Preventive Effects of Bee Pollen *Cistus ladaniferus* Extract on Bone Loss in Ovariectomized Rats *In Vivo*

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The effect of bee pollen *Cistus ladaniferus* extract on ovariectomy (OVX)-induced bone loss *in vivo* was investigated. The water-solubilized extracts were obtained from the bee pollen of *Cistus ladaniferus*. Cistus extract (5.0 or 10.0 mg/100 g body weight) was orally administered once daily for 30 days to OVX rats. The analysis using a peripheral quantitative computed tomography (pQCT) showed that OVX-induced a significant decrease in mineral content, mineral density, and polar strength strain index in the femoral-metaphyseal tissues. These decreases were significantly prevented after the administration of cistus extract (10.0 mg/100 g). Moreover, OVX-induced a significant decrease in calcium content in the femoral-diaphyseal and -metaphyseal tissues. This decrease was significantly prevented after the administration of cistus extract (5.0 or 10.0 mg/100 g). This study demonstrates that cistus extract has a preventive effect on OVX-induced bone loss *in vivo*.

Key words —— bee pollen, *Cistus ladaniferus*, bone, osteoporosis, ovariectomized rat

INTRODUCTION

Bone loss with aging induces osteoporosis, which is widely recognized as a major public health problem.[1–4] Bone loss may be due to decreased bone formation and increased bone resorption. A decrease in bone mass leads to bone fracture. Pharmacologic and nutritional supplements may prevent a decrease in bone mass with increasing age.[5–7] This may have a useful role in the prevention of osteoporosis.

Micronutrients and phytochemicals are found in food and plants. Our recent studies have shown that isoflavones,[6,8,9] vitamin K_2 (menaquinone-7),[10,11] carotenoid β-cryptoxanthin[12,13] and *p*-hydroxycinnamic acid[14,15] have stimulatory effects on osteoblastic bone formation and inhibitory effects on osteoclastic bone resorption, thereby increasing bone mass. Food chemical factors are found to have a regulatory role in the regulation of bone metabolism.

Bee pollen *Cistus ladaniferus* has been found to have anabolic effects on bone metabolism in rats *in vitro* and *in vivo*.[16] The extract of bee pollen cistus has stimulatory effects on bone formation and inhibitory effects on bone resorption *in vitro*.[16,17] The extract stimulates bone calcification as potently as propolis.[16] Royal jelly does not have an inhibitory effect on osteoclastogenesis.[18] The anabolic effect of bee pollen may thus be unique among bee-related products. The active component of bee pollen cistus extract, which stimulates bone formation and inhibits osteoclastic bone resorption, has been shown to be a fraction with molecular weight (MW) of less than 1000.[19]

The intake of bee pollen cistus extract has been shown to have preventive effects on bone loss in streptozotocin-diabetic rats, and the intake has partial restorative effects on serum biochemical findings with the diabetic state *in vivo*.[20]

This study was undertaken to determine whether the intake of bee pollen cistus extract has preventive effects on bone loss induced in ovariectomized (OVX) rats, a model of osteoporosis, *in vivo*.

MATERIALS AND METHODS

Chemicals —— All chemicals were reagent grade

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from Sigma Chemical (St. Louis, MO, U.S.A.) and Wako Pure Chemical Industries (Osaka, Japan). All water used was glass-distilled.

Bee Pollen Extracts —— Bee pollen was obtained from *Cistus ladaniferus*. The powder of bee pollen (5 g) was suspended in distilled water (20 ml) and mixed vigorously, and the suspension was centrifuged at 10000 *g* in a refrigerated centrifuge for 20 min.[16] The 10000 *g* supernatant fraction was collected and filtered, and the filtrate was freeze-dried. The powder of the water-solubilized extract was dissolved in ice-cold distilled water for use in experiments.

Animals —— Female Wister rats (8 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The six animals in each group were fed commercial laboratory chow (solid) containing 57.4% carbohydrate, 1.1% calcium, and 1.1% phosphorus at a room temperature of 25°C, and were given distilled water freely. Rats were given a sham OVX or bilateral OVX under ether anesthesia. The sham-operated animals were fed matched amounts of chow for 11 days, and then used in the experiment.

Administration Procedure —— Cistus extract was dissolved in distilled water. A concentration of 5 or 10 mg per 0.5 ml per 100 g body weight was orally administered to OVX rats through a stomach tube once daily for 30 days. Control (sham operated) rats received distilled water (0.5 ml per 100 g body weight) orally. The animals were sacrificed by cardiac puncture under light ether anesthesia at 24 hr after the last administration, and the blood and femur removed immediately.

Peripheral Quantitative Computed Tomography (pQCT) for the Femur —— The femur was removed, and immediately immersed in 70% ethanol solution. Femoral-diaphyseal and -metaphyseal mineral content, mineral density, and polar strength strain index were measured using pQCT (XCT Research SA$^+$ Stratec Medizintecnik GmbH, Pforzhein, Germany). The measurement with pQCT for femoral metaphysis was carried out at 3.0 mm from the growth plate. The measurement for femoral diaphyseal was carried out at half the femoral length.

Bone Calcium —— The diaphyseal or metaphyseal tissues were dried for 16 hr at 110°C. Calcium was determined by atomic absorption specrophotometry.[21] The calcium content in bone tissue was expressed as mg/g of dry bone.

Statistical Analysis —— The significance of differences between values was estimated by Student's *t*-test. We also used a multiple ANOVA to compare the treatment groups. $p < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Cistus extract (5.0 or 10.0 mg/100 g body weight) was orally administered to OVX rats at 11 days after OVX, and then once daily for 30 days. OVX induced a significant increase in body weight as compared with that of sham-operated (control) rats (data not shown). This increase was not significantly prevented after oral administration of cistus extract (5.0 or 10.0 mg/100 g body weight).

The morphological change in the femoral-diaphyseal and -metaphyseal tissues of OVX rats was examined using pQCT. Morphological change was observed in the femoral-metaphyseal tissue of OVX rats (Fig. 1). This change was significantly restored after oral administration of cistus extract (5.0 or 10.0 mg/100 g) once daily for 30 days. Morphological change was not seen in the femoral-diaphyseal tissue in OVX rats and cistus extract-administered OVX rats, as compared with that of control (sham operated) rats.

The change in mineral content, mineral density, and polar strength strain index in the femoral-diaphyseal and -metaphyseal tissues of OVX rats was examined using pQCT analysis. Mineral content in the femoral-metaphyseal tissues was significantly decreased in OVX rats (Fig. 2). This decrease was significantly prevented after oral administration of cistus extract (10.0 mg/100 g) for 30 days.

Mineral density in the femoral-metaphyseal tissues was significantly decreased in OVX rats as compared with that of control (sham operated) rats (Fig. 3). This decrease was significantly prevented after oral administration of cistus extract (5.0 or 10.0 mg/100 g).

The change in femoral polar strength strain index is shown in Fig. 4. The polar strength strain index in the femoral-diaphyseal tissues was not significantly changed after OVX, and it was significantly increased after oral administration of cistus extract (10.0 mg/100 g) to OVX rats for 30 days as compared with that of control (sham operated) rats. The polar strength strain index in the femoral-metaphyseal tissues was significantly decreased in OVX rats. This decrease was significantly pre-

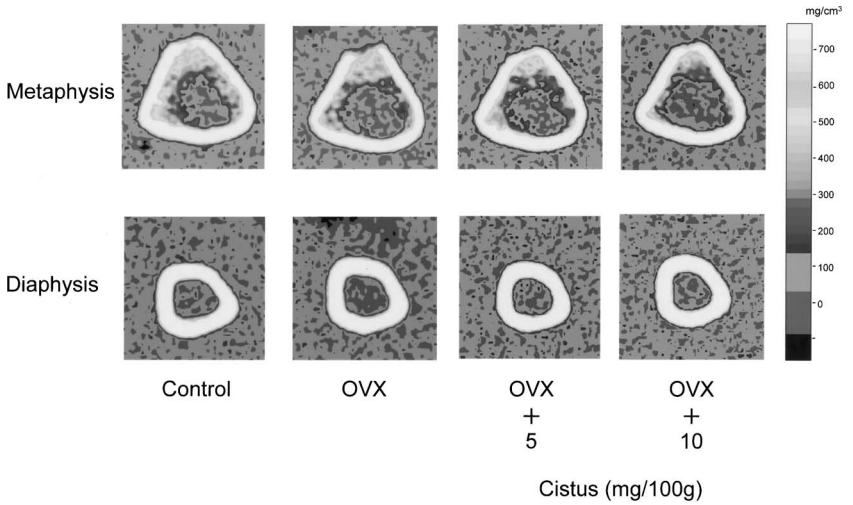

Fig. 1. Analysis of Bone Morphological Change in the Femoral-Diaphyseal and -Metaphyseal Tissues of OVX Rats Orally Administered Bee Pollen Cistus Extract

Cistus extract (5.0 or 10.0 mg/100 g body weight) was orally administered once daily for 30 days to OVX rats. pQCT analysis was carried out on the femurs of control (sham operated), OVX, or cistus extract-administered OVX rats. The figure shows one of six experiments with separate rats.

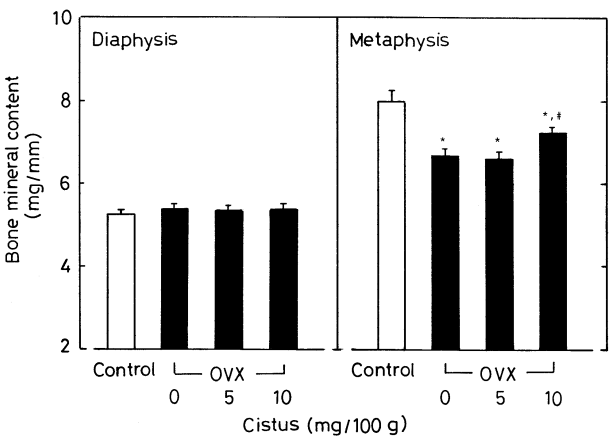

Fig. 2. Change in Bone Mineral Content in the Femoral-Diaphyseal or -Metaphyseal Tissues of OVX Rats. Orally Administered Bee Pollen Cistus Extract

Cistus extract (5.0 or 10.0 mg/100 g body weight) was orally administered once daily for 30 days to OVX rats. Bone mineral content was measured using pQCT. Each value is the mean ± SEM of six experiments with separate rats. $^{*}p < 0.01$ compared with the control value. $^{\#}p < 0.01$ compared with the value obtained from OVX (control) rats.

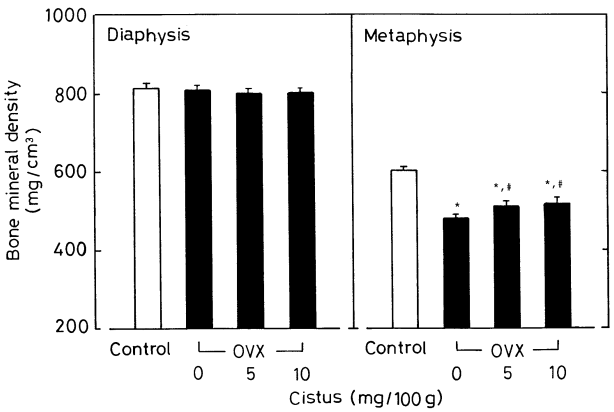

Fig. 3. Change in Bone Mineral density in the Femoral-Diaphyseal or -Metaphyseal Tissues of OVX Rats Orally Administered Bee Pollen Cistus Extract

Cistus extract (5.0 or 10.0 mg/100 g body weight) was orally administered once daily for 30 days to OVX rats. Bone mineral density was measured using pQCT. Each value is the mean ± SEM of six experiments with separate rats. $^{*}p < 0.01$ compared with the control value. $^{\#}p < 0.01$ compared with the value obtained from OVX (control) rat.

vented after oral administration of cistus extract (10.0 mg/100 g) for 30 days as compared with that of OVX (control) rats.

Calcium content in the femoral-diaphyseal and -metaphyseal tissues was significantly decreased in OVX rats as compared with that of control (sham operated) rats (Fig. 5). This decrease was significantly prevented after oral administration of cistus (5.0 or 10.0 mg/100 g) for 30 days.

DISCUSSION

A decrease in bone mass with aging may be due to decreased bone formation and increased bone resorption. The water-solubilized extract obtained from the bee pollen of *Cistus ladaniferus* has been shown to have stimulatory effects on bone formation and inhibitory effects on bone resorption *in vitro*,[16,17] thereby increasing bone mass. The oral administration of bee pollen cistus extract to streptozotocin-diabetic rats, which is shown to in-

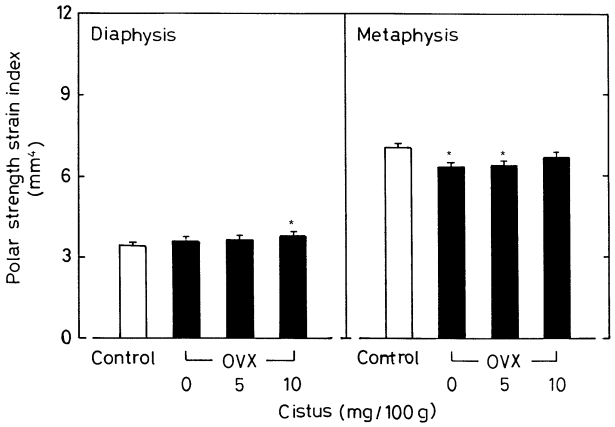

Fig. 4. Change in Pollen Strength Strain Index in the Femoral-Diaphyseal and -Metaphyseal Tissues of OVX Rats Orally Administered Bee Pollen Cistus Extract

Cistus extract (5.0 or 10.0 mg/100 g body weight) was orally administered once daily for 30 days to OVX rats. Bone polar strength stain index was measured using pQCT. Each value is the mean ± SEM of six experiments with separate rats. $^{*}p < 0.01$ compared with the control value.

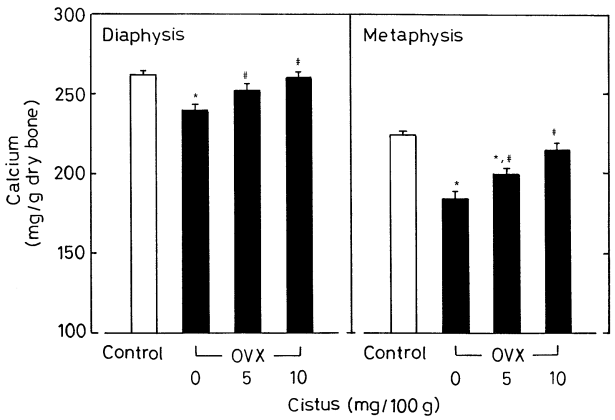

Fig. 5. Change in Calcium Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of OVX Rats Orally Administered Bee Pollen Cistus Extract

Cistus extract (5.0 or 10.0 mg/100 g body weight) was orally administered once daily for 30 days to OVX rats. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value. $^{\#}p < 0.01$ compared with the value obtained from OVX (control) rats.

duce bone loss,[22] has been shown to have preventive effects on bone loss with diabetes *in vivo*.[20] We examined the preventive effect of cistus extract on bone loss in OVX rats, a model of osteoporosis, *in vivo*, furthermore.

Prolonged oral administration was found to have a preventive effect on the decrease in mineral content, mineral density, and polar strength strain index in the femoral-metaphyseal (trabecular bone) tissues of OVX rats. OVX-induced morphological changes, moreover, was clearly restored after oral administration of cistus extract. These observations demonstrate that oral administration of cistus extract can prevent bone loss in OVX rats, suggesting a role in the prevention of osteoporosis.

Bone loss with morphological indexes was remarkable in the femoral-metaphyseal (trabecular bone) tissues of OVX rats. However, biochemical component (calcium content) was significantly decreased in the femoral-diaphyseal (cortical bone) and -metaphyseal tissues of OVX rats. Both decreases were also prevented after oral administration of cistus extract. Cistus extract can stimulate bone calcification in rat femoral tissues *in vitro*[16] and inhibit the release of calcium from femoral tissues cultured with bone-resorbing factor *in vitro*.[17] It is speculated that the active component of cistus extract stimulates bone formation and inhibits bone resorption in the femoral tissues of OVX rats *in vivo*, thereby preventing bone loss.

It has been demonstrated that the active component in a water-solubilized extract of bee pollen *Cistus ladaniferus*, which stimulates bone calcification and inhibits bone resorption *in vitro*, is a molecule of less than MW 1000.[19] The active component less than MW 1000 may be transported in the intestine after intake of cistus extract, and it may act on osteoblastic cells and osteoclastic cells in the bone tissues. The identification of the active component in bee pollen cistus extract remains to be elucidated, however.

In conclusion, it has been shown that the intake of bee pollen cistus extract by OVX rats has preventive effects on OVX-induced bone loss *in vivo*.

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