

A Efficacy of *Ginseng radix* on the Preservation of Spinal Bone Mineral Density and Bone Inorganic Substance of Oophorectomized Rats

Boohyeong Byun[†] and Bu-il Seo

Dept. of Oriental Medicine, Daegu Hanny University, Daegu 706-060, Korea

Abstract

This study was undertaken to investigate the effects of the *Ginseng radix* in osteoporosis of the oophorectomized rats. In this experiment, the rats were oophorectomized and administered the water extracts of the *Ginseng radix*. The spinal bone mineral density, calcium, phosphorus and ash weight of the bones were measured. The spinal bone mineral density was significantly increased in the ovariectomized (OVX)-*Ginseng radix* group at 8 weeks as compared to the OVX-saline group. Furthermore, the calcium and phosphorus contents of the femoral and fibula-tibia were significantly increased in the OVX-*Ginseng radix* group as compared to the OVX-saline group. The ash weights of the femoral and fibula-tibial bones were increases in the OVX-*Ginseng Radix* group, although it was not statistically significant. On reviewing these experiments, it appears that the *Ginseng radix* possess efficacy for the prevention of osteoporosis. Further study would be of value to confirm the efficacy of the *Ginseng radix* for the treatment and/or prevention of osteoporosis in humans.

Key words: *Ginseng radix*, oophorectomized rats, osteoporosis, bone mineral density

INTRODUCTION

Osteoporosis is a chronic illness characterized by the reduction of the bone mass and porous changes of the boney matrix. It is a common disorder of bone metabolism, and has recently increasing due to increased longevity and reductions in physical exercise. Ginseng has been demonstrated to promote energy, have beneficial effects on lung and spleen, and to have a calming effect; but its effects on bone metabolism have not been investigated (1). Traditional Asian healing teaches that the promotion of Qi energy has efficacy in the prevention or reversal of osteoporosis. Since ginseng is considered a powerful Qi energy booster, we investigated the ability of ginseng to prevent osteoporosis in rats. Accordingly, we used the methodology of Salville and others (2,3). The first step was create an artificial osteoporosis in oophorectomized white rats. We then administrated ginseng extract and subsequently measured bone density and alteration in the inorganic bone constituents. The results provide convincing evidence that ginseng delays the development of osteoporosis in the rat model used, and deserves further investigation in humans.

MATERIALS AND METHODS

Materials

The ginseng used in this experiment was purchased

through the pharmacy of Hanny University (6 years harvest, product of Korea) and inspected and processed in laboaltoty of herbal medicines. Experimental animals were healthy, female Sprague-Dawley white rats fed commercial rat diet and used when the body weight reached 240 g and beyond.

Preparation of *Ginseng radix* extract

10 g of the ginseng was mixed with 100 cc of purified water, heated for 2 hours, filtered, and condensed to 60 cc of ginseng extract.

Induction of osteoporosis

Animals were divided into sham group (sham-operation) and OVX group, and subjected to different surgical procedures; either just a skin incision or ooprectomy under pentobarbital sodium (50 mg/kg/BW) anesthesia. After the surgery, all the rats were maintained on purified water.

Allocation of experimental groups

After the surgery, the rats were divided into 3 groups of 7 rats each. The sham operation group (Sham Group) consisted of the 7 rats with just a skin incision; the OVX group is female of 7 rats with skin incision and oophrectomy. The ginseng extracts group (GR Group) consist of ooprectomized rats which were given ginseng extracts one day after the surgery.

[†]Corresponding author. E-mail: bhbyun@dhu.ac.kr
Phone: +82-53-770-2245. Fax: +82-53-768-6340

Administration of drugs

The GR group was given ginseng extracts 1 cc (0.17 g/100 g BW) each day for 8 weeks. The Sham and OVX group were given the same amount of 0.9% normal saline each day for 8 weeks.

Observation and check list

Measurement of bone density: Before the surgery and at 4 weeks and 8 weeks post-surgery, bone density of the spine (BMD) was measured by a bone densitometer (PIXI MUSTM, USA).

Measurement of bone ash and inorganic constituent: Rats were sacrificed after 8 weeks and the right femur bone was surgically removed and carefully cleaned to remove all the fat and surrounding tissues. The right thigh bone was used for ash determination by adding a few drops of 6 N-HCl and heated to 600°C for 24 hours (4). After completion of ash production it was cooled around 30 minutes, weighed. The ash was further assayed by adding the 3 N-HCl for complete resolution, diluting in normal saline and assaying for calcium and inorganic phosphorus (5).

Statistical analysis: The results were analyzed by students's t-test, and $p < 0.05$ was considered statistically significant.

RESULTS

Effect on spinal bone density

The spinal bone density was shown to be 111.81 ± 2.22 mg and 118.52 ± 4.03 mg on sham group at 4 and 8 weeks. On OVX group, it was 96.52 ± 2.36 mg and 96.90 ± 2.46 mg which was a highly significant decrease ($p < 0.001$). In the *Ginseng radix* group, it was that 100.24 ± 2.23 mg and 106.32 ± 3.28 mg, which demonstrated a significant effect in reducing bone loss due to the oophorectomies at $p < 0.05$ after 8 weeks (Table 1).

Table 1. Effects of *Ginseng radix* and thin root of *Panax ginseng* on spinal bone mineral density (BMD) in ovariectomized rats

Experimental group	Spinal BMD	
	4 week % of baseline ¹⁾	8 week % of baseline ¹⁾
Sham	111.81 ± 2.22 ²⁾	118.52 ± 4.03
OVX	96.52 ± 2.36 ⁺⁺⁺	96.90 ± 2.46 ⁺⁺⁺
GR ³⁾	100.24 ± 2.23	106.32 ± 3.28 [*]

⁺Significantly different from sham group (⁺⁺⁺ $p < 0.001$).

^{*}Significantly different from ovariectomized (OVX) group (^{*} $p < 0.05$).

¹⁾Percentage of 4 or 8 weeks to that of baseline (0 week).

²⁾Mean \pm standard error of 7 rats.

³⁾GR: Water extract of *Ginseng radix*.

Effect on changes of inorganic constituents of the bone

Effect on calcium content of the bone: Calcium content of the femur bone was 173.69 ± 8.08 mg in the sham group and 110.47 ± 5.63 mg in the OVX group, which was a significant decrease ($p < 0.001$). In contrast, *Ginseng radix* group has shown to be 196.56 ± 5.56 mg, exhibiting a significant increase ($p < 0.001$). The calcium content of the tibia and fibula bones was 150.21 ± 10.93 mg in the sham group, however, in the *Ginseng radix* group it was 179.20 ± 4.44 mg, which was a significant increase ($p < 0.001$) (Table 2).

Effect on phosphorous content of the bone: The phosphorus content of the femur bone was 51.21 ± 1.41 mg in the sham group and 42.64 ± 1.74 mg in the OVX group, which demonstrated a significant decrease at $p < 0.001$. In comparison, it was 53.04 ± 1.50 mg in the *Ginseng radix* group, which was significantly higher than in the OVX-saline group ($p < 0.001$). The phosphorus content of the tibia and fibula bones was 43.39 ± 2.61 mg on sham group and 37.29 ± 0.88 mg in the OVX group, which was significantly lower ($p < 0.05$). In contrast, the *Ginseng radix* group was 47.04 ± 1.58 mg, which was significantly higher ($p < 0.001$), as compared to the OVX group.

Effect on weight of the bone ash: The ash content of the femur bone was 0.4035 ± 0.0088 g on sham group and 0.39 ± 0.01 g on OVX group, showing a trend to decrease. In contrast, it was 0.41 ± 0.02 g in the *Ginseng radix* group, which suggested a tendency to increase but was not statistically significant. The ash weight of the fibula and tibia bones was 0.33 ± 0.01 g in the sham group and 0.33 ± 0.01 g in the OVX group, In comparison, it was 0.34 ± 0.01 g on *Ginseng radix* group, which suggested a possible tendency to increase, but was not statistically significant (Table 4).

DISCUSSION

The ginseng extract was made from thin root of *Panax ginseng* C. Meyer after removal of the branches. Ginseng

Table 2. Effects of *Ginseng radix* on bone calcium levels in ovariectomized rats

Experimental group	Bone calcium (mg)	
	Femur	Tibia and Fibula
Sham	173.68 ± 8.08 ¹⁾	150.21 ± 10.93
OVX	110.47 ± 5.63 ⁺⁺⁺	94.10 ± 5.65 ⁺⁺⁺
GR ²⁾	196.56 ± 5.56 ^{***}	179.20 ± 4.44 ^{***}

⁺Significantly different from sham group (⁺⁺⁺ $p < 0.001$).

^{*}Significantly different from ovariectomized (OVX) group (^{***} $p < 0.001$).

¹⁾Mean \pm standard error of 7 rats.

²⁾GR: Water extract of *Ginseng radix*.

has a broad range of effects on humans with demonstrated effectiveness for increasing energy, as a long spleen supplement, and for calming the spirit. It has demonstrated therapeutic efficacy for Qi deficiency disorders including: inflammation, poor appetite, lassitude, nausea, diarrhea, cough, sweating, fear, forgetfulness, dizziness, impotence, thirst fever, incontinence, child tremor. All types of Qi-blood deficiency osteoporosis is a degenerative disease characterized by reductions in the bony matrix and bony mass, and is one of the more common disease of bone metabolism (6,7). Osteoporosis is not only common in post-menopausal women but also among senile aging men, since longevity is increasing. According to the oriental medicine principles, osteoporosis is defined as a kidney deficiency. Therefore, it believed to be an imbalance in kidney Yin and Yang, and treatment should emphasize correction of the spleen and kidney Yang deficiency, Qi-blood enhancement, Qi-blood supplement etc (8,9). However, reviews of previous osteoporosis research revealed that there has been no previous on studies using spleen-Qi supplements, therefore we attempt to promote the spleen Qi with ginseng since the ginseng is known to be a specific herb for this purpose. Following removal of ovaries, the loss of the bony constituent is primarily due to the reduction of estrogen, resulting in decreased bone formation, increased bone absorption, and alteration of bone constituents (10). In this experiment we observed a reduction in bone mass throughout the body at 4 and 8 weeks, which was similar to the results reported by Ammann et al. (11). The percent change in spinal bone density of the GR group at 4 and 8 weeks were 100.24 ± 2.23 and 106.32 ± 3.28 , respectively; demonstrating a significant increase in comparison with OVX group that was statistically significant ($p < 0.05$) at 8 weeks (Table 1). The mechanism of the decreased calcium content on oophorectomized rats is considered to be the results of increased bony absorption (12). Phosphorus is a vital inorganic constituent of the bone that promotes bone formation. Approximately 90% of the phosphorus in our body is deposited in bone. As with calcium, the phosphorus contents of the bones was found to be higher in the ginseng group than in either of the other groups. The average phosphorus content in the femurs of the ginseng group was 53.04 ± 1.50 mg, which was significantly higher ($p < 0.001$) than the OVX group. Likewise, the phosphorus of the tibia and fibula bones was 47.04 ± 1.58 mg in the ginseng radius group, which was significantly higher than in the OVX group ($p < 0.001$) (Table 3). The ash weight of the greater femur was shown to be 0.405 ± 0.011 g/cm² in ginseng radius group, was slightly higher, but not statistically significant. The tibia, but not the femur, also was slightly higher, but not statistically significant (Table 4).

Table 3. Effects of *Ginseng radix* on bone phosphorus levels in ovariectomized rats

Experimental group	Bone phosphorus (mg)	
	Femur	Tibia and Fibula
Sham	51.21 ± 1.41 ¹⁾	43.39 ± 2.61
OVX	42.64 ± 1.74 ^{**}	37.29 ± 0.88 [†]
GR ²⁾	53.04 ± 1.50 ^{***}	47.04 ± 1.58 ^{***}

[†]Significantly different from sham group ($p < 0.05$, ^{**} $p < 0.01$).

^{*}Significantly different from ovariectomized (OVX) group (^{***} $p < 0.001$).

¹⁾Mean \pm standard error.

²⁾GR: Water extract of *Ginseng radix*.

Table 4. Effects of *Ginseng radix* on ash weight in ovariectomized rats

Experimental group	Ash weight (g)	
	Femur	Tibia and Fibula
Sham	0.404 ± 0.009 ¹⁾	0.330 ± 0.005
OVX	0.390 ± 0.004	0.332 ± 0.005
GR ²⁾	0.405 ± 0.011	0.337 ± 0.008

¹⁾Mean \pm standard error of 7 rats.

²⁾GR: Water extract of *Ginseng radix*.

These animal experiments showed positive effects of ginseng extract in the prevention of osteoporosis in oophorectomized rats. Therefore, further study is warranted to determine if ginseng can also provide meaningful therapeutic benefits in humans for the prevention of osteoporosis.

SUMMARY

In this osteoporosis study, we demonstrated that ginseng supplemented oophorectomized rats, retain greater bone density as well as calcium and phosphorus in comparison to the control group. Therefore, ginseng is considered to be an efficacious intervention for osteoporosis in rats, and deserves further study to confirm the efficacy of ginseng in humans.

REFERENCES

1. Rotella DP. 2002. Osteoporosis: challenges and new opportunities for therapy. *Curr Opin Drug Discov Devel* 5: 477-86.
2. Salville PD. 1969. Changes in skeletal mass and fragility with castration in the rat; A model of osteoporosis. *J Am Ger Soc* 17: 155-166.
3. Wronski TJ, Lowry PL, Walsh CC, Ignaszewski LA. 1985. Skeletal alterations on ovariectomized rats. *Calcif Tissue Int* 37: 324-328.
4. Yamazaki I, Yamaguchi H. 1989. Characteristics of an ovariectomized osteopenic rat model. *J Bone Min Res* 4: 13-23.
5. Goldberg H, Fernandez A. 1966. Simplified method for estimation of inorganic phosphorus in body fluid. *Clin Chem*

- 12: 871-872.
6. Riggs BL, Melton LJ. 1986. Involutional osteoporosis. *N Engl J Med* 314: 1676-1686.
 7. Oh HJ, Kim YH, Kim SU, Han YK. 1998. Bone mineral density of middle aged Korean men by QCT. *J Kor Bone Met* 5: 150-155.
 8. Shaoji H. 1992. *Present Chinese Internal Medicine*. Chinese Medicine Scientific Technique Publishing Company, Beijing. p 578-58.
 9. Jiangwei Z, Heming W. 1994. *Chinese Bone Pathology*. The Public Hygiene Publishing Company, Beijing. p 255-258.
 10. Gurkan L, Ekeland A, Gautvik KM, Langeland N, Ronningen H, Solheim LF. 1986. Bone changes after castration in rats. A model for osteoporosis. *Acta Orthop Scand* 57: 67-70.
 11. Ammann P, Rizzoli R, Slosman D, Bonjour JP. 1992. Sequential and precise *in vivo* measurement of bone mineral density in rats using dual-energy X-ray absorptiometry. *J Bone Miner Res* 7: 311-316.
 12. Parkinson I, Moore RJ, Poter S. 1990. Progressive cancellous bone loss in rats after oophorectomy. *Calcif Tissue Int* 47: 388-389.

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