Estrogenic Effects of Sedum sarmentosum Bunge in Ovariectomized Rats

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Summary The aim of this study was to evaluate the effects of Sedum sarmentosum Bunge (SS) on the lipid on serum and the collagen content of the connective tissues in ovariectomized estrogen-deficient rats. Three groups were surgically ovariectomized. The fourth group was sham operated. From day 2 until day 37 after the ovariectomy, Sprague-Dawley female rats were randomly assigned to the following groups: sham-operated rats (sham), ovariectomized control rats (OVX-control), ovariectomized rats supplemented with an ethyl ether fraction of SS at 10mg/kg bw/d (OVX-EE), ovariectomized rats supplemented an ethyl acetate fraction of SS at 10mg/kg bw/d (OVX-EA). The SS fractions were orally administered at 1 mL per day. The estrogenic effects of the ethyl ether and ethyl acetate fractions of SS, were investigated using one in vitro assay and two in vivo assays. The treatment of the partition of the ethyl ether and ethyl acetate layers of SS increased the transcriptional activity 0.7-fold and 0.5-fold compared to those that were given 17β-estradiol treatment, respectively. The OVX rats were significantly heavier than the sham-operated rats at all times, but supplementation with the SS extracts tended to result in less weight gain than OVX-control. The serum triglyceride levels were significantly decreased after supplementation with the SS portion EE and EA layers. Supplementation with the SS extracts prevented a decrease in the collagen level in bone and cartilage tissues. This result indicates that the SS affects the collagen synthesis in ovariectomized rats. These results are consistent with the conclusions based on the estrogenic activities of SS. Therefore, it may be used to possibly improve the quality of life in menopausal women.

Key Words Sedum sarmentosum Bunge, collagen, lipid, estrogenic effects, ovariectomized rats

An estrogen deficiency after menopause is associated with osteoporosis, and one or more symptoms such as hot flashes, depression, mood swings, sleeping disorders, vaginal dryness, and joint pain (1, 2). Hormone replacement therapy (HRT) has been used to relieve menopausal symptoms. In addition, HRT reduces the risk of osteoporosis, cardiovascular disease, dementia from Alzheimer’s disease, and certain types of cancer are reduced (3–7). Epidemiological data shows that a diet rich in phytoestrogens, such as those found in soy, reduce the number of hot flashes and the incidence of cancer in Oriental women (8). The ovariectomized rat is proposed as an experimental model for the rapid development of menopausal symptoms. An ovariectomy in rats has been widely used as a model, replicating many of the events associated with postmenopausal osteoporosis in humans (9–12).

Following loss of ovarian function, postmenopausal women display an increased incidence of hypertension and coronary heart disease, which is at least partially attributable to an increase in the total cholesterol, LDL-cholesterol and triglyceride levels, as well as a reduction in the HDL-cholesterol levels (13–17).

An ovarian hormone deficiency has a substantial influence on the skeletal metabolism, and a decline in the skeletal mass after the cessation of the ovarian function in humans is well recognized (18). There is evidence suggesting that skin collagen is affected by osteoporosis. McConkey et al. first reported the association between transparent skin and osteoporosis (19). In addition Foundos et al. and Lovett et al. reported significant changes in the structure of the inflamed rabbit bone and skin collagen fibrils (20, 21).

The aim of this study was to evaluate the effects of Sedum sarmentosum Bunge (crassulaceae, SS) on the lipid on serum and the collagen content of connective tissues in ovariectomized estrogen-deficient rats. In the present study, we had systematically evaluated the estrogenic activity of various partition layers of SS. In order to analyze the SS extracts on the treatment of menopausal symptoms, the estrogenic activity in...

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human breast cancer cell line MCF7 was measured using in vitro test system. The change in serum lipids and connective tissues collagen in ovariectomized rats were examined to further investigate the estrogenic effect of the SS extracts in vivo.

**MATERIALS AND METHODS**

**Preparation of materials.** The SS was purchased from the traditional market of Umgung-Dong. The plant (120 g) was macerated in EtOH (1.5 L) overnight. Following filtration, the marc was extracted twice using EtOH (1.5 L), with gentle heating (<45°C, 10 min). The extracts were combined, and the solvent was removed in vacuo. The plant extracts were redissolved in 20% aqueous EtOH (1.5 L) and partitioned against ethyl ether. The residual EtOH was removed in vacuo from the aqueous portion, and the latter was partitioned against EtOAc and BuOH successively. Removing the solvent yielded the ethyl ether, EtOAc, BuOH, and aqueous soluble fractions.

**Plasmid construction.** Complementary oligonucleotides spanning a minimal promoter composed of the TATA region of the adenovirus-2 major late promoter (Ad2MLP, −33 to +34) were synthesized. After annealing, a double-stranded oligonucleotide was subcloned between the PstI and XbaI sites in pCAT-Basic (Promega, USA) to yield pCAT-Ad2MLP. Complementary oligonucleotides spanning two consensus, perfectly palindromic Xenopus vitellogenin A2 genes EREs (ERE119; Table 1) were synthesized (22, 23). After annealing, the double-stranded oligonucleotide was subcloned between the HindIII and PstI sites in pCAT-Ad2MLP to yield pCAT-ERE119-Ad2MLP. The integrity of all constructs was verified by restriction analysis and sequencing.

**Cell culture and transient expression assays.** The human breast cancer cells line MCF7 was obtained from ATCC (Manassas, VA, USA). The MCF7 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, BioWhittaker, USA) supplemented with 10% dextran-coated charcoal stripped fetal bovine serum (Life Technologies, USA) at 37°C in a humidified atmosphere containing 10% CO2. Transfection was carried out using Lipofectamine plus reagent (Invitrogen BV, Netherlands) and 5 μg of the pCAT-ERE119-Ad2MLP plasmid in serum free medium in 60-mm dishes. A Plasmid Midi Kit (Qiagen, USA) was used to purify the plasmids. Three hours after transfection, either a pomegranate extract (40 μg/mL), 17β-estradiol (10−7 M; RBI, USA), or the vehicle (ethanol, 5 μL) was added to the corresponding dishes. The cells were harvested 48 h after adding the DNA. The lysates were prepared by four cycles of freezing and thawing of the harvested cells, which was followed by centrifugation.

The CAT assays were carried out using a CAT-enzyme linked immunosorbent assay (ELISA) kit (Boehringer Mannheim, Germany). All the CAT assay results were normalized to the protein concentration of the lysates measured by the BCA Protein Assay Reagent kit (Pierce, Rockford, IL, USA).

### Table 1. Oligonucleotides for plasmid construction.

| Oligonucleotide | Sequence |
|-----------------|----------|
| Ad2MLP Sense    | 5'-GCTATAAAAGGGGTGGGGCCCTTC GCCCTCATCTCTTGCGA CAGGCGACGTCT-3' |
| Antisense       | 5'-CTAGAGGCTGGCCCTGCAAGACAGCGAT CCGGAAAGAGTGAAGAGCAAGCGGCCC CACCCCTTTTTATAGCTGCA-3' |
| ERE119 Sense    | 5'-AGCTCTGAGATCTAGGTACATGACCT GACTGACATGATGACATGCAGTCAGTACCGAC TCTGCA-3' |
| Antisense       | 5'-GAGTCAGGTCAGTGAACCTGATCTCG AGTCAGGTCAGTGAACCTGATCTCGA-3' |

### Table 2. Experimental design.

| Groups | Ovariectomy | Ethyl ether fraction | Ethyl acetate fraction |
|--------|-------------|----------------------|-----------------------|
| Sham   | −           | −                    | −                     |
| OVX-control | +            | −                    | −                     |
| OVX-EE | +           | −                    | +                     |
| OVX-EA | +           | −                    | +                     |

1 Sham: sham-operated rats, OVX-control: ovariectomized rats, OVX-EE: ovariectomized rats supplemented ethyl ether fraction of Sedum sarmentosum Bunge at 10 mg/kg bw/d, OVX-EA: ovariectomized rats supplemented ethyl acetate at 10 mg/kg bw/d.

**Animals and diets.** Seven-week-old female rats (Sprague-Dawley) were obtained from Hyochang Science Co. (Daegu, Korea). They were fed a commercial diet for 1 wk in order to allow them to acclimatize to the new surroundings. At 8 wk of age, a bilateral ovariectomy was performed using the dorsal approach. The rats were divided into four groups of six or seven animals each, with similar mean body weights. The sham-operated rats (sham) were fed a control diet. The ovariectomized rats were assigned to the control (OVX-Control), the ethyl ether fraction of SS (10 mg/kg bw/d) (OVX-EE) and ethyl acetate fraction of SS (10 mg/kg bw/d) (OVX-EA) groups (Table 2). The temperature was maintained at 23±1°C and the animals were subjected to a 12-h light-dark cycle. Body weight and food consumption were recorded every 2 or 3 d. All rats were sacrificed under diethyl ether anesthesia at the end of the 5-wk feeding period. The uteri, skin, lungs, bones and cartilage were carefully removed. The weights were recorded for each organ, and the bone and cartilage were prepared as described below. All procedures were performed in accordance with the Silla University Guidelines for the Care and Use of Laboratory Animals.

**Preparation of blood and tissues for analysis.** Blood was collected from the aorta ventralis into tubes and separated by centrifugation at 2,000×g for 15 min at 4°C. The bone and cartilage were cut into small pieces, washed in saline and defatted with methanol: chloroform (1:2). The tissue samples were hydrolyzed with...
6 M HCl in sealed tubes at 110°C for 24 h and evaporated to dryness in vacuo.

**Determination of serum lipids.** The total cholesterol, HDL-cholesterol and triglyceride level in the serum were measured enzymatically using commercial kits (YD Diagnostics, Seoul, Korea).

**Determination of collagen.** The collagen was measured from its hydroxyproline content, assuming that the hydroxyproline content was 0.11 mol per mol collagen (24). The hydroxyproline contents of the resulting hydrolysates were determined using the method described by Woessner (25).

**Statistical tests.** The results are presented as a mean±SD. The statistically significant difference between the means of the two groups was evaluated by either a Student's t-test or the Cochran-Cox test, depending on whether variances were equal or different. A p value <0.05 was considered statistically significant.

## RESULTS

### Estrogenic activity of the Sedum sarmentosum Bunge extract

The estrogenic activity of the SS extracts (SS) was evaluated in the human breast cancer cell line, MCF7, using an in vitro test system. After treatment with the SS extracts or estrogen, the cell extracts were prepared and assayed for their chloramphenicol acetyl transferase (CAT) activity. Treatment with the ethyl ether and ethyl acetate layers of SS extract resulted in a 0.7-fold and 0.5-fold increase in the transcriptional activity compared to the 17β-estradiol treatment, respectively (Table 3). These observations indicated that SS extracts have effective estrogenic action.

### Food intake and body weight

The OVX rats were significantly heavier than the sham-operated rats at all times. There was no difference in the weight gained by any of the OVX groups at any time. Treatment with SS extracts tended to result in lower weight gain than the OVX-control, but the BW was not significantly altered in the OVX groups. The food intake in the OVX groups was higher than that in the sham-operated group (Table 4). No significant differences in food intake were observed between the OVX groups. The uteri of the OVX animals were markedly atrophic compared to the sham animals. The uterine weights in the OVX-EE and OVX-EA groups were the same as that in the OVX-control group.

### Change of lipid contents in serum

The ovariectomy caused an expected increase in the serum levels of total cholesterol and triglycerides (Fig. 1). The serum triglyceride levels of the OVX-EE and OVX-EA groups were significantly lower than that of the OVX-control group. The total cholesterol content of the ovariectomized rats in the serum was higher than in the sham-operated rats. Supplementation with the SS ethyl acetate extracts resulted in a decrease in the total cholesterol in the serum, but this was not significantly different than those from the OVX animals. The serum HDL-cholesterol in the OVX-EA group was significantly higher than in the OVX-control group (Fig. 1).

### Collagen contents in connective tissues

OVX caused a significant decrease in the amount of collagen in the connective tissues (Table 5). Supplemen-
tation with the SS extracts prevented the decrease in collagen in the bone and cartilage tissues.

Collagen from the lungs and skin from the ovariectomized rats treated with the SS extracts revealed a significant increase compared to the OVX-control.

**DISCUSSION**

The potential biological impact of environmental and dietary estrogens on human health has generated considerable interest (26–28). Since the side effects of traditional estrogen replacement therapy include a slight but significant increase in the risk of developing breast and endometrial cancer, women are increasingly using herbal remedies as an alternative (29–36). Zava et al. previously reported the estrogenic and progestin bioactivities of over 150 herbs (37). This study systematically evaluated the estrogenic properties of *Sedum sarmentosum* Bunge (SS) using one in vitro assay and two in vivo assays.

The estrogenic activity of the SS extracts was evaluated in human breast cancer cell line, MCF7, using the in vitro test system. Treatment with the ethyl ether and ethyl acetate layers of SS increased the transcriptional activity compared to 17β-estradiol treatment. The change of serum lipids and connective tissues collagen was examined in ovariectomized rats in order to further investigate the estrogenic effect of SS extracts in vivo.

The ovariectomy increased the BW by 4–9%, which is similar to the results reported by other researchers (38). This study observed that the OVX rats were significantly heavier than the sham-operated rats at all times, but treatment with the SS extracts tended to result in lower weight gain than the OVX-control. The food intake in the OVX groups was higher than that in the sham group. It has been reported that an ovariectomy resulted in an increase in the food intake in rats but not in hamsters (39, 40). The uteri weights in the OVX-EE and OVX-EA groups were the same as that in the OVX-control group. The SS extracts did not affect the uterus weight in the ovariectomized rats, as was reported in other papers (41).

The risk of hypertension and stroke is lower in postmenopausal women relative to men of the same age. However, the incidence of cerebrovascular events rapidly increases in women after menopause (42, 43). Stevenson et al. reported that a change from a pre-menopausal to postmenopausal status results in an increase in the total cholesterol, the LDL-cholesterol and triglyceride levels, as well as a reduction in the HDL2-cholesterol levels (17). It is known that an ovariectomy induces an increase in the serum total cholesterol levels in rats, rabbits and hamsters (44–47). In this study using rats, there is a tendency for a higher serum cholesterol and triglyceride after an ovariectomy. The supplementation of SS ethyl ether and ethyl acetate layers resulted in a significant decrease triglyceride level in the serum.

The collagen level is altered in osteoporosis and it is important that these changes are recognized in studies of the bone metabolism in osteoporosis because they may play a role in the pathogenesis of the disease (48). An ovarian hormone deficiency has a substantial influence on the skeletal metabolism, and the decline in the skeletal mass after the cessation of the ovarian function in humans is well recognized (18). Compared to the OVX-control, a change in the collagen contents was observed in the lung and skin of the ovariectomized rats with the SS extracts. Supplementation with the SS extracts prevented the collagen decrease in the bone and cartilage tissues.

These results are consistent with these conclusions based on the estrogenic activities of SS. A further investigation into SS is necessary in order to evaluate its potential estrogenicity and to fully understand its mechanisms.

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