Nutraceuticals & Food
Vol. 8, p. 46~53 (2003)

Nutraceuticals and Food

Supplementation of Safflower Seed Powder and Extracts Enhances Bone Metabolism in Rib-Fractured Rats

Hyun-Ju Seo, Kwang-Deog Moon*, Seon-Min Jeon, Jun-Han Kim* and Myung-Sook Choi†

*Department of Food Science and Nutrition, *Department of Food Science and Technology, Kyungpook National University, Daegu 702-701, Korea*

Abstract

The current study investigated the effect of Korean safflower (*Carthamus tinctorius* L.) seed powder and its water and ethanol extracts on bone metabolism during recovery from rib-fracture induced by surgical operation in rats. 10-week-old male Sprague-Dawley rats weighing about 320 g were divided into 9 groups after arrival: 10d control (AIN 76 semi-purified diet), 10d safflower seed powder (10d SS-powder), 10d safflower seed ethanol extract (10d SS-EtOH), 10d safflower seed water extract (10d SS-H_2O), 20d control (AIN-76 semi-purified diet), 20d safflower seed powder (20d SS-powder), 20d safflower seed ethanol extract (20d SS-EtOH), 20d safflower seed water extract (20d SS-H_2O), and 20d sham-operation (20d sham). The dietary level for all the supplements was 5% based on the raw material weight. The rats were fed the experimental diets for 10 days before the rib fracture operation and for a further 10 or 20 days after the operation. A number 9 rib was fractured surgically and a sham-operation also performed. The rats were then sacrificed on the 10th or 20th day after the operation. The body weight initially decreased after the operation in all the rib-fractured groups, then gradually recovered. The concentrations of plasma osteocalcin were higher in the control group than in all the safflower-supplemented groups 10 and 20 days after the rib-fracture ($p < 0.05$). The bone-specific ALP (alkaline phosphatase) activity was significantly higher in the SS-EtOH group than in the other groups 20 days after the rib-fracture ($p < 0.05$). The level of urinary DPD (deoxypridinoline) was significantly higher in the SS-EtOH and SS-H_2O groups than in the other groups 10 days after the rib-fracture. When comparing the PTH (parathyroid hormone) and calcitonin levels, the SS-H_2O group exhibited the highest PTH level among the groups 10 and 20 days after the rib-fracture. Thus, it was concluded that the bone turnover during the fracture-healing period was more rapid in the rats supplemented with safflower seed powder or its fractions than in the control rats. Furthermore, the SS-H_2O fraction was identified as the most effective in stimulating bone remodeling, as bone resorption and bone formation were both significantly increased during fracture healing when compared to the control group.

Key words: safflower seed, rib-fracture, bone resorption, bone metabolism indices

INTRODUCTION

The skeleton serves several functions as a support for the body, protector of internal organs, attachment site for muscles, cavity for bone-forming cells, and reservoir for minerals (1-4).

Bone constantly undergoes the process known as remodeling or bone turnover, which is a coupled reaction of resorption and formation. This remodeling process is part of the normal maintenance and development of bone. In the remodeling process, old bone is resorbed (dissolved) by enzymes released from osteoclasts (bone-resorbing cells), resulting in a bone cavity, which is then filled with a new bone matrix through osteoblasts (bone-forming cells) (5). Under normal circumstances, the bone resorption and formation processes are tightly coupled and balanced. Remodeling is regulated by both local and systemic factors, including electrical and mechanical forces, hormones (e.g., parathyroid hormone, thyroid hormone, vitamin D and its metabolites, estrogen, androgens, cortisol, calcitonin, and growth hormones), growth factors [e.g., insulin-link growth factor 1 (IGF-1) and transforming growth factor ß], and cytokines (e.g., interleukins 1 and 6) (1).

Recently, there has been an increase in the use of functional foods and/or nutraceuticals for preventing or treating chronic diseases, such as cancer, hyperlipidemia, diabetes, and osteophoresis. Among medicinal herbs, the inhibitory effect of "Honghwain-Jahage (HJ)" is very similar to that of calcitonin treatment, indicating a similar role in inhibiting osteoclast-mediated bone resorption. In Korean herbal

†Corresponding author. E-mail: mschoi@knu.ac.kr
Phone: +82-53-950-6232, Fax: +82-53-950-6229

medicine, HJ is known to be effective for treating inflammation, hyperlipemia, arteriosclerosis, and gynecological diseases, such as osteophoresis and bone resorption. According to ancient Chinese and Korean medicinal and herbal literature (6), the formula of HJ is an effective biological response modifier for augmenting the host homeostasis of body circulation (6). HJ consists of crude ingredients from two medicinal herbs, *Carthamus tinctorius* L. seeds and *Hominis placenta*. The chemical constituents of *C. tinctorius* L. seeds are carthamin ($C_{21}H_{22}O_{11}$) (7) and various fatty acids (8,9), while the constituents of *H. placenta* are annexin (10) and placenta protein 4 (11). This herbal medicine has also been shown to express diverse activities, such as immunomodulating, anti-infarction, anti-allergic, and anti-inflammatory effects (12).

Safflower (*Carthamus tinctorius* L.) seeds have a long history of clinical use in Korea related to promoting bone formation and preventing osteoporosis (13). The dried flower of safflower is also used to promote blood circulation and treat angina pectoris and cerebral thrombosis (14). Safflower seeds contain three serotonin derivatives that exhibit antioxidative activity (15).

In preliminary experiments, the supplementation of safflower seed powder (10%) has been found to influence bone fracture recovery by accelerating the process of bone repair (16,17). Accordingly, the current study investigated the effect of Korean safflower (*Carthamus tinctorius* L.) seed powder and its water & ethanol extracts on bone metabolism during recovery from rib-fracture induced by surgical operation in rats.

MATERIALS AND METHODS

Preparation of safflower seed extract and chemical analysis

One hundred grams of safflower seeds was extracted using water or ethanol. The powder from roasted safflower seeds was extracted twice using either 500 mL of hot distilled water (90°C) or 80% ethanol (80°C) for 6 hr. The extracts were then filtered through Whatman No. 2 filter paper, evaporated under a vacuum at 40°C, and further dried to a powder using a freeze-dryer at -50°C. The extraction method used in the current study yielded 10.1 g of SS-H_2O or 3.0 g of SS-EtOH/100 g safflower seeds. Different types of safflower seed were analyzed for their general composition. The crude protein, crude fat, and crude ash contents were determined using the Kjedahl method, Soxhlet extraction method, and wet-dry ashing, respectively. The total and reducing sugar contents were analyzed using the AOAC method (18), while the total phenolic content was analyzed by the Folin-Denis method (19). The fatty acids were analyzed using gas chromatography (20). The major compositions of general nutrients and fatty acids in the safflower seeds and extracts are shown in Table 1.

The crude fat content was higher in the ethanol preparation than in the water preparation, but the opposite was true for the crude ash content. The total sugar content was 7 times higher in the ethanol preparation than in the water preparation, while the total phenolic content in the ethanol preparation was 5 times higher than that in the water preparation based on the dried weight of each extract (Table 1). The total phenolic content included in the SS-powder, SS-EtOH, and SS-H_2O diets was about 155 mg, 7.45 mg, and 5.0 mg per 100 g of diet, respectively. Among the fatty acid profiles for the safflower seed preparations, the contents of caprylic acid and erucic acid were higher in the ethanol preparation than in the water preparation. The major fatty acid in all the safflower seed preparations was linoleic acid. However, the contents of palmitoleic acid and stearic acid were higher in the water preparation than in the ethanol preparation (Table 1).

Animals and diets

Sixty male Sprague-Dawley rats weighing between 300 g and 320 g were obtained from Bio Genomics (Seoul, Korea). The animals were individually housed in stainless steel cages in a room at 20~23°C with a 12-h light:dark cycle. All the animals were fed a pelletized commercial chow diet for eleven days after arrival, then randomly

Table 1. Composition of safflower seeds and extracts

Component	SS-Power	SS-EtOH	SS-H_2O
		%, dry basis	
Crude protein	18.82	41.22	34.24
Crude fat	14.61	14.52	6.84
Crude ash	3.87	8.51	16.39
Crude fiber	10.46	0.29	trace
N-free extract	52.23	35.45	42.52
		mg/g, dry basis	
Total sugar	21.01	322.16	46.07
Reducing sugar	0.99	5.68	6.81
Total phenolics	31.00	49.65	10.01
Fatty acid		Area %	
Caprylic acid	trace	8.3	4.3
Palmitoleic acid	7.8	1.2	6.0
Stearic acid	5.1	0.7	2.4
Oleic acid	14.1	12.6	12.1
Linoleic acid	69.1	72.9	72.9
Arachidic acid	0.9	0.4	0.7
Heneicosanoic acid	trace	0.3	0.3
Behenic acid	1.8	1.0	1.2
Erucic acid	trace	2.6	trace
TSFA[1)]	12.9	10.2	12.7
TUSFA[2)]	87.1	89.8	87.3

[1)]Total saturated fatty acids.
[2)]Total unsaturated fatty acids.

divided into 9 groups: 10d control (AIN 76 semipurified diet), 10d safflower seed powder (10d SS-powder), 10d safflower seed ethanol extract (10d SS-EtOH), 10d safflower seed water extract (10d SS-H_2O), 20d control (AIN-76 semipurified diet), 20d safflower seed powder (20d SS-powder), 20d safflower seed ethanol extract (20d SS-EtOH), 20d safflower seed water extract (20d SS-H_2O), and 20d sham-operation (20d sham) (Table 2). The experimental diets were supplied for 10 days prior to the rib fracture operation and for a further 10 or 20 days after the operation. A number 9 rib was fractured surgically and a sham-operation also performed. The rats were then sacrificed on the 10th or 20th day after the operation. The level of all the dietary supplements was 5% based on the raw material (safflower seed) weight. The experimental diets were made isoenergetic and isonitrogenous by adjusting the diet composition based on the information in Table 1. The diet compositions are shown in Table 3; the control diet was based on an AIN-76 semi-synthetic diet (21,22). The animals were given free access to food and distilled water during the experiment. The food consumption and weight-gain were measured every third day. At the end of the experimental period, the rats were anesthetized with Ketamine following a 12h fast. Blood was drawn from the inferior vena cava into a heparin-coated tube, and the plasma obtained by centrifuging the blood at 1,000 g for 15 min at 4°C. Urine was collected for 2 days before sacrificing. The plasma and urine were stored at -60°C until analyzed.

Biochemical markers analyses

The concentrations of plasma calcium and phosphorus were determined using a commercial kit (Asan, Korea). The level of alkaline phosphatase (Total-ALP), a bone formation index, was determined according to the spectroscopic luminous method using a commercial kit (Asan, Korea). The activity of bone-specific alkaline phosphatase (BAP) in the plasma was determined by an immunoradiometric assay (IRMA) using an ALKPHASE-B kit (Metra Biosystems, Inc., USA). The concentration of osteocalcin in the plasma was also determined by an immunoradiometric assay (IRMA) using a Human Osteocalcin kit (Immutopics Inc., USA), where the sample containing osteocalcin is incubated simultaneously with an antibody-coated bead and ^{125}I labeled antibody, then the radioactivity bound to the bead is measured using a gamma counter (23).

The amount of deoxypyridinoline (DPD) in the urine, as an indicator of bone resorption, was quantitatively determined using a PYRILINKS®-D kit (Metra Biosystems, Inc., USA), which performs a competitive enzyme immunoassay. The parathyroid hormone (PTH) level and concentration of calcitonin, both of which regulate the concentration of plasma calcium, were determined by an immunoradiometric assay using an INTACT PTH kit (Nichols Institute Diagnostics, USA) and Calcitonin Double Antibody kit (Diagnostic products corporation, USA), respectively. Then the radioactivity was measured using a gamma counter. The protein concentration was determined according to Bradfords method (24) using bovine albumin as the standard.

Table 2. Experimental design to determine effect of different safflower seed (SS) fractions on repair of rib-fracture

Duration after rib-fracture	Symbols for groups	N[1)]	Type of safflower seed
10 days	Control	7	-
	SS-powder	7	Powdered seed
	SS-EtOH	7	Powdered EtOH extract
	SS-H_2O	6	Powdered H_2O extract
20 days	Control	7	-
	SS-powder	7	Powdered seed
	SS-EtOH	7	Powdered EtOH extract
	SS-H_2O	6	Powdered H_2O extract
	Sham	6	-

[1)]Number of animals.

Table 3. Composition of experimental diets (%) fed to rib-fractured animals

Ingredients	Dietary groups			
	Control	SS-powder	SS-EtOH	SS-H_2O
Casein	20.0	19.06	19.97	19.82
D,L-methionine	0.3	0.3	0.3	0.3
Corn starch	15.0	14.64	14.99	14.98
Sucrose	50.0	48.79	49.99	49.93
Cellulose powder	5.0	3.43	4.98	4.90
Corn oil	5.0	4.27	4.93	4.95
Mineral mixture[1)]	3.5	3.31	3.49	3.42
Vitamin mixture[2)]	1.0	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2	0.2
SS-powder	-	5.0	-	-
SS-EtOH extract	-	-	0.15	-
SS-H_2O extract	-	-	-	0.5
Total	100	100	100	100

[1)]AIN-76 mineral mixture

[2)]AIN-76A vitamin mixture contained (in g/kg mixture): thiamin HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7: nicotinic acid, 0.003; D-calcium pantothenate, 0.0016; folate, 0.2; D-biotin, 0.02; cyanocobalamin (vitamin B_{12}), 0.001; retinyl palmitate premix, 0.8; DL-alpha tocopheryl acetate, premix, 20; cholecalciferol (vitamin D_3), 0.0025; menaquinone (vitamin K), 0.05; antioxidant, 0.01; sucrose, finely powdered, 972.8.

Statistical analyses

The parameter values were all expressed as the mean ± SE. Statistical differences among the groups were determined by one-way ANOVA using the SPSS package program. The results were considered significant if the value

of p was less than 0.05. Duncan's multiple-range test was performed to determine any differences between the 10 or 20-day groups. The differences between the 10 and 20-day experimental groups were also compared by Student's *t* test using standard social science statistical packages.

RESULTS AND DISCUSSION

Effect on food intake, weight gain, and organ weights

The body weight and food intake were unaffected by the safflower seed extract supplementation, as shown in Table 4. The body weight initially decreased after the operation in all the rib-fractured groups, then gradually recovered. Generally, the body weight and dietary intake are lower during fracture healing after rib-fracture (25-27). However, in the current study, the food intake, weight gain, and organ weights for the safflower seed and extract groups did not differ significantly from those for the control group (Table 5).

Bone formation and resorption markers

Plasma bone-specific alkaline phosphatase, total alkaline phosphatase, and osteocalcin : The safflower seed supplemented diets did result in certain changes in the markers of bone formation (Fig. 1, Table 6). After the rib-fracture, the concentration of plasma osteocalcin, a bone formation index, was significantly higher in the control group than in the SS-powder and SS-EtOH groups among the 10-day groups, and significantly higher in the control group than in the SS-H_2O group among the 20-day groups. When comparing the length of time after the rib-

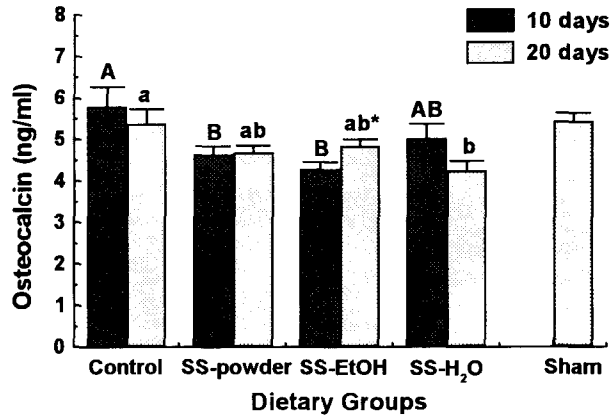

Fig. 1. Concentration of osteocalcin in plasma of rib-fractured rats supplemented with different safflower seed fractions.[1)]
[1)]Mean ± SE.
[AB]Different letters indicate significant differences ($p<0.05$) among 10-day groups.
[ab]Different letters indicate significant differences ($p<0.05$) among 20-day groups.
*Symbol indicates significant difference ($p<0.05$) between 10-day and 20-day groups.

Table 4. Food intake and weight gain in rib-fractured rats fed different safflower seed fraction diets

Duration after rib-fracture	Dietary groups	Food intakes (g/day)	Weight gain (g/day)
10 days	Control	25.71 ± 0.94[1)NS2)]	0.30 ± 0.42[NS]
	SS-powder	24.50 ± 1.23	-0.09 ± 0.40
	SS-EtOH	27.79 ± 0.88	-0.64 ± 0.37
	SS-H_2O	25.54 ± 1.15	-0.27 ± 0.24
20 days	Control	26.93 ± 0.82	1.06 ± 0.17
	SS-powder	26.89 ± 0.82	0.94 ± 0.12
	SS-EtOH	28.18 ± 0.58	1.16 ± 0.23
	SS-H_2O	26.63 ± 0.49	0.85 ± 0.50
	Sham	27.46 ± 0.46	1.34 ± 0.08

[1)]Mean ± SE.
[2)]NS: not significant.

Table 5. Liver, kidney, and heart weights for experimental rats fed different safflower seed fraction diets

Duration after rib-fracture	Dietary groups	Liver (g)	Heart (g)	Kidney (g)
10 days	Control	11.03 ± 0.60[1)NS2)]	1.16 ± 0.05[NS]	2.20 ± 0.06[NS]
	SS-powder	10.84 ± 0.34	1.19 ± 0.04	2.40 ± 0.07
	SS-EtOH	11.53 ± 0.19	1.12 ± 0.03	2.25 ± 0.06
	SS-H_2O	10.77 ± 0.23	1.21 ± 0.03	2.29 ± 0.04
20 days	Control	11.46 ± 0.37	1.19 ± 0.04	2.27 ± 0.03
	SS-powder	11.06 ± 0.27	1.15 ± 0.05	2.30 ± 0.05
	SS-EtOH	13.23 ± 0.45	1.24 ± 0.04	2.45 ± 0.09
	SS-H_2O	12.46 ± 0.63	1.23 ± 0.06	2.26 ± 0.07
	Sham	11.81 ± 0.38	1.06 ± 0.03	2.21 ± 0.06

[1)]Mean ± SE.
[2)]NS: not significant.

Table 6. Activity of bone-specific alkaline phosphatase and total alkaline phosphatase in plasma of rib-fractured rats supplemented with different safflower seed fraction diets

Duration after rib-fracture	Dietary groups	Bone specific alkaline phosphatase (U/L)	Total alkaline phosphatase (K-A)
10 days	Control	0.51±0.06[1)]	35.18±2.85[NS2)]
	SS-powder	0.68±0.08	31.75±3.83
	SS-EtOH	0.50±0.05	39.25±2.56
	SS-H_2O	0.64±0.14	33.63±1.97
20 days	Control	0.55±0.05[a3)]	36.14±2.69
	SS-powder	0.54±0.06[a]	33.28±2.16
	SS-EtOH	0.95±0.20[b]	36.96±1.91
	SS-H_2O	0.73±0.08[ab]	34.08±3.52
	Sham	0.54±0.08[a]	42.39±7.22

[1)]Mean±SE.
[2)]NS: not significant.
[3)]Different letters indicate significant differences ($p<0.05$) among 10-day or 20-day groups.

fracture, the osteocalcin concentration in the SS-EtOH group only increased significantly after 20 days rather than after 10 days.

Bone-specific alkaline phosphatase is a tetrameric glycoprotein found on the cell surface of osteoblasts (28) that are responsible for new bone formation. The detailed function of bone-specific ALP is still not clearly understood, although it would appear to play a role in skeletal mineralization (28-30). The total-ALP activity in the plasma did not differ among the dietary groups or 10 and 20-day groups. The bone-specific ALP activity, which is apparently related to the calcification of bone, exhibited no difference 10 days after the rib-fracture, yet became significantly higher in the SS-EtOH group and slightly higher in the SS-H_2O group compared to the other groups 20 days after the rib-fracture. This result was also consistent with preliminary experiments (16).

Urinary deoxypyridinoline (DPD) : The level of urinary DPD, a bone-resorption index, was significantly higher in the SS-EtOH and SS-H_2O groups than in the other groups 10 days after the rib-fracture. In addition, the urinary DPD was significantly increased in the control and SS-powder groups 20 days after the rib-fracture compared to 10 days after, yet no different to the other groups 20 days after the rib-fracture (Table 7). DPD, an amino acid derivative found primarily in mature type I collagen in bone, forms crosslinks between adjacent collagen molecules and provides tensile strength to the collagen matrix in bone (5). During bone resorption, DPD is released from the matrix and eventually excreted into urine. Therefore, since DPD is released during the breakdown of mature bone, a variation in the amount of excreted DPD reflects a quantitative change in the rate of bone resorption. Thus, measuring the urinary DPD concentration, which is not influenced by diet or physical exercise, reflects the true bone turnover rate (31). In the current study, supplementation with the safflower seed ethanol extract and safflower seed water extract seemed to initiate bone resorption earlier than in the SS-powder group or control group, as revealed by the DPD values 10 and 20 days after the rib-fracture.

Table 7. Levels of urinary deoxypyridinolin in rib-fractured supplemented with different safflower seed fractions

Duration after rib-fracture	Dietary groups	Deoxypyridinoline (nM/mM creatine)
10 days	Control	876.59+41.79[1)a2)]
	SS-powder	764.46±21.34[a]
	SS-EtOH	2855.80±158.75[b]
	SS-H_2O	2917.67±51.51[b]
20 days	Control	2475.50±190.04***[3)]
	SS-powder	2553.57±155.28***
	SS-EtOH	2848.29±76.87
	SS-H_2O	2644.80±159.98
	Sham	2833.50±124.79

[1)]Mean±SE.
[2)]Different letters indicate significant differences ($p<0.05$) among 10-day or 20-day groups.
[3)]Symbol indicates significant difference ($p<0.001$) between 10-day and 20-day groups.

Plasma levels of hormone, calcium, phosphorus and protein

Parathyroid hormone and calcitonin : When comparing the PTH and calcitonin levels that regulate the concentration of plasma calcium, the SS-H_2O group exhibited the highest level of PTH among the 10 and 20-day groups after the rib-fracture. The PTH levels were significantly higher in the SS-powder and SS-H_2O groups compared to the other groups 20 days after the rib-fracture (Fig. 2). However, the levels of calcitonin did not differ among the dietary groups or between the 10 and 20-day groups (Fig. 3).

PTH plays an important role in maintaining the concentration of ionized calcium within the limits needed to achieve normal metabolic functions. When the serum cal-

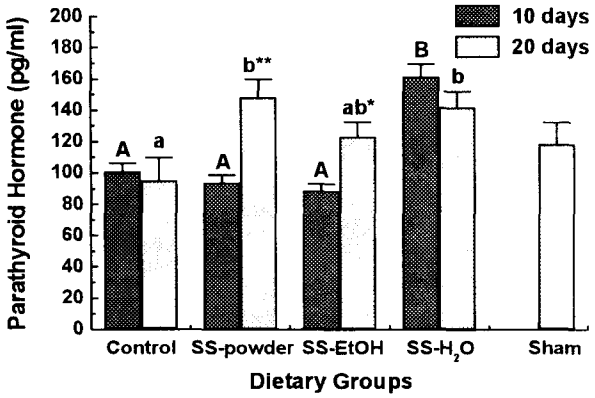

Fig. 2. Levels of plasma parathyroid hormone in rib-fractured rats supplemented with different safflower seed fractions.[1]
[1]Mean ± SE.
[a,B]Different letters indicate significant differences ($p < 0.05$) among 10-day groups.
[a,b]Different letters indicate significant differences ($p < 0.05$) among 20-day groups.
[*,**]Symbol indicate significant difference between 10-day and 20-day groups at $p < 0.05$ and $p < 0.01$, respectively.

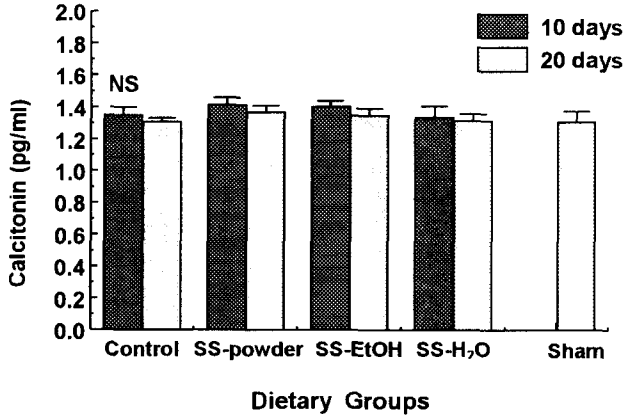

Fig. 3. Concentrations of plasma calcitonin in rib-fractured rats supplemented with different safflower seed fractions.[1]
[1]Mean ± SE.
[2]NS: not significant.

cium levels are decreased, the parathyroid gland increases the secretion of PTH, resulting in an increased mobilization of calcium from the skeletal reserves into the circulation. Conversely, when the levels of serum calcium are increased, the secretion of PTH is reduced (32). However, recent studies have shown that daily subcutaneous injections of relatively low doses of PTH stimulate bone formation in osteopenic animals and osteoporotic men and women (33-36). As indicated by PTH levels that stimulate bone resorption and the urinary DPD, a bone resorption index, bone resorption was stimulated in the SS-H_2O group 10 days after the rib-fracture and in the SS-powder, SS-EtOH, and SS-H_2O groups 20 days after the rib-fracture. As such, bone resorption was continuously active, especially in the SS-H_2O group (Fig. 2, Table 7)

Plasma protein, calcium, and phosphorus: The concentrations of plasma protein were significantly lower in the SS-EtOH group and exhibited a lower tendency in the SS-H_2O group compared to the other groups 10 days after the rib-fracture. The plasma protein concentration also tended to be lower in the safflower seed supplemented groups than in the control group 20 days after the rib-fracture (Table 8). Pyridinium crosslinks are not metabolized or absorbed from the diet, thus approximately 60% of the crosslinks released during resorption are bound to protein, while the remaining 40% are free (not protein bound) (37). As such, the lower concentration of plasma protein in the SS-EtOH and SS-H_2O groups 10 days after the rib-fracture may have been due to the increased urinary excretion of DPD mediated by the supplementation of the safflower seed extracts (Table 7).

When comparing the plasma calcium and phosphorus levels, the concentration of calcium was no different 10 days after the rib-fracture, yet significantly higher in the SS-EtOH group compared to the other groups 20 days after the rib-fracture (Table 8). This result was consistent with

Table 8. Concentration of plasma protein, calcium, and phosphorus in rib-fractured rats supplemented with different safflower seed fraction diets

Duration after rib-fracture	Dietary groups	Protein (mg/mL)	Calcium (mg/dL)	Phosphorus (mg/dL)	Ca/P ratio
10 days	Control	46.13 ± 3.38[1)a(c)2)]	9.97 ± 0.25[1)a2)]	7.06 ± 0.46[a]	1.44 ± 0.08[a]
	SS-powder	47.39 ± 2.73[a]	10.18 ± 0.15[a]	9.41 ± 1.44[ab]	1.29 ± 0.04[a]
	SS-EtOH	29.44 ± 3.08[b]	10.38 ± 0.31[a]	11.68 ± 1.08[b]	0.92 ± 0.09[b]
	SS-H_2O	37.52 ± 2.88[bc]	10.03 ± 0.25[a]	9.91 ± 1.06[ab]	1.08 ± 0.13[bc]
20 days	Control	41.53 ± 2.54[a]	9.80 ± 0.15[a]	6.43 ± 0.21[a]	1.53 ± 0.05[a]
	SS-powder	38.47 ± 4.57[a]	10.24 ± 0.29[ab]	8.58 ± 0.58[b]	1.22 ± 0.08[b]
	SS-EtOH	32.54 ± 1.16[a]	10.73 ± 0.44[b]	8.05 ± 0.48[b*3)]	1.37 ± 0.11[ab**]
	SS-H_2O	35.03 ± 3.52[a]	9.90 ± 0.09[ab]	7.78 ± 0.33[b]	1.29 ± 0.06[b]
	Sham	46.83 ± 2.48	10.67 ± 0.66	8.80 ± 0.35	1.22 ± 0.08

[1)]Mean ± SE.
[2)]Different letters indicate significant differences ($p < 0.05$) among 10-day or 20-day groups.
[3)]*,**Symbols indicate significant difference between 10-day and 20-day groups at $p < 0.05$ and $p < 0.01$, respectively.

the levels of PTH that increase the serum calcium level. The concentration of phosphorus was significantly higher in the SS-EtOH group than in the control group 10 days after the rib-fracture, significantly higher in the safflower-supplemented groups than in the control group 20 days after the rib-fracture, and significantly lower in the SS-EtOH group 20 days after the rib-fracture, rather than 10 days after. The Ca/P ratio was significantly lower in the SS-EtOH and SS-H_2O groups than in the other groups 10 days after the rib-fracture. However, this ratio became significantly lower in the SS-powder and SS-H_2O groups than in the control group 20 days after the rib-fracture. In the SS-EtOH group, the Ca/P ratio was significantly lower 10 days after the rib-fracture, rather than 20 days after. Generally, bone resorption is lowered when the plasma calcium level is high, whereas bone resorption and bone formation are both stimulated when the plasma phosphate level is higher than the normal concentration (38). As such, the current results were consistent with preliminary experiments (16).

In conclusion, the supplementation of safflower seed powder and its extracts was found to accelerate bone turnover during recovery from bone fracture. The SS-H_2O extract, identified as the most effective extract, seemed to stimulate bone remodeling, as it increased both bone resorption and bone formation biomarkers during the healing period in rib-fractured rats. However, further investigation is needed to determine whether safflower seeds have potential clinical applications for bone fractures.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of the Woori Safflower Seed Farmers Association in 1998.

REFERENCES

1. Watts NB. 1999. Clinical utility of biochemical markers of bone remodeling. *Clin Chem* 45 (8B): 1359-1368.
2. Heaney RP, Gailagher JC, Johnston CC, Neer R, Parfitt AM, Bchir MB, Whedon GD. 1982. Calcium nutrition and bone health in the elderly. *Am J Clin Nutr* 36: 986-1013.
3. Raise LG. 1988. Local and systemic factors in the pathogenesis of osteoporosis. *N Engl Med J Med* 318: 818-828.
4. Christenson RH. 1997. Biochemical markers of bone metabolism: an overview. *Clinical Biochemistry* 30: 573-593.
5. Seibel MJ, Robins SP, Bilezikian JP. 1992. Urinary pyridinium crosslinks of collagen. *Trends Endocrinol Metab* 3: 263-270.
6. Shi XC. 1983. *Chinese-English terminology of traditional Chinese medicine*. Hunan Science Publishing Corporation, Peoples Republic of China. p 71-98.
7. Kim NM, Kim KH. 1992. Researchs for analgesic and hepatoprotective action of Carthamin. *Pusan Bull Pharmaceut Sci* 26: 32-35.
8. Noh WS, Park JS. 1992. Lipid composition of Korean safflower seeds. *J Korean Agric Chem Soc* 35: 110-115.
9. Kim SK, Cha JY, Jeong SJ, Chung CH, Choi YR, Cho YS. 2000. Properties of the chemical composition of safflower (*Carthamus tinctorius L.*) sprout. *Korean J Life Sci* 10: 68-73.
10. Huber R, Romisch J, Paques EP. 1990. The crystal and molecular structure of human annexin V, an anticoagulant protein that binds to calcium and membranes. *EMBO J* 9: 3867-3874.
11. Romisch J, Heimburger N. 1990. Characterization of placental protein 4. *Biological Chemistry Hopper-Seyler* 5: 383-388.
12. Hong HT, Kim HJ, Lee TK, Kim DW, Kim HM, Choo YK, Park YG, Lee YC, Kim CH. 2002. Inhibitory effect of a Korean traditional medicine, Honghwain-Jahage (water extracts of *Carthamus tinctorius* L. seed and *Hominis placenta*) on interleukin-1-mediated bone resorption. *J Ethnopharma* 79: 143-148.
13. Kim HJ, Bae YC, Park RW, Choi SW, Cho SH, Choi YS, Lee WJ. 2002. Bone-protecting effect of safflower seeds in ovariectomized rats. *Calcif Tissue Int* 71: 88-94.
14. Huang KC. 1993. *The pharmacology of Chinese herbs*. CRC Press, Boca Raton. p 249-250.
15. Zhang HL, Nagatsu A, Sakakibara J. 1996. Novel antioxidants from safflower (*Carthamus tinctorius* L.) oil cake. *Chem Pharma Bull* 44: 874-876.
16. Jeon SM, Kim JH, Lee HJ, Lee IK, Moon KD, Choi MS. 1998. The effects of Korean safflower (*Carthamus tinctorious* L.) seed powder supplementation diet on bone metabolism indices in rats during the recovery of rib fracture. *Korean J Nutr* 31: 1049-1056.
17. Kim JH, Jeon SM, An MY, Ku SK, Lee JH, Choi MS, Moon KD. 1998. Effect of diet of Korean safflower (*Carthamus tinctorius* L.) seed powder on bone tissue in rats during the recovery of rib fracture. *J Korean Soc Food Sci Nutr* 28: 625-631.
18. AOAC. 1995. *Official Methods of Analysis*. 16th ed. Association of Official Analytical Chemists, method number 931.02, Washington, DC, USA. p 22-24.
19. Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybic-phosphotungstic acid reagents. *Am J Enol Vitic* 16: 144-158.
20. Metcalf LD, Schmitz AA, Pelka JR. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal Chem* 38: 514-515.
21. American Institute of Nutrition. 1977. Report of the American Institute of Nutrition. Ad Hoc Committee on standards for nutritional studies. *J Nutr* 107: 1340-1348.
22. American Institute of Nutrition. 1980. Report of Ad Hoc Committee on standards for nutritional studies. *J Nutr* 110: 1717-1726.
23. Minisola S, Rosso R, Romagnoli E, DErasmo E, Manfredi G, Damian C. 1997. Serum osteocalcin and bone mineral density at various skeletal sites: a study performed with three different assays. *J Lab Clin Med* 129: 422-429.
24. Bradford MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
25. Sarmiento A, Schaeffer JF, Beckerman L, Latta LL, Enis JE. 1977. Fracture healing in rat femora as affected by functional weightbearing. *J Bone Joint Surg* 59: 369-375.
26. Einhorn TA, Bonnarens F, Burstein AH. 1986. The contributions of dietary protein and mineral to the healing of experimental fracture. *J Bone Joint Surg* 68: 1389-1395.

27. Geinoz G, Rapin CH, Rizzoli R, Kraemer R, Buchs B, Slosman D, Michel JP, Bonjour JP. 1993. Relationship between bone mineral density and dietary intakes in the elderly. *Osteoporos Int* 3: 242-248.
28. Price CP. 1993. Multiple forms of human serum alkaline phosphatase: detection and quantification. *Ann Clin Biochem* 30: 355-372.
29. Epstein E, Kiechle FL, Artiss JD, Zak B. 1986. The clinical use of alkaline phosphatase enzymes. *Clin Lab Med* 6: 491-505.
30. Price CP, Thompson PW. 1995. The role of biochemical tests in the screening and monitoring of osteoporosis. *Ann Clin Biochem* 32: 244-260.
31. Robin SP, Woitge H, Hesley D. 1994. Direct, enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption. *J Bone Mineral Res* 9: 1643-1649.
32. Finch JL, Rapp N, Marin KJ, Slatopolsky E. 1992. A new sensitive homologous radioimmunoassay for amino-terminal parathyroid hormone in the rat. *J Bone Mineral Res* 7: 229-233.
33. Lindsay R, Nieves J, Formica C, Henneman E, Woelfert L, Shen V, Dempster D, Cosman F. 1997. Randomised controlled study of the effect of parathyroid hormone on verterbral-bone mass and fracture incidence among postmenopausal women on esterogen with osteoporosis. *Lancet* 350: 550-555.
34. Reeve J, Bradbeer JN, Arlot M, Davies UM, Green JR, Hampton L, Edouard C, Hesp R, Hulme P, Ashby JP. 1991. Hpth 1-34 treatment of osteoporosis with added hormone replacement therapy: biochemical, kinetic, and histological responses. *Osteoporosis Int* 1: 162-170.
35. Neer R, Slovik DM, Daly N, Potts JT Jr, Nussbaum SR. 1993. Treatment of postmenopausal osteoporosis with daily parathyroid hormone plus calcitriol. *Osteoporosis Int* 1 (Suppl): 204-205.
36. Slovik DM, Rosenthal DI, Doppelt SH, Protts JT Jr, Daly MA, Campbell JA, Neer RM. 1986. Restoration of spinal bone in osteoporotic men by treatment with human parathyroid hormone (1-34) and 1,25 dihydroxy vitamin D. *J Bone Miner Res* 1: 377-381.
37. Colwell A, Russell RGG, Eastell R. 1993. Factors affecting the assay of urinary 3-hydroxy pyridinium crosslinks of collagen as markers of bone resorption. *Eur J Clin Investig* 23: 341-9.
38. Watson RC, Grossman H, Meyers MA. 1994. Radiologic findings in nutritional disturbances. In *Modern nutrition in health and disease.* Shil ME, Olson JA, Shike M, eds. 8th ed. Lea and Febiger, Philadelphia. p 861-908.

(Received December 18 2002; Accepted February 27, 2003)