Arch Pharm Res Vol 28, No 1, 106-110, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Bone Loss Preventing Effect of Sophorae Fructus On Ovariectomized Rats

Jae Geul Shim, Seung Hwan Yeom, Hyun Jung Kim, Young Wook Choi, Do Ik Lee, Kye Yong Song[1], Suk Hyung Kwon[2], and Min Won Lee

College of Pharmacy, Chung-Ang University, Korea, [1]College of Medicine, Chung-Ang University, Seoul 156-756, Korea, and [2]Rexgene Biotech Co., Ltd.

(Received October 1, 2004)

The preventive effects of Sophorae Fructus extracts (I: hot water extract and II: combination product using I) on bone loss in ovariectomized (OVX) rats were investigated. Sophorae Fructus extracts were orally administrated to OVX rats for 9 weeks. Ovariectomy caused the increase of body weight and deoxypyridinoline (Dpd: bone resorption marker) and decrease of calcium (Ca: bone formation marker) level in serum. Dpd level were significantly decreased and Ca levels were elevated at 9 weeks in Sophorae Fructus extracts administered groups after ovariectomy at a dose of 0.556 g/kg/day compared with control group. In administered groups, trabecular bone area (TBA) in the tibia and lumbar were also increased compared with control group in histomorphological analysis. The preventive or treatment effects of Sophorae Fructus extracts on bone loss in OVX rats appears to be due to suppression of bone turnover.

Key words: Sophorae fructus, Osteoporosis, Ovariectomy, Deoxypyridinoline, Calcium level, Trabecular bone area

INTRODUCTION

Osteoporosis associated with ovarian hormone deficiency after menopause is the most common cause of aged-related bone loss. A sharp decrease in ovarian estrogen production is predominant cause of the rapid hormone-related bone loss during the first decade after menopause (Gruber *et al.*, 1984). Traditional therapies for postmenopausal osteoporosis have used agents that inhibit bone resorption such as estrogen, calcitonin and bisphosphonates. Estrogen replacement therapy, recommended only for women who are at high risk of osteoporosis and who have no contraindications, seems to be the most effective method to reduce the rate of postmenopausal bone loss, but may be accompanied by side-effects (Genant *et al.*, 1989).

Phytoestrogens are naturally occurring plant-derived nonsteroidal estrogen which are present in the human diet. Their chemical structure is to that of estrogen, what enables them to bind the estrogen receptor thus acting as estrogen agonists or antagonists (Setchell, 1998; Belcher and Zsarnovszky, 2001). Interest in phytoestrogens comes from the observation that people from East Asian countries showed a lower incidence of cardiovascular diseases and osteoporosis than people from Western countries. Since Asians consume 20-50 times more soy-derived food per capita than Americans do, and soybeans are a concentrated source of phytoestrogens (particularly isoflavones), it has been postulated that phytoestrogens could be responsible for the health-promoting effect of soy consumption (Glazier and Bowman, 2001; Bhathena and Velasquez, 2002; Stark and Madar, 2002; Wang, 2002).

Sophora japonica L. (Leguminosae) has common name as Chinese scholar tree or Japanese pagoda tree (Kim, 1995) and is distributed throughout Korea, China and Japan. The flower and flower bud are used for the treatment of hematuria, hemorrhinia, conjunctivitis, hemorrhoid, leukorrhea, uterine or intestinal hemorrhage, pyodermas, metrorrhagia, arteriosclerosis and hypertension (Han *et al.*, 1996). The fruits of this plant also used for hemorrhoid, dysentery, hematochezia, headache, dizziness and congestion of the eyes due to heat in the liver and the roots are used for the treatment of pruitus, hemorrhoid and ascaridiasis (Han *et al.*, 1996).

Rutin, sophoraflavolonoside, kaempferol-3-rhamnodiglucoside, isorhamnetin-3-rutinoside, genistein-7-D-cellobioside,

Correspondence to: Min Won Lee, College of Pharmacy, Chung Ang University, Seoul 156-756, Korea
Tel: 82-2-820-5602, Fax: 82-2-822-9778
E-mail: mwlee@cau.ac.kr

sophoricoside, sophorabioside, betulin and sophoradiol were isolated from the flower bud of this plant (Kimura and Yamada, 1984; Kariyone *et al.*, 1956; Takahashi *et al.*, 1960). Genistein, sophoricoside, sophorabioside, sophoraflavolonoside, isorhamnetion-3-rutinoside and isorhamnetin-3-rutinoside were isolated from the fruit of this plant (Balbaa *et al.*, 1974; Akhmedkhodzhaeva *et al.*, 1986).

In the present study, we examined Sophorae Fructus which was known to rich source of isoflavone (Kim *et al.*, 2004) on bone loss preventive or treatment effects against OVX rats.

MATERIALS AND METHODS

Samples

The extracts of Sophorae Fructus I (hot water extract; lot # 0301S2) and II (composed of 47% of I and supplemented with shark cartilage and calcium; Isocal) were prepared and supplied by Rexgene Biotech Co. Ltd., and soybean isoflavone (composed of 33% of total isoflavonoid; SDB) was used for comparative control. All samples dissolved in distilled water.

Animal

Adult sexually native female Sprague-Dawley (SD) rats, weight 230-250 g, aged 12 weeks, were maintained in cages on a 12 h light: 12 h dark regimen at a room temperature of 25 °C for 9 weeks. Water and food were available *ad libitum*. The rats were divided into 7 groups of 7 animals after adaptation time for 1 week. Animals in group 1 was a normal group, those in group 2 was subjected to sham operation, and those in group 3 (control group), group 4 (17β-Estradiol injected group), 5 (Iextract of Sophorae Fructus I administered group), 6 (Iextract of Sophorae Fructus II administered group) and 7 (SDB administered group) underwent bilateral ovariectomy under ether anesthesia (Table I). In the sham-operated animals, both ovaries were handled, but not removed. All animals were had convalescence for 1 week. From 14 weeks of age, after blood collection at angular vein, all animals were allowed free access to a commercial diet (pellet) and to water for 9 weeks. Throughout this period, the rats in groups 4 were given intraperitoneal injection (0.1 17β-estradiol/ body weight per day) and those in groups 5, 6 and 7 received orally samples I, II and SDB (0.556 g/kg body weight per day). Sham-operated and control rats received the vehicle solution orally. On the day after the last dose, the rats were bled from the abdominal vein after cardiac puncture under light anesthesia with ether. Blood were used to determine deoxypyridinoline (Dpd) and Calcium (Ca). Also, the tibia and lumbar were removed immediately after bleeding for histopathological analysis.

Table I. Dietary formula, dose and administration method in each group

Group	Content	Administration (amount and method)
1	-	-
2	sham-operation	edible water
3	ovariectomy	edible water
4	ovariectomy	1 μg/kg/day (i.p.)
5	ovariectomy	0.556 g/kg/day (p.o.)
6	ovariectomy	0.556 g/kg/day (p.o.)
7	ovariectomy	0.556 g/kg/day (p.o.)

Analytical procedure

Serum Deoxypyridinoline (Dpd) and Calcium (Ca)

Blood samples were centrifuged 30min after collection. The serum was separated and analyzed immediately. Serum Dpd was measured spectrophotometrically at 405 nm, using commercial kit (Pyrilinks-D, Quidel Corporation, USA). Also, serum Ca was measured by OCPC method (Riley, J. P., 1959).

Trabecular bone area

In the experiment, tibia and lumbar were removed to measure trabecular bone area (TBA). Sections of decalcified tibia and lumbar fixed in 10% neutral buffered formalin and embedded in paraffin wax were taken using a microtome and stained with haematoxyline and eosin. The sections were mounted and observed for histopathological changes.

Statistical analysis

Measurements are expressed as means ± S.E.M. Comparison between two groups was performed using Student's *t*-test. A *P* value less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Body weight

At the first stage of the study, the OVX groups gained weight drastically than the normal and sham-operated group after the operation. But group 4, 5, 6, and 7 had tempered to increase body weight by administration each samples for 9 weeks. Final weight of these groups appeared equal to normal group at the last of the study (Fig. 1 and Table II).

Serum Deoxypyridinoline (Dpd) and Calcium (Ca) level

Deoxypyridinoline (Dpd) make cross-link in bone matrix and stabilize the Type I collagen chain (Seyedin and

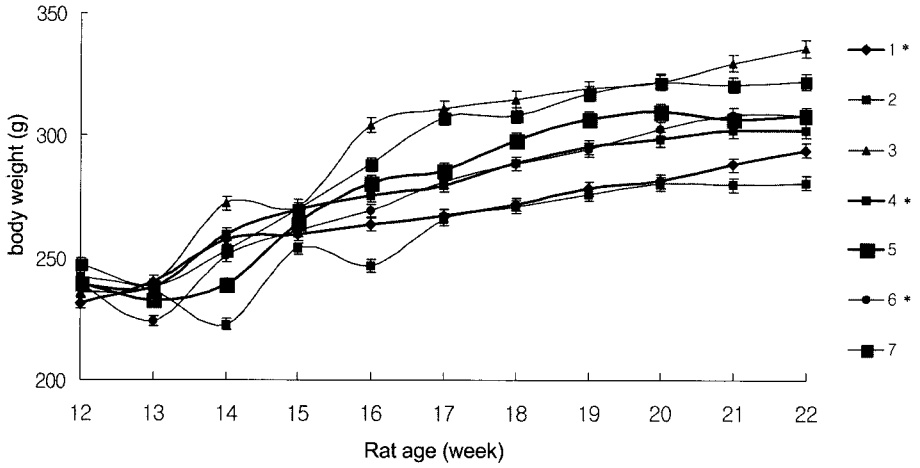

Fig. 1. The changes in body weight in each group during 10 weeks. *; P<0.05

Table II. The growth rate in each group

Group	Weight(g)		Growth rate (g/day)
	Start	Final	
1	231.4 ± 12.303	294.2 ± 21.869	0.9968 ± 0.1*
2	242.1 ± 17.520	280.8 ± 14.336	0.6142 ± 0.2
3	236.0 ± 8.5147	335.6 ± 34.112	1.5809 ±0.2
4	238.8 ± 16.742	302.4 ± 29.885	1.0095 ± 0.1*
5	241.2 ± 7.159	306.9 ± 11.662	1.0428 ± 0.1*
6	243.2 ± 12.903	307.2 ± 25.772	1.0158 ± 0.1*
7	245.6 ± 9.341	322.1 ± 19.548	1.2142 ± 0.2

(*; P<0.05)

Rosen, 1990; Garnero and Delmas, 1996). Serum Dpd is excreted in urine when started the hydrolysis of bone matrix by osteoclast (Eastell *et al.*, 1997). Inhibition of Dpd level means the possibility of prevention or treatment activity of osteoporosis (Riggs, 1991; Hesley *et al.*, 1998).

In the experiment, the bone resorption marker-serum Dpd levels were increased in control group (group 3). Sample groups (group 5 and 6) showed 77.4% and 93.5% reduction in Dpd levels at the end of 9 weeks following ovariectomy compared with control group. Comparative control group (group 7) also showed a 50% reduction in Dpd levels at the last of the study (Fig. 2).

Most of the calcium in the human body is located in the skeleton as hydroxyapatite crystals. Intracellular calcium homeostasis must be maintained within very narrow margins. Prolonged calcium deficiency stimulates the release of parathyroid hormone, which increases bone resorption, thereby releasing calcium into the plasma (Van Staveren *et al.*, 1999).

In the experiments, the bone formation marker-Ca levels were significantly decreased at 9 weeks after ovariectomy in control group. E2 and samples administered

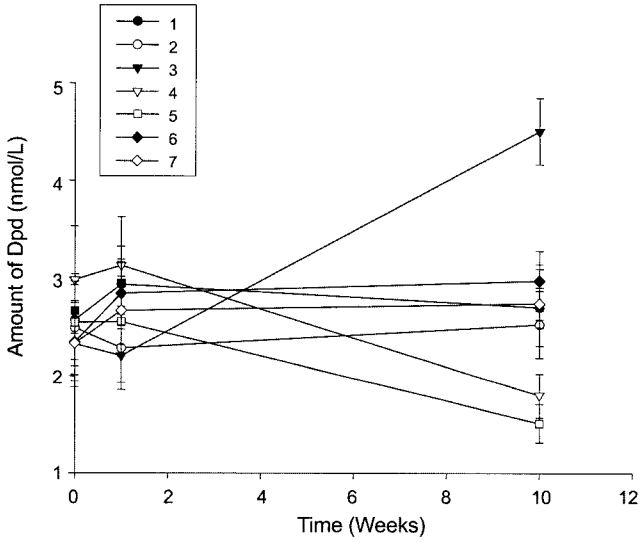

Fig. 2. The changed amount of Dpd in each group during 10 weeks.

groups (group 4, 5, 6, and 7) showed an increase (186.1, 169.1, 145.4, and 116.4%) respectively of calcium level compared with the control group (Fig. 3).

Trabecular bone area

Trabecular bone area is an indicator of many changes in bone formation and resorption. Recent reports indicate that the loss of the trabecular bone mass is more marked than the loss of cortical bone mass (Omi and Ezawa, 1995).

In the experiment, ovariectomy resulted in significantly decreased trabecular bone area (TBA) of tibia and lumbar in control group. TBA in the tibia group 2 (413%), 4 (209%), 5 (210%), 6 (278%), and 7 (203%) increased compared with the control group ($p < 0.05$). Also, TBA in the lumbar of the group 2 (135%), 4 (124%), 5 (122%), 6 (133%), and 7 (110.8%) increased compared with the control group ($p < 0.05$).

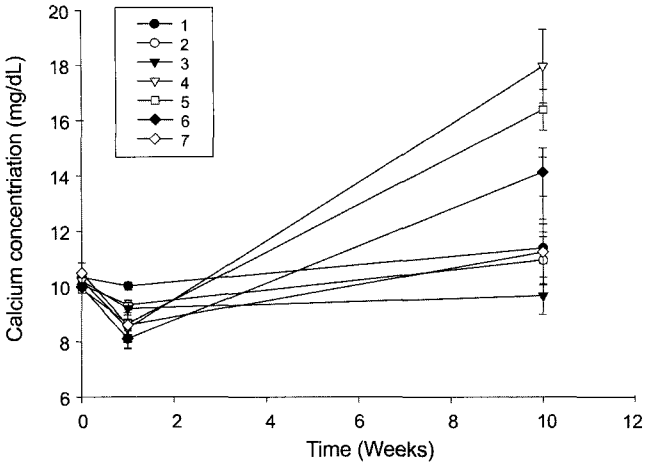

Fig. 3. Changes of Calcium concentration in each group during 10 weeks.

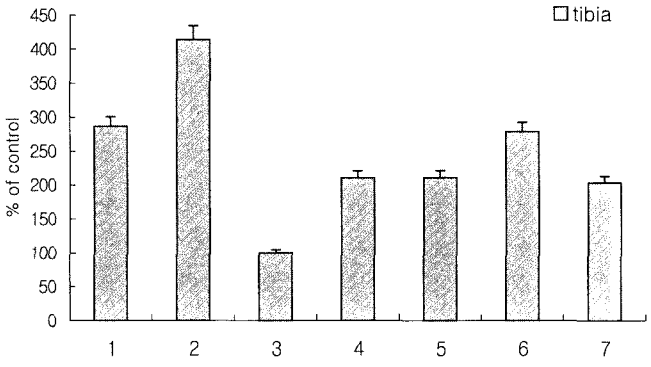

Fig. 4. Trabecular bone area in the tibia in each group after 10 weeks. *; P<0.05.

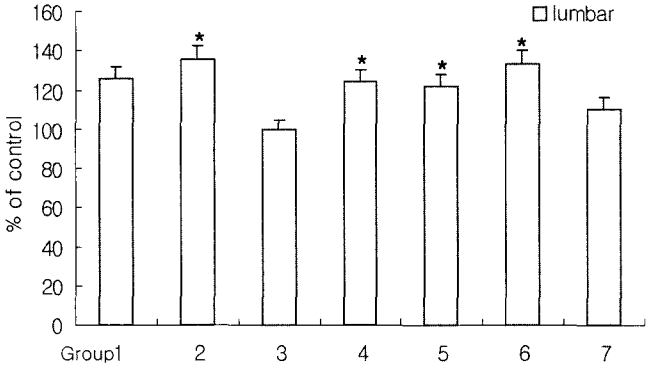

Fig. 5. Trabecular bone area in the lumbar in each group after 10 weeks. *; P<0.05.

CONCLUSION

Administrations of Sophorae Fructus extracts significantly inhibited declines in serum Ca and TBA, and elevation of Dpd level in OVX rats. These results indicated that the Sophorae Fructus extracts is effective for bone loss in OVX rats and also suggested that the Sophorae Fructus may be developed as potential agent in preventing and treatment of osteoporosis.

ACKNOWLEDGMENT

This study was supported by Rexgene Biotech Co., Ltd. in 2003.

REFERENCES

Akhmedkhodzhaeva, N. M., Svechnikova, A. N., Bandyukova, V. A., and Kambarova, D. M., Determination of flavones in Sophora japonica fruits. *Farmatsiya*, 35, 60-61 (1986).

Balbaa, S. I., Zaki, A., Y., and Eln Shamy, A. M., Qualitative and quantitative study of the flavonoid content of the different organs of Sophora japonica at different stages of development. *Planta Medica*, 25, 325-330 (1974).

Belcher, S. M. and Zasrnovszky, A., Estogenic actions in the brain: estrogen, phytoestrogen, and rapid intracellular mechanisms. *J. Pharmacol. Exp. Ther.*, 299, 408-414 (2001).

Bhathena, S. M. and Velasquez, M. T., Beneficial role of dietary phytoestrogens in obesity and diabetes. *AM. J. Clin. Nutr.*, 76, 1191-1201 (2002).

Eastell, R., Colwell, A., Hampton, L., and Reeve, J., Biochemical markers of bone resorption compared with estimates of bone resorption from radiotracer kinetic studies in osteoporosis. *J. Bone Miner. Res.*, 12, 59-65 (1997).

Garnero, P. and Delmas, P. D., New developments in biochemical markers for osteoporosis. *Calcified tissue international*, 59, S2-9, (1996).

Genant, H. K., Bay link, D. J., and Gallagher, J. C., Estrogens in the prevention of osteoporosis in post menopausal women. *American Journal of Obsteterics and Gynaecology*, 161, 1842-1846 (1998).

Glazier, M. G. and Bowman, M. A., A review of the evidence for the use of phytoestrogens as a replacement for traditional estrogen replacement therapy. *Arch. Intern. Med.*, 161, 1161-1172 (2001).

Gruber, H. E., Ivey, J. L., Baylink, D. L., Mathews, M., Nelp, W. B., Sison, K., and Chestnut, C. H., Lomg-term calcitonin therapy in postmenopaual osteoporosis. *Metabolism*, 33, 295-303 (1984).

Han, B. H., Takeatsu, K., Paul, P. H. But, Guo, J.-X., and Sung, C. K., International Collation of Traditional and Folk Medicine: Northeast Asia-Part I, World Scientific Publishing Co., Singapore, p.p. 68-69 (1996).

Hesley, R. P., Shepard, K. A., Henkins, D. K., and Riggs, B. L., Monitoring estrogen replacement therapy and identifying rapid bone losers with an immunoassay for deoxypyridinoline. *Osteoporosis int.*, 8, 159-164 (1998).

Kariyone, T., Ishimasa, S., and Shiomi, T., Triterpenoids. VIII. Triterpenoids contained in Sophora japonica. *Yakugaku Zasshi.*, 76, 1210-1211 (1956).

Kim, H. J., Kim, M. G., Shim, J. G., Yeom, S. H., and Lee, M. W., Quantitative Determination of Isoflavonoids and Flavonoids from Sophorae Fructus. *Chung-Ang Journal of Pharmacal Sciences*, 18, 37-43 (2004).

Kim, T. W., The Woody Plants of Korea in Color, Kyo-Hak Publishing Co., Seoul, Korea, p. 399 (1995).

Kimura, M. and Yamada, H., Interaction in the antibacterial activity of flavonoids from Sophora japonica L. to Propionibacterium. *Yakugaku zasshi.*, 104, 340-346 (1984).

Omi, N. and Ezawa, I., The effect of ovariectomy on bone metabolism in rats. *Bone*, 17, 163S-168S (1995).

Riggs, B. L., Overview of osteoporosis. *West J. Med.*, 154, 63-77 (1991).

Riley, J. P., The use of continuous extraction for the removal of interfering elements in the determination of calcium and magnesium. *Analytica Chimica Acta*, 21, 317-323 (1959).

Setchell, K. D. R., Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *AM. J. Clin. Nutr.*, 68, 1333S-1346S (1998).

Seyedin, S. M. and Rosen, D. M., Matrix Proteins of the Skeleton. *Curr. Opin. Cell Biol.*, 2, 914-919 (1990).

Stark, A. and Madar, Z., Phytoestrogens: a review of recent findings. *J. Pediatr. Endocrinol. Metab.*, 15, 561-572 (2002).

Takahashi, M., Ishimasa, S., and Koyama, Y., Components of Sophora japonica. IV. Optical rotary dispersion curve of the derivative of sophoradiol. *Yakugaku Zasshi.*, 80, 698-699 (1960).

Van Staveren, W. A., de Groot, L. C., Blanco, Y. H., and Van der Wilen, R. P., Assessing diets of elderly people: problems and approaches. *Am. J. Clin. Nutr.*, 59, 2215-2235 (1999).

Wang, L.-Q., Mammalian phytoestrogens: enterodiol and enterolactone. *J. Chromatoar.*, B 777, 289-309 (2002).