum IGF-II levels determined. Values are mean ± S.E.M. The number of animals is 10/group. *P*-values denote significant difference from the OVX group and *C. japonicum* treated group. $^{**}p < 0.01$ vs. the OVX group, $^{*}p < 0.05$ vs. the OVX group. Normal; Normal and saline treated group, OVX; ovariectomized and saline treated group, *C. japonicum*; ovariectomized and *C. japonicum* treated group. 17-β estradiol; ovariectomized and 17-β estradiol (30 ㎍/㎏) treated group. $^{**}p < 0.01$ vs. the OVX group.

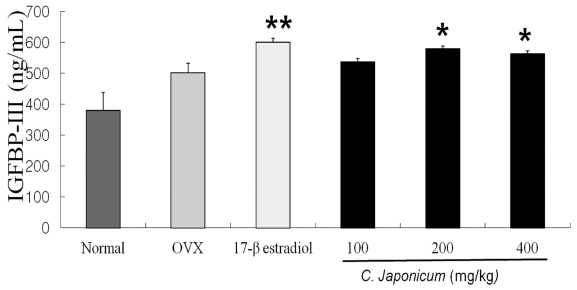

Fig. 4. Effects of OVX and *C. japonicum* on serum IGBPF-III levels determined. Values are mean ± S.E.M. The number of animals is 10/group. *P*-values denote significant difference from the OVX group and *C. japonicum* treated group ($p < 0.05$). Normal; Normal and saline treated group, OVX; ovariectomized and saline treated group, *C. japonicum*; ovariectomized and *C. japonicum* treated group. $^{*}p < 0.05$ vs. the OVX group. 17-β estradiol; ovariectomized and 17-β estradiol (30 ㎍/㎏) treated group. $^{**}p < 0.01$ vs. the OVX group.

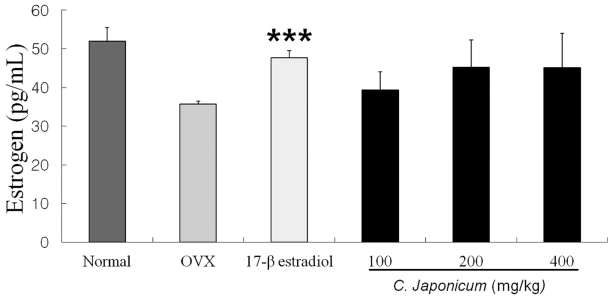

Fig. 5. Effects of OVX and *C. japonicum* on serum estrogen levels determined. Values are mean ± S.E.M. The number of animals is 10/group. Normal; Normal and saline treated group, OVX; ovariectomized and saline treated group OVX+, *C. japonicum;* ovariectomized and *A. japonica* treated group. 17-β estradiol; ovariectomized and 17-β estradiol treated group. $^{***}p < 0.001$ vs. the OVX group.

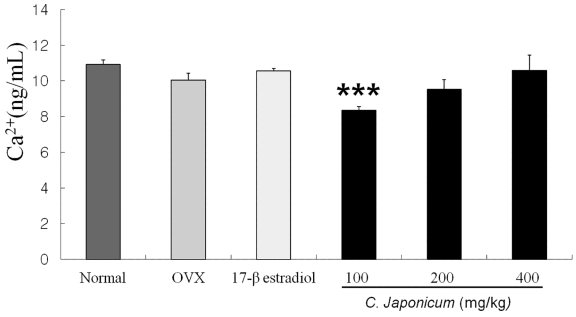

Fig. 6. Effects of OVX and *C. japonicum* on serum Ca^{2+} levels determined. Values are mean ± S.E.M. The number of animals is 10/group. Normal; Normal and saline treated group, OVX; ovariectomized and saline treated group OVX+, *C. japonicum*; ovariectomized and *C. japonicum* treated group. $^{***}p < 0.001$ vs. the OVX group.17-β estradiol; ovariectomized and 17-β estradiol (30 ㎍/㎏) treated group.

that IGFBP-III also could be a biomarker of bone growth. We studied the increase of IGF-I during *C. japonicum* treated osteoporosis compared with the OVX group after ovariectomy. IGF-I induces early osteoblast gene expression in human mesenchymal stem cells (Koch *et al*., 2005) and increased bone remodeling in transgenic mice with osteoblast targeted insulin-like growth factor-I (Jiang *et al*., 2006).

Estrogen play a major role acts in mineral homeostasis, 'the deficiency of estrogen is recognized as a major factor in loss of bone minerals in postmenopausal osteoporosis (Danielsen and Flyvbjerg, 1996). IGF-I induces cell proliferation via the action in the granulose cells of the ovary and the Leydig cells of the testis and increases the synthesis of the female hormones (estradiol, progesterone) and the male hormone (testosterone) under the control of gonadotropic hormone (Kim *et al*., 2003b) (Fig. 5). IGFBP-3, the quantitatively predominant IGFBP in circulation, is positively regulated by growth hormone and potentiates IGF action under most conditions. IGFBP-III has the unique property of being able to associate with an acid-labile subunit (ALS) after IGF binding, thus forming a 150 kd complex (Fig. 6, 7).

Longitudinal bone growth is concern with the expression of various growth hormons (GH). GH induces the IGF-I expression in the liver, and circulating IGF-I in serum

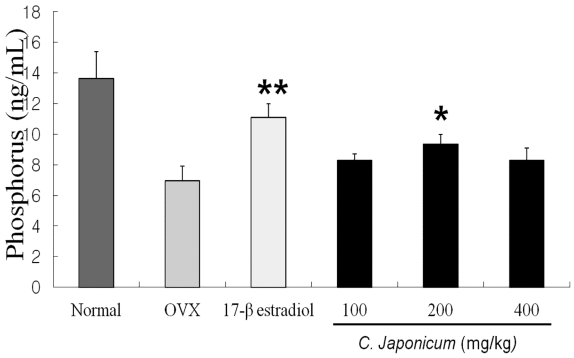

Fig. 7. Effects of OVX and *C. japonicum* on serum phosporus levels determined. Values are mean ± S.E.M. The number of animals is 10/group. Nornal; Normal and saline treated group, OVX; ovariectomized and saline treated group, *C. japonicum;* ovariectomized and *C. japonicum* treated group. *$p < 0.05$ vs. the OVX group.17-β estradiol; ovariectomized and 17-β estradiol treated group. **$p < 0.01$ vs. the OVX group.

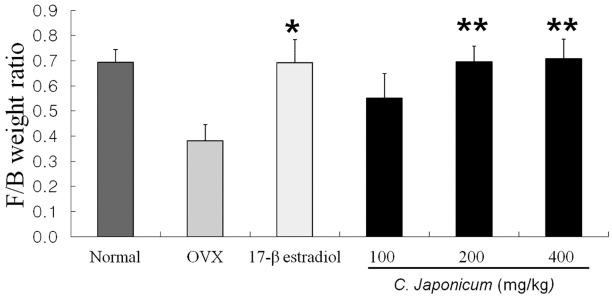

Fig. 8. Effects of OVX and *C. japonicum* on F/B weight ratio levels determined. Values are mean ± S.E.M. The number of animals is 10/group. Normal; Normal and saline treated group, OVX; ovariectomized and saline treated group, *C. japonicum*; ovariectomized and *C. japonicum* treated group. **$p < 0.01$ vs. the OVX group. 17-β estradiol; ovariectomized and 17-β estradiol (30 ㎍/㎏) treated group. *$p < 0.05$ vs. the OVX group.

stimulates skeletal growth (Yakar *et al*., 2002) and affects the expression of IGF-I in the bone (Roith *et al*., 2001). In this study, OVX dramatically increases body weights, while *C. japonicum* treatment presents almost normal levels. Although the mechanisms by which OVX induces an increase in body weight are not clear, estrogen deficiency induced body fat accumulation and subsequently caused an increase in body weight (Dang *et al*., 2002) (Fig. 8). Therefore, this study indicates that effects of serum IGFs expression by treated *C. japonicum* may be related to growth rate in OVX rats. In conclusion, *C. japonicum* is able to prevent OVX-induced in bone loss, suggesting that *C. japonicum* may be a reasonable natural alternative for the prevention of postmenopausal osteoporosis.

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REFERENCES

Ammann P, Rizzoli R, Meyer JM and Bonjour JP. (1996). Bone density and shape as determinants of bone strength in IGF-I and/or pamidronate-treated ovariecto-mized rats. Osteoporos International. 6:219-227.

Baxter RC. (1993). Circulating binding proteins for the insulin like growth factors. Trends in Endocrinology and Metabolism. 4:91-96.

Binoux M and Hossenlopp P. (1988). Insulin-like growth factor(IGF) and IGF-binding proteins: Comparison of human serum and lymph. Journal of Clinical Endocrinolgy Metabolism. 67:509-14.

Christiansen C. (1993). Consensus development conference: Diagnosis, prophylaxis, and treatment of osteoporosis. The American Journal of Medicine. 94:646-650.

Chung HY, Sung B, Jung KJ, Zou Y and Yu BP. (2006). The molecular inflammatory process in aging. Antioxidant & Redox Signaling. 18:572-581.

Dang ZC, Van Bezooijen RL, Karperien M, Papapoulos SE and Lowik CW. (2002). Exposure of KS483 cells to estrogen enhances osteogenesis and inhibits adipogenesis. Journal of Bone and Mineral Research. 17:394-405.

Dinielsen CC and Flyvbjerg A. (1996). Insulin-like growth Factor I as a predictor of cortical bone mass a long-term study of ovariectomized an estrogen-treated rats. Bone. 19:493-498.

Epstein S. (2006). Epstein update of current therapeutic options for the treatment of postmenopausal osteoporosis. Clinical Therapeutics. 28:151-173.

Jiang J, Lichtler AC and Gronowicz GA. (2006). Transgenic mice with osteoblast targeted insulin-like growth factor-I show increased bone remodeling. Bone. 39:494-504.

Jiro F, Shumpei N, Yuji Y, Akira Y and Mitsuo O. (2011). Wear debris stimulates bone-resorbing factor expression in the fibroblasts and osteoblasts. Hip International. 21:231-237.

Juul A. (2001). The effects of oestrogens on linear bone growth. Human Reproduction Update. 7:303-313.

Kim DY, Kang SH and Ghil SH. (2010). *Cirsium japonicum* extract induces apoptosis and anti-proliferation in the human breast cancer cell line MCF-7. Molecular Medicine Reports. 3:427-432.

Kim SH, Ku SY and Jee BC. (2003b). Clinical efficacy of serum insulin-like growth factor-I(IGF-I), IGF-II, and IGF binding protein-3(IGFBP-3) in predicting the prognosis of in vitro fertilization and embryo transfer. Korean Journal of Obstetrics

& Gynecology. 46:802-809.

Koch H, Jadlowiec JA and Campbell PG. (2005). Insulin-like growth factor-I induces early osteoblast gene expression in human mesenchymal stem cells. Stem Cells and Development. 14:621-631.

Lee HK, Kim JS, Kim NY, Kim MJ, Park SU and Yu CY. (2003). Antioxidant, antimutagenicity and anticancer activities of extracts from *Cirsium japonicum* var. *ussuriense* KITAMURA. Korean Journal of Medicinal Crop Science. 11:53-61.

McLean RR. (2009). Proinflammatory cytokines and osteoporosis. Current Osteoporosis Reports. 7:134-139.

Nasu M, Sugimoto T, Chihara M, Hiraumi M, Kurimoto F and Chihara K. (1997). Effect of natural menopause on serum levels of IGF-I and IGF-binding protein : Relationship with bone mineral density and lipid metabolism in perimenopausal woman. European Journal of Endocrinology. 136:608-616.

Nilsson A, Ohlsson C, Isaksson OG, Lindahl A and Isgaard J. (1994). Hormonal regulation of longitudinal bone growth. European Journal of Clinical Nutrition. 48:150-158.

Park MK, Rhyu MR, Yoon BK, Kwon H, Jang S and Lee YJ. (2008). Modulation of the genomic estrogen receptor pathway by water extracts of *Cirsium japonicum*. Archives of Pharmacal Research. 31:225-230.

Park YC, Lee JS, Kim DY, Son HY, Lee JW, Cheoi YS, Kim KK, Yu CY, Chung IM, Im MH, Lee KJ, Choi RN, Shim HS and Lim JD. (2013). A 90 day repeated dose-oral toxicity study of extracts from *Astragalus membranaceus*-aboveground parts in rats. Korean Journal of Medicinal Crop Science. 21:474 -485.

Roith DL, Bondy C, Yakar S, Liu JL and Butler A. (2001). The somatomedin hypothesis. Endocrine Reviews. 22:53-74.

Rosen CJ, Donahue LR and Hunter SJ. (1994). Insulin-like growth factors and bone: the osteoporosis connection. Proceedings of the Society for Experimental Biology and Medicine. 206:83-102.

Rosen CJ and Pollak M. (1999). Circulating IGF-I: New perspectives for a new century. Trends in Endocrinology and Metabolism. 10:136-141.

Stewart CE and Rotwein P. (1996). Growth, differentiation and survival: Multiple physiological functions for insulin-like growth factors. Physiological Reviews. 76:1005-1026.

Turner RT, Maran A, Lotinun S, Hefferan T, Evans GL, Zhang M and Sibonga JD. (2001). Animal models for osteoporosis. Review of Endocrinology and Metabolism. 2:117-127.

Yakar S, Rosen CJ and Beamer WG. (2002). Circulating levels of IGF-I directly regulate bone growth and density. Journal of Clinical Investigation. 110:771-781.

Yin J, Heo SI and Wang MH. (2008). Antioxidant and antidiabetic activities of extracts from *Cirsium japonicum* roots. Nutrition Research Practice. 2:247-251.