J Vet Clin 20(3) : 263-273(2003)

Effects of Safflower Seeds on the Serum Levels of Insulin-like Growth Factors, Insulin-like Growth Factor Binding Protein-3 and BALP in Osteoporosis Induced-ovariectomized Rats

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Abstract : This study was carried out to investigate the effects of the Korean Safflower (*Carthamus inctorius L*) seed powder on serum level of hormones and trabecula area during the recovery from osteoporosis induced ovariectomized rats. Four month-old rats were ovariectomized (OVX), remained untreated for 8 weeks, and were subsequently administered safflower seed (0.03 g/kg) every other day 30 for days. We examined the effects of treated safflower seed every 10 days on ovariectomy-related changes in Insulin-like Growth Factors, Insulin-like Growth Factor binding protein-3 (IGFBP-3), Estrogen, Bone-specific alkaline phosphotase, Calcium, and Phospotase in the serum, and also histomorphology of the proximal fibula metaphysis and femur/body weight rate. Ten and 20 days after safflower seed treatment in OVX rats, serum levels of IGF-I, -II and IGFBP-3 were not different from the Sham and OVX groups. In 30 days, serum levels of IGF-I,-II and IGFBP-3 were higher after safflower seed treatment in OVX rats as compared to the other two groups ($p<0.05$). Bone alkaline phosphatase levels were increased through safflower seed treatment in OVX rats compared to the other two groups in 30 days. There were no differences between OVX and safflower seed treated OVX rats in serum levels of estrogen and femur/body weight rate, but estrogen levels for the sham group were higher than for the other two groups. The safflower seed is increased to serum levels of IGFs, IGFBP-3 and BALP of osteoporosis induced by ovariectomized rats. Thus, we conclude that the safflower seed is a possible role for improvement of osteoporosis induced-ovariectomized rats.

Key words **:** Safflower seed, Ovariectomy, IGFs-IGFBP-3, Bone Alkaline phosphatase

Introduction

Osteoporosis is a metabolic bone disease associated with unequilibrated bone remodeling due to decreased bone formation or accelerated bone resorption. Its regulators are changed by ages, ions, ca^{2+}, hormones and growth factors *et al*[15,20,23,34,62]. The most abundant growth factors, produced by bone cells, are essential for promoting bone cell differentiation and proliferation[32]. One of these growth factors, insulin-like growth factors, play an important role in maintaining bone mass[5,13,,2]. IGFs are potent stimulators of cell proliferation and differentiation, and fetal growth and development[5,9,30]. Circulating IGFs are believed to act as a systemic agent produced in the liver[6,7,16]. Also, IGFs are recognized as being synthesized in other tissues, where they are believed to function as an autocrine or paracrine and endocrine regulator of cell growth and assumed to be necessary for normal bone remodeling[8-10]. The physiological effects of insulin-like growth factors (IGFs) are modulated by several insulin-like growth factor binding proteins (IGFBPs)[9]. IGFs in serum are bound to the various kinds of IGF-binding proteins (IGFBPs), mainly to IGFBP-3[3,5,52]. Serum levels of IGFBP-3 are modulated by hormones, nutrition, age, stress and diseases *et al*[7,27]. Its functions are to transport IGFs from the circulation to peripheral tissues and to maintain a reservoir of IGFs in the circulation as well as promote or inhibit the growth of bone cells. Also, they are postulated to play a key role in bone metabolism[18], which is tightly regulated at the local level by networks of estrogen, BALP, $Ca^{2+}$and P[28,33,34,49,64,65].

Carthamus tincturius L, which is called safflower, is a perennial plant and belongs to the *Chrysanthemum*[63] family. Safflower was well known as an accelerator for cleaning the blood, ataralgesia, sanguimotor, and safflower seed have had effect on bone diseases such as fracture, osteoporosis, osteogenesis impergecta and bone metabolism in Chinese medicine[55,60,67,68]. The supplementation of Korean safflower seed powder influences recovery from bone fractures by accelerating the process of bone repair. Thus, safflower seed has been related to bone cell proliferation, bone matrix synthesis, and longitudinal bone growth.

Currently, therapy for osteoporosis is aimed at preventing further bone loss, primarily by inhibiting bone resorption. This in turn, influenced factors which have been involved in IGFs axis, but there is no study of safflower seed and osteoporosis induced-ovariectomy rats which involved IGFs/IGFBPs.

Therefore, we determined the serum levels of IGFs, IGFBP-3, TALP, BALP, estrogen, Ca^{2+}, P, histomorphology and f/b weight ratio in osteoporosis induced-ovariectomized

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rats. Then, this study demonstrates the effects of safflower seed on the IGFs/IGFBP axis in osteoporosis induced-ovariectomized rats.

Materials and Methods

Experimental Animals

Female Sprague-Dawley rats, 4 months aged, were purchased from the bio-safety research institute of Chon-buk National University Korea. Rats were housed 4 animals per cage in a light-controlled (12 : 12 hr light-dark cycle), constant temperature at 25°C, and had free access to food and water. Ovariectomies of the rats were performed through bilateral flank incision under thiopental sodium (25 mg/kg). Ovarian blood supply was ligated, and ovaries were sectioned from the uterine horns and removed. For the sham operation, ovaries were accessed through flank incision, lifted out of the animal, and replaced. Muscle and skin incisions were sewn separately with 4.0 silk. Sham and OVX Groups received solvent vehicle daily. The Safflower seed treated group received 0.03 g/kg (safflower seed ingredients; Table 1) safflower seed body weight every other day for 30 days. All animals were sacrificed after each 10 day period for a total of 30 days, and fibula were dissected for our histomorphology analysis. Blood was collected and serum separated, and stored at -80°C until required for analysis.

IGFs Radioimmuno assay (RIA)

(1) Pre-treatment sample. Serum was treated using the acid-ethanol method as described by Daughaday *et al.* Briefly, 100 ml serum was mixed with 400 ml acid-ethanol (12.5% 2 mol HCl/1-87.5%, Ethanol; V/V) and the mixture kept at room temperature for 30 min. The acid-ethanol extract was centrifuged for 30 min at 1850× g at 4°C and 250 ml supernatant was removed and neutralized with 100 ml 0.855 mol Tris/L and then diluted with RIA buffer (0.03 mol phosphate/L, PH 7.5; 0.02% (w/v) protamine sulphate; 0.05% (v/v) Tween-20; 0.01 mol EDTA/L and 0.02% (w/v) NaN_3) so that 100 ml aliquots for IGF-I RIA would contain 0.5 ml native serum equivalent. All drugs were purchased from the Sigma company.

(2) Iodination. Recombinant human IGFs (Calbiochem-novabiochem Co, USA) was iodinated by the modification of the chloramine-T method. Briefly, insulin-like growth factors (1 μg) were iodinated with 1 mCi ^{125}I(Amersham, England) and 2.5 μg chloramine-T (Kodak, USA) for 40s by shaking in an ependorf tube, after which the reaction was quenched with 0.5% BSA. Specific activity of the iodinated IGFs was approximately 150-300 Ci/g protein. The iodination mixture was purified on a Sephadex G-50 column (1× 50 cm) pre-equilibrated with phosphate-buffered saline (0.1 mol/L; pH 7.5) containing 0.2% (w/v) saline azide. These drugs were purchased from the Sigma company. Purified hormone was kept at 4°C and was usable for up to 2 months.

(3) IGFs radioimmuno assay (RIA). Serum was separated using the acid-ethanol method, as described by Daughaday *et al.*. Immunoreactive IGFs assays were performed through the method developed by Kang *et al.* Briefly, 50 μg of polyclonal IGFs antibody (Santa Cruz Biotechnology Inc, USA) diluted to the 1: 1000 were added to 100 μl of sample/standard and then incubated for 1 hr at room temperature. ^{125}I-IGFs 20,000 cpm was added to the samples/standard, and they were then incubated for 18 hrs at 4°C. All samples were centrifuged at 3,000× g for 30 minutes. The supernatant was discarded, and radioactivity of the precipitate containing bounding [^{125}I]-IGFs was counted in a gamma scintillation counter (Wallac, Finland). All assays were performed in duplicate. Inter and intra assay coefficients of variation for IGF-1 were 8% and 10%, respectively.

IGFBP-3, Estrogen, Ca^{2+} and P Assay

IGFBP-3 and estrogen were assayed using an immunoradiometric assay kit (Diagnostic Systems Laboratories, Inc, USA) and radioimmunoassay kit (Diagnostic Products Co, USA). The Ca^{2+} and P assays used kits (Embiel Co, Korea).

TALP and BALP Assay

This was measured spectrophotometrically on a Tecnicon RA 1000 analyzer using p-nitrophenyl phosphate as substrate according to the method recommended by the Scandinavian Committee on Enxummes. The intraassay CV was 1.8% and the interassay CV 3.0% at a mean value of 489 U/L[24].

Table 1. Diet compositions of safflower seed fed to osteoporosis induced-ovariectomized rats.

Ingredients	Composition	Ingredients	Composition
Br (ppm)	5.15 ± 0.05	K (ppm)	15500 ± 600
Ca (ppm)	3260 ± 168	Mg (ppm)	2980 ± 170
Cl (ppm)	2096 ± 17	Mn (ppm)	27.6 ± 0.2
Co (ppm)	45.7 ± 2.0	Na (ppm)	126 ± 2
Cr (ppm)	0.17 ± 0.04	Rb (ppm)	7.1 ± 0.3
Cu (ppm)	51.4 ± 14.2	Ru (ppm)	28.1 ± 10.1
Fe (ppm)	350 ± 6	Sb (ppm)	22.4 ± 4.6
Sc (ppm)	1.5 ± 0.3	Zn (ppm)	61.7 ± 0.3

The samples were pretreated by incubating 300 μl of serum with 30 μl Triton X(20 g/L) for 30 minutes at 37°C. An aqueous solution of wheat germ lectin (300 μl) (Sigma -9640, 5 g/L in distilled water) was then added, and the samples were mixed and incubated for 30 minutes at 37°C. After centrifugation at 2000 g for 10 minutes, the AP-activity in the supernatant was determined as above, and S-L-AP was calculated as the difference between total and supernatant activity after correction for sample dilution. The intraassay CV was 5.1% and the interassay CV 6.8% at a mean value of 244 U/L.

This was measured spectrophotometrically in the supernatant after precipitation with wheat germ lectin.

Measurement of Femur/body Weight

Body and femur weight was measured every 10 days after safflower seed treatment in osteoporosis induced-ovariectomized rats using chemical balance (Saturous, U.S.A).

Histological Analysis

Specimens of harvested fibula were decalcified with 10% EDTA, embedded in paraffin, cut into 6-μm thick sections through the long axis, and stained with Hematoxilin and Eosin staining.

Statistical Anlysis

Data are shown as Means ± SE. Two factor analysis of variance (ANOVA) was used to examine the individual effects and interactions among safflower seed treatments. The differences among the Sham, OVX rats, and safflower seed treated OVX rats were assessed by Students't-test. $p < 0.05$ was considered statistically significant.

Results

Effect of Ovariectomy and Safflower Seed on Serum IGF-I Levels

Changes in serum level of IGF-I after safflower seed treatment in osteoporosis induced-OVX rat groups over time are shown in Fig 1. After 10 and 20 days, serum level of IGF-I was significantly different between the sham and other groups ($p < 0.05$), but there was no difference between the OVX and safflower seed treated OVX groups. After 30 days, serum level of IGF-I was significantly higher in the safflower seed treated OVX group than in the OVX and sham groups ($p < 0.05$) whereas no changes were observed between the sham and OVX groups.

Effect of Ovariectomy and Safflower Seed on Serum IGF-II Levels

Serum level of IGF-II after safflower seed treatment in osteoporosis induced-OVX rat groups over time are shown in Fig 2.

After 10 days, serum level of IGF-II in sham group tended to decrease compared to other groups, but this finding was not statistically significant. After 20 days, serum level of IGF-II on the safflower seed treated OVX group significantly increased compared with the sham group ($p < 0.05$). But there was no statistically significant difference between the sham and OVX groups. After 30 days, serum level of IGF-II on the safflower seed treated OVX group significantly increased compared with the sham group($p < 0.05$), but there was no difference between the sham and OVX groups.

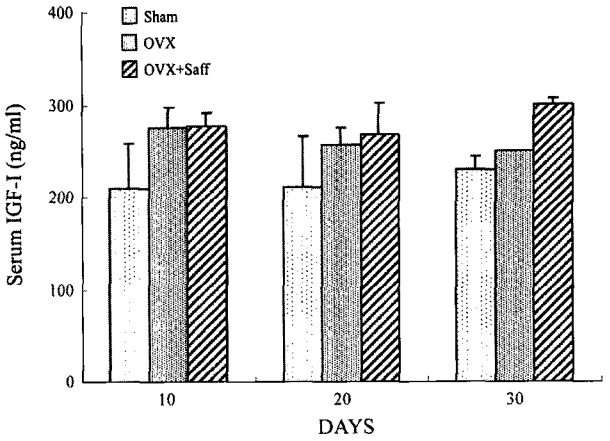

Fig 1. Effects of ovariectomy(OVX) and Safflower seed on serum IGF-I levels determined. (0.03 g/kg body wt./every other day thereafter until day 30) Data are mean± S.E. The nember of animals is 6/group. P-values denote significant difference from the sham-operated group and ovariectomized group (*$p < 0.05$)
Sham: normal and saline treated group.
OVX: ovariectomized and saline treated group.
OVX+Safflower seed treatment: ovariectomized and safflower seed treatment.

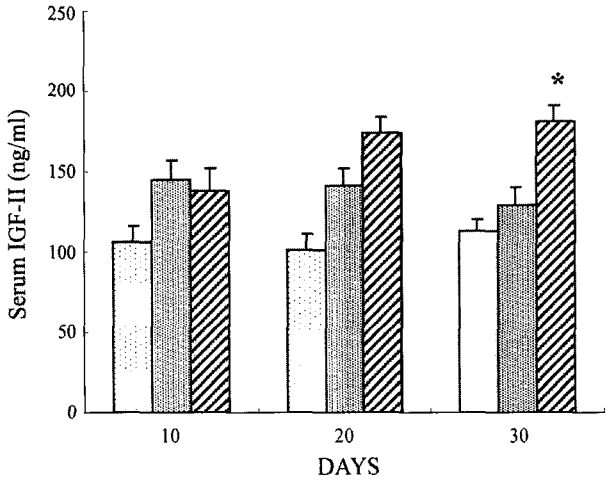

Fig 2. Time course of effects of sham, ovariectomy(OVX) and safflower seed on serum IGF-II levels. Data are mean± S.E. The number of animals is 6/group. P-values denote significant difference with the corresponding sham-operated and OVX groups.

Effect of Ovariectomy and Safflower Seed on Serum IGFBP-3 and Estrogen Levels

Serum levels of IGFBP-3 after safflower seed treatment in osteoporosis induced-OVX rat groups over time are shown in Fig 3.

After 10 days, serum levels of IGFBP-3 in the sham group significantly increased compared with the other groups, but OVX and safflower seed treated OVX groups showed no difference. After 20 days, serum levels of IGFBP-3 on the safflower seed treated OVX group significantly increased compared with the other groups ($p < 0.05$), but there was no difference between the sham and OVX groups.

After 30 days, serum levels of IGFBP-3 on the sham group significantly decreased compared with the other group ($p < 0.05$), but there were no statistically significant differences between the safflower seed treatment OVX and OVX groups.

Changes in serum level of estrogen after safflower seed treatment in osteoporosis induced-OVX groups over time are shown in Fig 4.

After 10, 20 and 30 days, serum estrogen levels differentiation was not observed as a consequence of OVX or the safflower seed treatment group, but there were statistically significant differences between sham and other groups ($p < 0.05$).

Effect of Ovariectomy and Safflower seed on Serum BALP, Ca^{2+} and P Levels

Serum levels of BALP after safflower seed treatment in osteoporosis induced-OVX rat groups over time are shown in Fig 5.

After 10 and 20 days, serum level of BALP was not different among the groups, but the sham group showed significant decrease compared to the safflower seed treated OVX group ($p < 0.05$).

Changes in serum level of Ca^{2+} and P after safflower seed treatment in osteoporosis induced-OVX groups over time are shown in Fig 6 and 7. The serum levels of Ca^{2+} and P in all

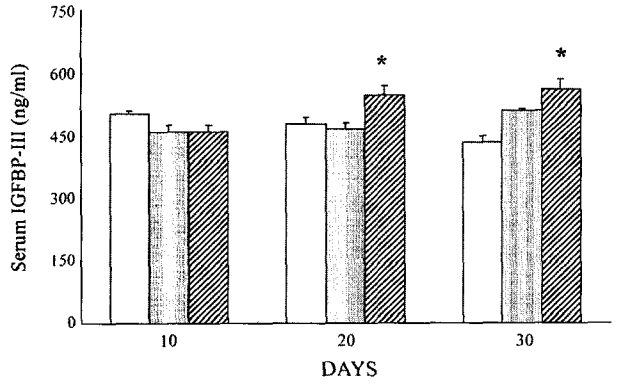

Fig 3. Time course of effects of sham, ovariectomy(OVX) and safflower seed on serum IGFBP-3 levels. Data are mean±S.E. The number of animals is 6/group. P-values denote significant difference with the corresponding sham-operated and OVX groups.

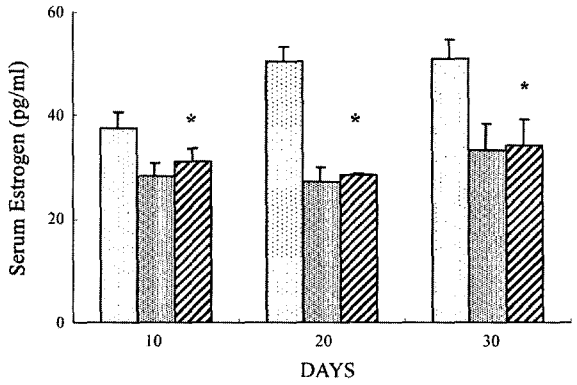

Fig 4. Time course of effects of sham, ovariectomy(OVX) and safflower seed on serum Estrogen levels. Data are mean±S.E. The number of animals is 6/group. P-values denote significant difference with the corresponding sham-operated and OVX groups.

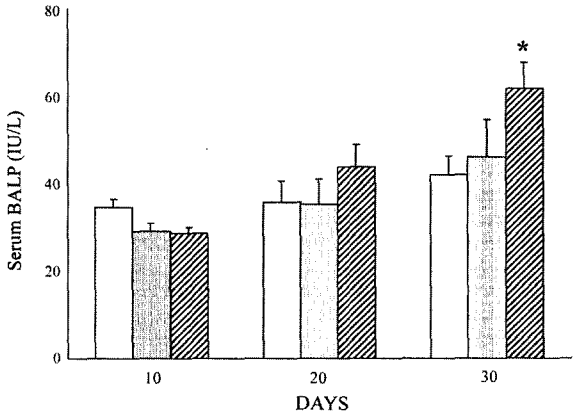

Fig 5. Time course of effects of sham, ovariectomy(OVX) and safflower seed on serum BALP levels. Data are mean±S.E. The number of animals is 6/group. P-values denote significant difference with the corresponding sham-operated and OVX groups.

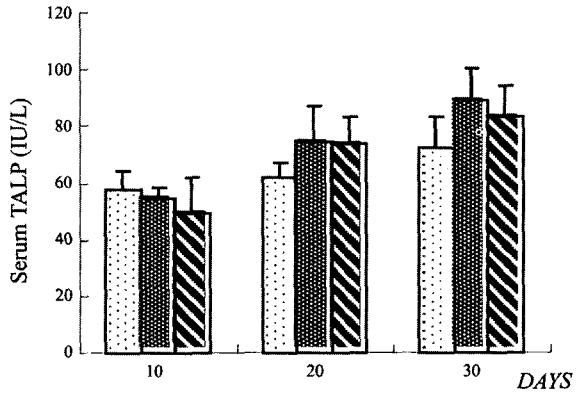

Fig 6. Time course of effects of sham, ovariectomy(OVX) and safflower seed on serum TALP levels. Data are mean±S.E. The number of animals is 6/group. P-values denote significant difference with the corresponding sham-operated and OVX groups.

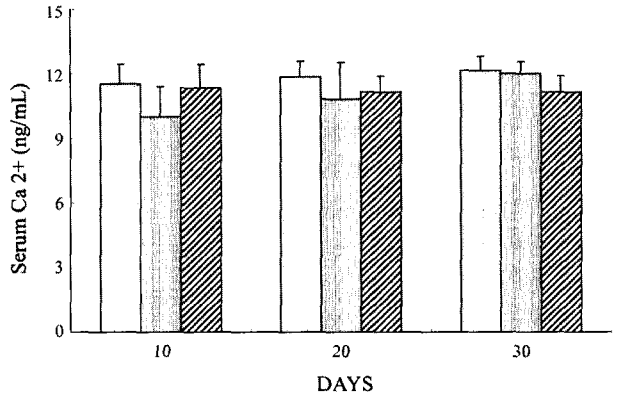

Fig 7. Time course of effects of sham, ovariectomy(OVX) and safflower seed on serum Ca2+ levels. Data are mean± S.E. The number of animals is 6/group. P-values denote significant difference with the corresponding sham-operated and OVX groups.

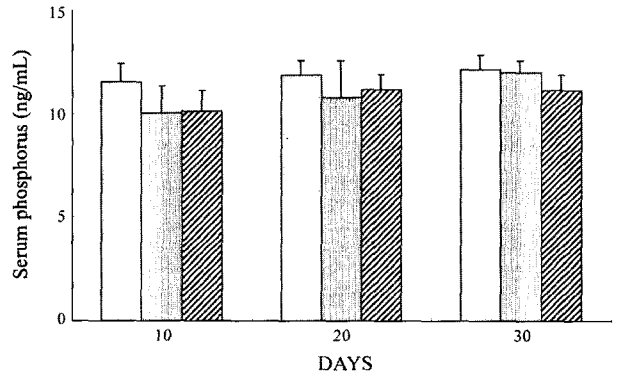

Fig 8. Time course of effects of sham, ovariectomy(OVX) and safflower seed on serum phosphorus levels. Data are mean ± S.E. The number of animals is 6/group. P-values denote significant difference with the corresponding sham-operated and OVX groups.

groups were not different an all days.

Femur/body Weight after Safflower Seed Treatment in Osteoporosis Induced OVX Rats

There were no statistically significant differences in femur/body weight among the three groups an all days(Fig 8).

Histomorphology after Safflower Seed Treatment in Osteoporosis Induced OVX Rats

Ovareictomy decreased cancellous bone volume in the proximal fibula metaphysis. The decrease could perhaps be accompanied by a significant rise in the number of trabecular osteoclasts and osteoblasts. Safflower seed treated OVX rats 'positive determinant of proximal fibula metaphysis trabecula bone mass in ovariectomized rats' is show in Fig 9. After 10 and 20 days, trabecular bone mass in the safflower seed

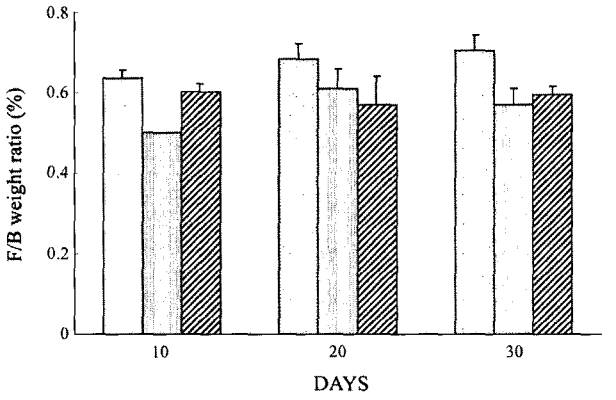

Fig 9. Time course of effects of sham, ovariectomy(OVX) and safflower seed on serum Femur/Body weight ratio. Data are mean± S.E. The number of animals is 6/group. P-values denote significant difference with the corresponding sham-operated and OVX groups.

treated OVX group increased, and after 30 days, proximal fibular metaphysis trabecular bone mass in the safflower seed treated OVX group significantly increased compared with the OVX groups, it was almost similar to normal status(Fig 11).

Discussion

This study demonstrates that the effect of safflower seed augments IGFs/IGFBPs axis on osteoporosis induced-ovariectomy rats. Moreover, the effects of osteoporosis and safflower seed therapy on the mature rat model of postmenopausal state were found after the rats were ovariectomized.

Above all, the level of osteoporosis in rats was shown to rise 6 weeks after removing the ovaries. Other groups reported that osteoporosis was induced by ovariectomized rats in 2-4 weeks[62]. According to several other reports, our results observed that osteoporosis was induced after 8 weeks in ovariectomized rats as evidenced by decreased proximal fibula metaphysis trabecular bone mass.

Estrogen deficiency induced accelerated bone turnover with an early increase in resorption markers occurring within 6-8 weeks after cessation of gonadal function, followed by a later increase in formation markers accurring up to 6 months later[6,10,18,41]. Estrogens play a major role in mineral homeostasis, 'the deficiency of estrogen is recognized as a major factor in loss of bone minerals in postmenopausal osteoporosis[4,25,50]. These results suggested that ovariectomized rats' status after 8 weeks was one of already induced osteoporosis. Our results observed that ovariectomy rats after 66 and 76 days showed increased serum IGFs levels and decreased serum IGF-I level on the 86th day. Other groups reported that ovariectomy rats have shown increases in serum IGF-1 early post ovariectomy, but the level of IGF-1 decreased approximately 60 days post-ovariectomy in rats and 1 or 2 years after menopose in humans[11,12,29]. Circulating levels of IGF-I, -II is lowed in men

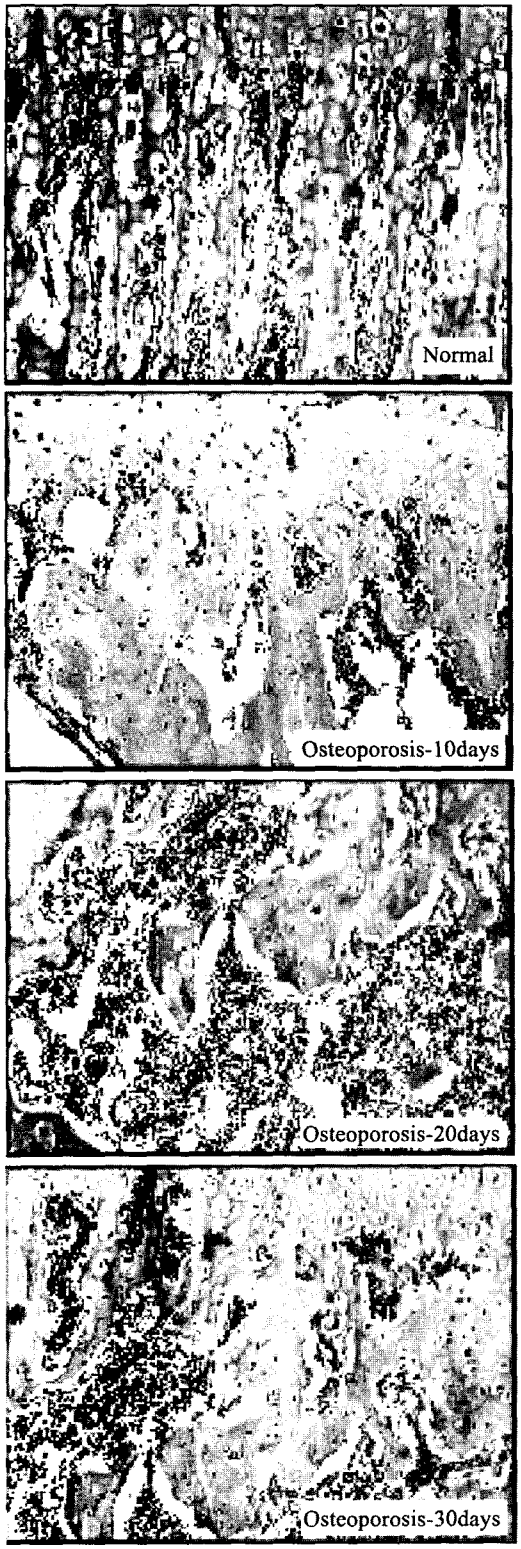

Fig 10. Histological changes of the poximal fibula metaphysis normal and osteoporosis induced-ovariectomy(Hematoxilin and Eosin ×40 except)

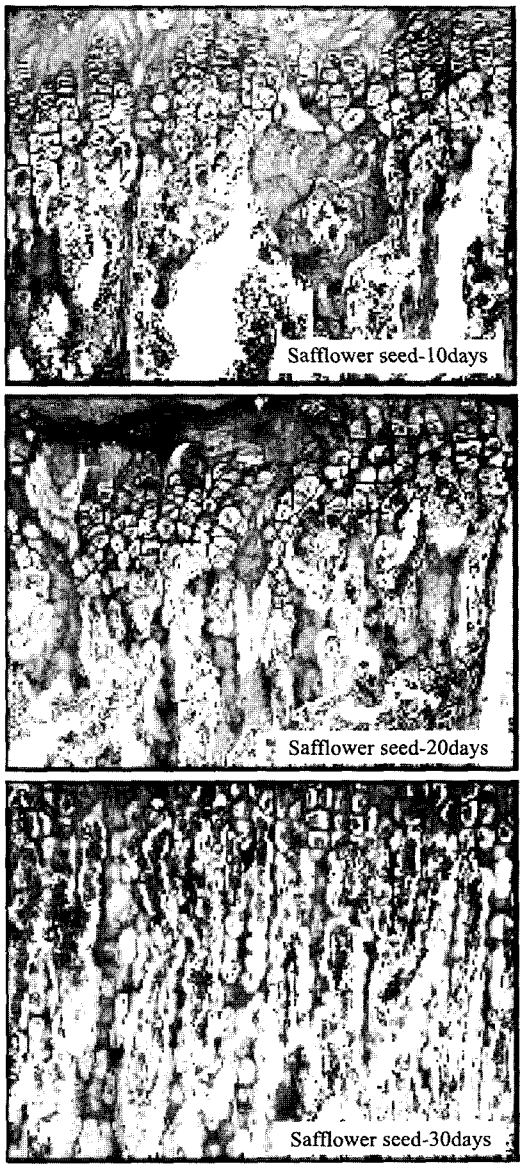

Fig 11. Histological changes of the proxinal fibula metaphysis after 10, 20, and 30 days safflower seed treatment(Hematoxilin and Eosin ×40 except)

and women with osteoporosis[1,13,21]. Circulating level of IGF-I and IGFBP-3 were also lowed in males and females with osteoporosis due to decreased bone formation or accelerated bone resorption[12,29,31]. Addition, increased serum IGF-I levels after ovariectomy over time by which ovariectomized rats have been attenuated[8]. It has been demonstrated that changes in serum IGF-I levels in ovariectomized rats are time-dependent[15] and increased serum IGF-I level stimulates interleukin-6 production by mouse long bones[21]. Cytokine has also been implicated in the increased osteoclastogenesis that occures following ovariectomy[41]. IL-6 is produced by bone marrow stromal cells and osteoblastic cells, both of which influence osteoclastogenesis in cultures of mouse bone cells[2,37].

Thus, these results suggest that no change in serum IGF-I

levels may already contribute to both the osteoclastic and ostoblastic components of the concomitant finish in bone turnover in ovariectomy rats.

We observed the increase in serum IGF-I during safflower seed treated osteoporosis compared with the OVX group after 30 days. In vitro studies have indicated that IGF-I stimulates the synthesis of DNA, collagen and non-collagen proteins in cultured rat calvaria and in cultured osteoblasts[17]. Kim *et al* reported that significant increases in spinal, pelvic, right femoral and tibial BMD after safflower seeds water extract (1250 mg/kg BW) treatment in ovariectomized rats suggest a possible protective effect of safflower seeds water extract (1250 mg/kg BW) against bone loss in OVX rats[55]. Jang *et al* also reported that aqua-acupuncture Laenec.N.HO *Carthami semen* prevented the increase in bone turnover and the decrease in bone mass induced by OVX in rats[68]. The supplementation of Korean safflower seed powder influences the recovery of bone fracture by accelerating the process of bone repair[55]. Also, IGF-I correlates to a modest extent with bone mineral density. Furthermore IGF-I has been related to bone cell proliferation[3], bone matrix synthesis[18], and longitudinal bone growth[19,36,54]. Elevated levels of IGF-I seen in safflower seed treated OVX rats might have been caused by the increased bone formation rate. Take together, the results of this study indicate that 30 days treatment with safflower seed had significant effects on bone metabolism in osteoporosis induced-ovariectomized rats.

Insulin-like growth factor II is the principal IGF present in bone and fetuses[38]. Its physiological function is fetal growth and development[9]. Especially IGF-II rate in bone of production by osteoblasts is several-fold higher than that of IGF-II[31]. It is possible that bone is a contributor to the IGF-2 serum pool[6,43].

Safflower seed treated OVX rats positive determinant of proximal fibula metaphysis trabecula bone mass in ovariectomized rats in 30 days. Therefore, it is of note that the increase we observed in serum IGF-2 levels in safflower seed treated OVX rats augmented in 30 days just as osteoblast differentiation and activity aid. The observed increase in IGF-2 appears to be due to the stimulatory effect of safflower seed on osteoblast and osteoclast bone formation in osteoporosis induced-ovariectomized rats.

Six IGFBPs have been described so far and osteoblasts have been shown to produce IGFBP-2, -3, -4, -5 and -6[23-25]. We observed IGFBP-3, which is the most abundant IGFBP in serum. Its function is regulation of IGFs and GH secretion[5]. Ernst *et al.* showed an important role for IGFBP-3 on osteoblastic cells in vitro[10]. Decreased IGFBP-3 concentrations may contribute to impaired osteoblastic function by decreases in local concentrations of IGF-I[21]. These effects are regulated by IGFs actions in bone. Serum IGFBP-3 levels did significantly increase after safflower seed treatment in 20 and 30 days in our studies. Thus, on the increase in serum IGFBP-3 safflower seed treatment can be related to regulate of IGFs activity as well as suppression of osteoclastogenesis, and may also be accompanied by decreased bone turnover.

The pattern of B-ALP response to safflower seed in our surgically postovariectomy rats is predicted from the bone remodeling cycle. Our results are supported by other studies, in which a corresponding increased level of B-ALP was found in safflower seed treatment[55]. Furthermore, increased B-ALP levels represent the biochemical marker of bone turnover[22,26,40]. Bone remodeling proceeds in an orderly fashion, with bone resorption always being followed by bone formation[22,39]. Both IGF-I and IGF-II stimulate alkaline phosphatase activity[9]. Xuezhohg reported that treatment with IGF-I increases alkaline phosphatase activity in primary rat calvaria cell culture, and IGF-II increases alkaline phosphatase activity in human osteoblast (Hob) cultures[47,52], suggesting that IGFs promote not only bone cell proliferation but also differentiation[13,44,46]. Concerning the B-ALP relation of IGF-I, II, we found that the increase in IGF-I, II might have been affected by a change in level between the BALP and IGFBP-3[18,48,51]. Furthermore tit has been reported that IGF-I, IGF-II, IGFBP-3 and ALS showed a similar pattern of change associated with age. Thus, the effect of safflower seed on bone has a positive correlation with IGFs and IGFBPs. However, we found neither ovariectomy nor safflower seed treatment to be a determinant to serum estrogen and femur/body weight, though estrogen was found to temporarily increase 30 days after safflower seed treatment.

In Chinese herbal medicine, safflower(*Carthamus tinctorius L*) seed has long been conidered effective fractures, osteoporosis, osteogenesis imperfecta, and other bone diseases. It is also used to release the extravasated blood to decrease cholesterol in plasma and neutralize fat in women as well as lengthen bleeding time and stop platelet coagulation. Safflower has so much linoleic acid that it could possibly play on important role in the bone[14,61]. It has been reported that aqua-acupuncture containing Hirudo nipponica Whitman (AA-H) and Carthamus tinctorius L (AA-L) have some effects on intravascular coagulation[50]. Previous studies have shown that carthami semen aquacupuncture (CSA) extract exerts a protective effect against oxidants in the kidney tissues of rabbits[59]. Results from Kim's study indicated that Carthami Flos Oil. Acupuncture can improve the recovery of the Gout in rats induced by Microcrystalline Sodium Urate[62,63]. Additionally, Han and yoo reported that Cartgami Flos inhibited the proliferation of Balb/c 3T3 cells and stimulated the proliferation of thymocytes[68]. Kim and Jang reported that Carthami Semen (CS) extration exerts a protective effect against tBHP-induced inhibition of Na-K-ATPase activity, and this effect may be due to an antioxidant action in microsomal fraction isolated from rabbit kidney cortex[57,63]. Lee reported that Cathami Flos aqua-acupuncture inserted into yangnungchon (GB34) appeared to have an effect on Adunvabt Arthritis in rats[66].

Therefore, this study shows that the administration of safflower seed prevents the decline in trabecular bone mass in the proximal fibular metaphysis with OVX rats. It also appears possible that the effection of serum IGFs secretion by treated safflower seed may be related to growth rate in OVX rats.

Further, the effects of osteoporosis and safflower seed therapy on the mature rat model of postmenopausal bone loss are areas where more longitudinal studies are needed to elucidate the pathological changes in safflower seed treated-ovariectomy rats developing osteoporosis.

Conclusion

In the present study, we aimed to examine how the characteristics of safflower seed affects serum levels of IGF-I, IGF-II, IGFBP-3, BLAP, P, Ca^{2+}, Estrogen, Femur/body weight ratio and histomorphology in OVX rats. Additionally, we sought to investigate the possible role of safflower seed in the regulation of bone metabolism in ovariectomized rats. According to the results, we concluded:

1) Serum levels of IGF-I, IGF-II and IGFBP-3 in safflower seed treated OVX rats increased.

2) BALP on safflower seed treated rats in serum increased compared with that of OVX, but Estrogen, Ca^{2+}, P, Femur/body weight ratio were unchanged compared to control.

3) Safflower seed treated OVX rats, positive determinant of proximal fibula metaphysis trabecula bone mass in ovariectomized rats.

These results suggest that safflower seed affects the serum levels of IGFs, IGFBP-3 and BALP in OVX rats. Further, change of serum IGFs and IGFBP-3 levels in osteoporosis indicated that it can be used as a parameter.

Acknowledgement

This study was supported by a grant form the Korean Research Foundation grant fund (KRF-99-003-G00102G7012).

References

1. Abel Lopez-Bermejo, Caroline K. Buckway and Ron G. Rosenfeld Genetic Defects of the Growth Hormone-Insulin-like Growth Factor Axis TEM 2000, 11(2) 39-49.
2. Ann Verbruggen, Luc S. De Clerck, Chris H. Bridts, Jan F.Van Flow cytometrical determination of Interleukin 1b, Interleukin 6 and Tumour necrosis factor a in monocytes of rheumatoid arthritis patients ; relation with parameters of osteoporosis. Cytokine November, 1999: 869-874.
3. Atsushi Kawakami, Tomoki Nakashima, Mashiko Tsuboi, Satoshi Insulin-like Growth Factor I Stimulates Proliferation and Fas-Mediated Apoptosis of Human Osteoblasts. *Biochemical and Biophysical Research Communications* 1998 247: 46-51.
4. C. C. Danielsen and A. Flyvbjerg Insulin-like Growth Factror I as a Predictor of Cortical Bone Mass in a Long-term Study of Ovariectomized and Estrogen-treated Rats. *Bone* 1996: 493-498.
5. Christoph Schmid, Claudia Keller, Irene Schlapfer, Christian Calcium and Insulin-like Growth Factor I Stimulation of Sodium-Dependent Phosphate Transport and Proliferation of Cultured Rat Osteoblasts. *Biochemical and biophysical research communications* 1998; 245: 220-225.
6. Claes ohlsson, Bengt-ake bengtsson, olle g.p.isaksson. Growth Hormone and Bone. *Endocrine Reviews* 1998, 19(1): 55-79.
7. Claire E. H. Stewart and peter rotwein. Growth, Differentiation, and Survival: Multiple Physilolgical Functions for Insulin-Like Growth Factors. physiological Reviews 1996; 76(4): 1005-1026.
8. Clifford J.Rosen and Michael Pollak Circulating IGF-I: New Perspectives for a New Century. *Elsevier Science* 1999; 10(4): 136-141.
9. Clifford J. Rosen, Leah Rae Donahue, and susan J. Hunter Insulin-like growth factors and bone: The ostpeoporosis connection. *IGFs and Osteoporosis*, minireview 83-102, 1994.
10. Craig H. Selzman, Jaime S. Gaynor, A. Simon Turner, Estrogen replacement inhibits intimal hyperplasia and the accumulation and effects of transforming growth factor β_1 *Jurnal of surgical research* 1998; 80: 380-385.
11. C. Wuster, W.F.Blum, S.Schlemilch, M.B.Ranke & R. Ziegler. Decreased serum levels of insulin-like growth factors and IGF binding protein 3 in osteoporosis. *Journal of Internal Medicine* 1998; 234: 249-255.
12. Dike N.Kalu, Bahram H.Arjmandi, Chung-Ching Liu, Mohammed A.Salih. Effects of ovariectomy and estrogen on the serum levels of insulin-like growth factor-I and insulin-like growth factor binding protein-3. *Bone and Mineral* 1994; 25: 135-148.
13. Douglas S. Steinbrech, MD Babak J. Mehrara, MD Norman Gene Expression of Insulin-like Growth Factors I and II in Rat Membranous Osteotomy Healing. *Inc.* 1999: 481-487.
14. EC Staley, GS Smith and JA Greenberg. Effects on Reproduction in Female Offspring from Sprague-Dawley Rats Fed 10% Snakeweed (Gutierrezia Microcephala) Throughout Prenancy and Concurrent Treatment with Safflower Oil *Vet Human Toxicol* 1995; 35(5): 440-442.
15. E.I.Barengolts, T.Kouznetsova, A.Segalene, P.Lathon, C. Odvina, Effects of Progesterone on Serum Levels of IGF-1 and on Femur IGF-1 mRNA in Ovariectomized rats. *Journal of bone and mineral research.* 1996; 11(10): 1406-1412.
16. Han-Jung Chae, Jun-Gi Lee, Jang-Sook Kang, Young-Chul Park, Synergistic Cooperation Between Camp and IFN-r for induction of nitric oside synthesis Ros 17/2.8 osteoblasts. *Research Communications in Biochemistry, Cell & Molecular Biology* 1992: 50-62.
17. Hans-Peter Guler, Juren Zapf, Eroka Scheiwiller, and E. Rudolf Recombinant human insulin-like growth factor I stimulates growth and has distinct effects on organ size hypophysectomized rats (growth hormone/body-weight gain/longitudinal bone growth/kidneys/spleen) *Proc. Natl Acad Sci. USA* July 1988; 85: 4889-4893.
18. I.M.Dick, A.St. John, S. Heal, R. L. Prince The effect of estrogen deficiency on bone mineral density, renal calcium and phosphorus heandling and calcitropic hormones in the rat. *Calcif tissue int* 1996; 59: 174-178.
19. JAMJL Janssen, H Burger, R P Stolk, D E Grobbee, F H de Jong. Gender-specific relationship between serum free and total IGF-I and bone mineral density in elderly men and women. *European Journal of Endocrinology* 1998; 138: 627-632.
20. Jonathan S. Fisher, Wendy M. Kohrt, and Marybeth brown.

Food restriction suppresses muscle growth and augments osteopenia in ovariectomized rat *Journal appl. physiology* 2000; 88: 265-271.
21. Jan Frystyk, Christian Skjaerbaek, Bo Dinesen, Hans Orskov Free insulin-like growth factors (IGF-I and IGF-II) in human serum *FEBS Letters* 1994; 348: 185-191.
22. Jon P. van Straalen, Edward Sanders, Mark F. Prummel. Bone-alkaline phosphatase as indicaator of bone formation. *Clinica Chemica Acta* 1991; 201: 27-34.
23. J.Pfeilschifter, L.Bonewald, and G.R.Mundy Role of Growth Factors in Cartilage and Bone Metabolism *Text book of growth factors* 371-400.
24. Junji Takaya, Hirohiko Higashino, Rika Miyazaki, and Yohnosuke Kobayashi Effects of Insulin and Insulin-like Growth Factor-1 on Intracellular Magnesium of Platelets *Experimental and Molecular Pathology* 1998; 65: 104-109.
25. KALU DN The ovariectomized rat model of postmenopausal bone loss. *Bone miner* 1991; 15: 175-192.
26. Kim Brixen, Henning K, Nielsen, Erik F. Erikse Efficacy of wheat germ lectin-precitated alkaline phosphatase in serum as an estimator of bone mineralisation rate: comparison to serum total alkaline phosphatase and serum bone gla-protein *Calcif Tissue Int* 1989; 44: 93-98.
27. Lawrence A.Wetterau.Michael G.Moore, Kuk-Wah Lee, Melanie L.Shim, and Pinchas Cohen. MINIREVIEW Novel Aspects of the Insulin-like Growth Factor Binding Proteins. *Molecular Genetics and Metabolism* 1999; 68: 161-181.
28. Lorenz C. Hofauer, Colin R. Dumstan, Thomas C. Spelsberg Osteoprotegerin production by human osteoblast lineage cell stimulated by vitamin D, bone morphogenetic protein-2, and cytokines. *Biochemical and biophysical research communications* 1998; 250: 776-781.
29. Masamichi Nasu, Toshitsugu Sugimoto, Mieko Chihara, Mitsuo Effect of natural menopause on serum levels of IGF-I and IGF-binding proteins:relationship with bone mineral density and lipid metabolism in perimenopausal women. *European Journal of Endocrinology* 1997; 136: 608-616.
30. M Kassem, K Brixen, W Blum, L Mosekilde Effects of growth hormone treatment on serum levels of insulin-like growth factors(IGFs) and IGF binding protein 1-4 in postmenopausal women *Clinical endocrinology* 1998; 49: 747-756.
31. M Kassem, K Brixen, W Blum, L Mosekilde and EF Eriksen No evidence for reduced spontaneous or growth - hormone - stimulated serum levels of insulin-like growth factor (IGF)-I, IGF-II or IGF binding protein 3 in women with spinal osteoporosis. *European journal of endocrinology* 1994; 131: 150-155.
32. Michael H.Raymond, Brian C.Schutte, James C.Torner, Trudy Mapping of the Human Gene BGLAP and Its Potential Role in Postmenopausal Osteoporosis. *Genomics* 1999; 60: 210-217.
33. Murray W. Tilard, M. B., Ch. B. George F. S. Spears, M. Sc., B. A., B. Com., Treatment of postmenopausal osteoporosis with calcitriol or calcium. *The New England Journal of Medicine,* 1992: 357-362.
34. P.A.J. Baldock, H. A. Morris, R. J.Moore, A. G. Need, Prepubertal oophorectomy limits the accumulation of camcellous bone in the femur of growing rats with long-term effects on metaphyseal bone architecture *Calcif Tissue int* 1998; 62: 244-249.
35. Paul E. Belhets, M.D. Hormonal treatment of postmonopausal women. *The new england journal of medicine* 1994; 14: 1062-1071.
36. Peter A. Hill, John J. Reynolds, and Murrary C. Meikle Osteoblasts mediate insulin-like growth factor-I and -II stimulation of osteoclast formation and function *Endocrinology* 1995; 136(1): 124-131.
37. Peter Vestergaard, Anne Pernille Hermann, Haans Orskov, Leif Mosekilde, Effect of Sex Hormone Replacement on the Insulin-Like Growth Factor System and Bone Mineral: A Cross-Sectional and Longitudinal Study in 595 Perimenopausal Women Participating in the Danish Osteoporosis Prevention Study. *The Journal of Clinical Endocrinology & Metabolism* 1990; 84(7): 2286-2290.
38. P Ravn, K Overgaard, EM Spencer and C Christiansen Insulin-like growth factors I and II in healthy women with and without established osteoporosis. *European Journal of Endocrinology,* 1995; 132: 313-9.
39. Ralph J. Duda JR, John F. Obrien, Jerry A. Katzmann, James M. Concurrent Assays of Circulating Bone Gla-Protein and Bone Alkaline Phosphatase: Effects of Sex, Age, and Metabolic Bone Disease *Journal of Clinical Endocrinology and Metabolism* 1988, 66(5): 951-957.
40. R. Dresner-pollak, M. mayer, D. Hochner-Celiniker. The decrease in serum bone-specific alkaline phosphatase predicts bone mineral density response to hormone replacement therapy in early postmenopausal women. *Calcif Tissue int* 2000; 66: 104-107.
41. Robert L.Jilka, Giao Hangoc, Giuseppe Girasole, Giovanni Passeri, Daniel C, Increased Osteoclast Development After Estrogen Loss: Mediation by Interleukin-6 *Science* 1992; 257(3).
42. Rousselle Anne-Valerie, Damiens Christelle, Fortun Yannick, Passuti Norbert, Padrines Marc, and Heymann Dominique. Human Growth Hormone Stimulates Proteinase Activities of Rabbit Bone Cells via IGF-I. *Biochemical and Biophysical Research Communications* 2000; 268: 875-881.
43. Sandra D.C.Dell, Ian N.M. Day. Molecules in focus imsulin-like growth factor II (IGF-II) *The international journal of biochemistry and cell biology* 1998; 30: 767-771.
44. Steven Boonen,subburaman mohan, Jan dequeker, Jeroen aerssens, Insulin-like Growth Factor(IGF) System (IGF-I, IGF-II Binding Protein [BP]-3, and IGFBP-5) in Age-Related (Type II) Femoral Neck Osteoporosis *Journal of Bone and mineral reseach* 1999; 14(12): 2150-2158.
45. Sundeep Khosia, Ahmed A.K. Hassoun, Bonita K. Baker, Frances Liu, Insulin-like Growth Factor System Abnormalities in Hepatitis C-associated Osteosclerosis. *The Journal of Clinical Investigation* 1998; 101(10): 2165-217.
46. Takashi Shimakata and Paul K. Stumpf. The procaryotic nature of the fatty acid synthetase of developing *Carthamus tinctorius L*(Safflower) seeds. *Archives of Biochemistry and baioophysics* 1982; 217(1): 144-154.
47. Thomas A.Linkhart, Subburaman Mohan and David J.Baylink Growth Factors for Bone Growth and Repair: IGF,TGFB and BMP *Bone* 1996; 19(1): 1S-12S.
48. Thomas L.Mccarthy, Sandra Casinghino, Michael Centrella, and Ernesto Canalis. Complex Pattern of Insulin-Like

Growth Factor Binding Protein Expression in Primary Rat Osteoblast Enriched Cultures: Regulation by Prostaglandin E2, Growth Hormone, and the Insulin-Like Growth Factors. *Journal of cellular physiology* 1994; 160: 163-175.

49. T. J. Wronski, C-F. Yen, H. Gi, and L. M. Dann Parathyroid Hormone is More Effective Than Estrogen or Bisphosphonates for Restoration of Lost Bone Mass in Ovariectomized Rats. *Endocrinology* 1993; 132(2): 823-831.
50. Tommy storm, Gorm thamsborg, Torben steiniche, Harry K, Effect of intermittentcyclical eticronate therapy on bone mass and fracture rate in women with postmenopausal osteoporosis. *The new england journal of medicine.* 1990; 322(18): 1265-1271.
51. Urban Lindgren and Hector F. Deluca Role of Parathyroid Hormone and 1,25-Dihydroxyvitamin D3 in the Development of Osteopenia in Oophorectomized Rats *Calcif Tissue Int* 1982; 34: 510-514.
52. Xuezhong qin, Dongwonbyun, Donna D. strong Studies on the role of human insulin-like growth factor - II(IGF-II) - Dependent IGF binding protein(hIGFBP)-4 protease in human osteoblasts using protease-resistant IGFBP-4 analogs. *Journal of bone and mineral research* 1999; 14(12): 2079-2088.
53. Yong li, Mark F. Seifert, Denise M. Ney, Mike Grahn, Dietary conjugated linoleic acids alter serum IGF-I and IGF binding protein concentrations and reduce bone formation in rats fed (n-6) or (n-3) fatty acids. *Journal of bone and mineral research* 1999; 14(7): 1153-1162.
54. Yu H.; Mistry J.; Khosravi M.J; Diamandis A.; Van Doorn J.; Juul A. Insulin-like growth factors (IGF-I, free IGF-I, and IGF-II) and insulin-like growth factor binding proteins IGFBP-2,IGFBP-3,IGFBP-6,and ALS) in blood circulation *Physiologica reviews* 1996; 76(4): 1005-1026.
55. Mi Ryeo Kim, Choe Ha Yang, Bu-il Seo. Effects of Safflower Seeds on Bone Mineral Density in Ovariectomy-Induced Pstmenopausal Osteoporotic Rats *Kor. J. Herbology* 1998; 13(2): 37-43.
56. Sun Hyuk Kim, Joon Moo Lee, Effects of Aqua-acupuncture with Carthami Flos Oil on Gout in rats induced by Microcrystalline Sodium Urate *The Journal of korea Acupuncture on Moxibustion society* 1998; 15(1): 483-491.
57. Yeo Jin Kim, Kyung jeon Jang, Chun ho Song, Chang beom Ahn, The Effects of Carthami Semen Extraction on Oxidant-Indeced Impariment of Na-K-ATPase Activity in Kidney *The Journal of korea Acupuncture on Moxibustion society* 1998; 15(1): 493-502.
58. Jun Han Kim, Seon Min Jeon, Mi Young An, Sae-Kwang Ku, *et al.* Effects of Diet of Korean Safflower(*Carthamus tinctorious L.*) Seed Powder on Bone Tissue in Rats during the Recovery of Rib Fracture. *J. Korean Soc. Food Sci. Nutr* 1998; 27(4): 698-704.
59. 백남인, 방면화, 송정춘, 이상양, 박남규 홍화(Carthamus tinctoriusL)씨의 항산화활성물질, N-feruloylserotonin. 한국농화학회지 1999; 제42권 제4호: 366-368.
60. Seo, Hyun Ju. Kim Jun Han, Kwak, Dong Yun, Jeon, Seon Min. *et al.* The Effects of Safflower Powder and Its Fraction on Bone Tissue in Rib-Fractured Rats during the Recovery *The Korean Nutrition Society* 2000; 33(4): 411-420.
61. Sang Ae Seung, Soo Youn Chung et al Studies on the Pharmacological Assay of the Fracture Healing in Long Bone *The annual Report of KFDA* 456-469, 1997.
62. O, Ju Hwan Lee, Yeon Sook Effects of Dietary Calcium Levels on the Reduction of Calcium Availability in Ovariectomized Osteoporosis Model Rats *Korean J Nutrition* 1993; 26(3): 277-285.
63. Hyoun-min, Youn. chang-belhm, Ahn Effect of Carthami semen aquacupuncture on oxidant induced cell death and DNA damage in renal tubular cells *The Journal of korea Acupuncture on Moxibustion society* 1999; 16(4): 293-305.
64. Yeon Sook Lee, O, Ju Hwan Effects of Bovine Bone Ash and Calcium Phosphate on Calcium Metabolism in Postmenopausal Osteoporosis Model Rats *Korean J Nutrition* 1995; 28(5): 434-441.
65. Seng Kil Inm Therapy of osteoporosis *Korean J Nutrition* 1993; 26(2): 213-219.
66. Hee Tae, Lee: O. M. D., Hak In, Lee: O. M. D., Ph.D. Effects of Cathami Flos aqua-acupuncture on the arthritis induced by injecting Freunds complete adjuvant *The journal of east-west medicine:* 6-20.
67. Seon Min Jeon, Jun Han Kim, et al The Effects of Korean Safflower (*Carthamus tinctorius L.*) Seed Powder Supplementation Diet on Bone Metabolism Indiees in Rats during the Recovery of Rib Fracture. *Korean J Nutrition* 1998; 31(6): 1049-1056.
68. Su Jin Jang, Chang Hyun Lee. Effects of Laennec. N.HO (*Hominis placenta. Cervi pontorionum cornu. Carthami semen*) aqua-acupuncture on the ovariectomized osteoporotic Rats *The Journal of Korean Oriental medical Society* 1998; 19(1): 5-18.
69. Jong Hyun Han, Kwang Suk Yoo et al Cytotoxicity of Carthami Flos on Human cancer cell-lines(I) The Journal of Korean Oriental Medical Society 1996; 17(2): 303-310.

흰쥐의 난소제거로 유발한 골다공증에 대한 홍화씨의 IGFs, IGF binding protein-3 그리고 BALP에 대한 혈청내 효과

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요 약 : 본 연구는 흰쥐의 난소제거로 유발한 골다공증에 대한 홍화씨(Carthamus inctorius L)의 투여 효과를 알아보기 위하여 혈청내 호르몬과 골소주의 변화를 관찰하였다. 실험동물은 4개월 령의 흰쥐를 난소절제를 실시하여 골다공증을 유발 시킨 후 실험에 이용하였으며 30일간 격일 간격으로 0.03g/kg의 용량을 투여 하였다. 혈청 내에서 Insulin-like Growth Factors, Insulin-like Growth Factor binding protein-3 (IGFBP-3), Estrogen, Bone-specific alkaline phosphotase, Calcium, and Phospotase를 매 10일 간격으로 측정하였으며 비골의 골간을 채취하여 조직 형태학적인 검사를 실시하였고 체중에 대한 대퇴골 무게를 측정하였다. 홍화씨 투여 10일과 20일에서는 혈청내 IGF-I, IGF-II 그리고 IGFBP-3의 변화는 대조군에 비하여 유의성 있는 변화를 관찰할 수 없었으나 홍화씨 투여 30일에 있어서는 IGF-I, IGF-II 그리고 IGFBP-3의 변화가 대조군에 비하여 현저히 높은 유의서 있는 변화를 관찰할 수 있었다($p<0.05$). Bone alkaline phosphatase(BALP)에 있어서도 홍화씨 투여 30일 에 가장 많은 변화가 있었으나 estrogen과 체중에 대한 대퇴골의 무게에 있어서는 유의성 있는 변화를 관찰하지 못했다. 오히려 이시기에 난소를 절제하지 않는 대조군의 estrogen치가 높게 나타났다. 난소절제로 골다공증을 유발시킨 흰쥐에 있어서 홍화씨의 투여는 혈청내 IGFs,. IGFBP-3 and BALP을 높임으로서 골다공증 치료에 효과가 있는 것으로 사료됩니다.

주요어 : 골다공증, 홍화씨, 난소절제술, 혈청내 IGF-I, IGF-II