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Antiosteoporotic Activity of OST-6(Osteocare), a Herbomineral Preparation in Calcium Deficient Ovariectomized Rats

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The preventive effect of the herbal formulation OST-6 (Osteocare) on the progress of bone loss induced by ovariectomy and concurrent calcium deficiency was studied in rats. Ovariectomy (OVX) and calcium deficiency (CD) resulted in bone loss as evident from decreased femoral weight and density. Treatment with OST-6 at a dose of 250 mg/kg body wt twice a day orally for a period of 16 weeks significantly restored the femoral weight and density. The biomechanical strength of the tibia was decreased by OVX and CD and this was significantly suppressed by the administration of OST-6. Histologically decalcified bone sections revealed narrowed, and the disappearance of, trabeculae and widened medullary spaces. The total numbers of tartrate-resistant acid phosphates (TRAP) positive cells were increased significantly in OVX animals. Treatment with OST-6 significantly inhibited these histopathological changes and strongly suggested that OST-6 was effective in preventing the progress of bone loss induced by ovariectomy and concurrent calcium deficiency. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: osteoporosis; ovariectomy; herbal preparation; OST-6; osteocare; impact test.

INTRODUCTION

Osteoporosis is a major public health problem that is predicted to worsen over the next decade (Kannus *et al.*, 1995) because of an expanding aging population. It comprises a group of conditions characterized by a net loss of bone mass and architectural abnormalities leading to the increased risk of skeletal fractures (Lindsay and Cosman, 1992). Postmenopausal osteoporosis is a major health problem with significant morbidity and mortality (Cummings *et al.*, 1990). The decline in oestrogen in postmenopausal women is at least in part responsible for the decrease in bone mass and the increase in the incidence of osteoporotic fractures. Oestrogen replacement therapy (ORT) in postmenopausal women remains the mainstay for the prevention of bone loss. However, ORT is not accepted universally due to the contraindication in some patients, low compliance and because of many undesirable side effects, the most serious being breast and uterine cancers (Lobo, 1995).

Considering the disadvantages in the conventional system of medicine in the management of postmenopausal bone loss, there is an increasing demand in the alternative system of medicine to prevent and cure this devastating ailment. From time immemorial, a large number of remedies based on herbal and mineral resources have been used in Ayurveda, an ancient system of Indian medicine, for the correction of bone metabolic disorders and fractures in particular. Herbal remedies are gaining popularity as the public becomes disinterested with conventional medicine and resorts to remedies with the reputation of being both safe and efficacious. This resurgence of interest in traditional medicine could also arise from the encouragement received from the recent reports of the bone sparing effects of many phytoestrogens (Messina, 1995) and other naturally occurring bioactive molecules.

OST-6 is a herbomineral preparation comprising mainly *Terminalia arjuna* W & A, *Withania somnifera Dunal*, *Commiphora mukul* Hook Ex stock and *Praval bhasma* that are well known for their beneficial effects in rickets (Mitra *et al.*, 2000) and osteoporosis (Mitra *et al.*, 2001). Each gram of OST-6 contains *Terminalia arjuna* W & A (250 mg), *Withania somnifera Dunal* (250 mg), *Commiphora mukul* (280 mg) Hook Ex stock and *Praval bhasma* (220 mg). The present study was designed to evaluate and demonstrate further the antiosteoporotic activity of OST-6 in a calcium deficient ovariectomized rat model.

MATERIALS AND METHODS

Animals and diets. The study was conducted in accordance with current legislation on animal experiments in India. Thirty, 3-month-old, virgin female Sprague-Dawley rats were obtained from the animal facility at the Himalaya Drug Company, Bangalore and housed in standard laboratory conditions with a 12 h light/dark cycle. The rats were acclimatized to the local vivarium

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Table 1. Composition of synthetic calcium deficient diet

Ingredient	Content (g/kg)
Casein	200
DL-Methionine	3
Sucrose	512
Corn starch	150
Corn oil	50
Alpha cellulose	50
Ca-free minerals	35
Vitamin mix	1 kg 100 lbs of diet

With a total calcium level of 0.04%.

conditions for 2 weeks and allowed free access to water and pelleted commercial diet (Lipton India Ltd, Mumbai).

Study procedure. Ten rats were sham-operated and treated orally with vehicle (0.5% sodium carboxy methyl cellulose in water), while the remaining rats were bilaterally ovariectomized and treated with either vehicle or OST-6 at 250 mg/kg body weight, twice a day orally (Mitra *et al.*, 2001). The OVX animals were fed with casein based synthetic diet containing a low quantity of calcium (0.04%) and distilled water *ad libitum*. The composition of the calcium deficient diet is shown in Table 1. The body weights of all the animals were recorded at the beginning and at weekly intervals throughout the 16 week experimental period. At the end of the experimental period, the animals were bled from the carotid artery under ether anaesthesia and killed. The right and left femora along with tibias were dissected out. The left femurs were thawed, autoclaved for 15 min at 110 °C and divested of soft tissue for the measurement of weight, length, volume and density. The cleaned right femurs were immediately fixed in 10% neutral buffered formalin (NBF) for histological examination. The right tibias were carefully separated from the femurs and stored in 50% normal saline ethanol for biomechanical testing using impact test apparatus.

Measurement of femur parameters. The femur length, defined as the distance between the greater trochanter and the medial condyle, was measured in the left femurs using digital slide callipers (Mitotoyo Corp., Japan). The same femurs were then dried in an evacuated oven at 110 °C for 48 h and weighed using a digital weighing balance (Sartorius, Germany). The femur bone density was determined by the method described by Kalu *et al.* (1989) and the bone volume was calculated by the method of Donahue *et al.* (1988).

Histology of femur bone. The right femurs were fixed in 10% NBF for 12 h at 4 °C, decalcified in 5% ethylenediamine tetraacetictacid (EDTA) for 7 days, embedded in paraffin and cut into longitudinal sections of 5 μm thickness. The sections were stained with haematoxylin and eosin (H&E) and tartrate-resistant acid phosphatase (TRAP), a cytochemical marker for osteoclasts and finally counterstained with haematoxylin (Bancroft and Cook, 1988; Drury, 1980). The number of positively stained osteoclasts in the sections of the median portion of the whole femora was enumerated for the three groups.

Biomechanical strength of tibia by impact test. The biomechanical strength of the tibia was determined using an impact test apparatus (Tinius Olsen, PA, USA). The total energy (fracture energy) required to break each tibia was determined by using a software program (Instron Dynatup, 930-I). Briefly, each sample was placed in a specially designed sample holder and held firmly by means of two screws one on each end of the sample. The hammer, i.e. collider, was dropped from a fixed and predetermined angle and impact test parameters were picked up by a sensing flag that was placed perpendicular to the collider. Using the software, various parameters such as impact velocity, impact energy, maximum load, energy to maximum load, time to maximum load and the total fracture energy were calculated.

Statistical analysis. The results were expressed as the mean with the standard error. All data were analysed using Graphpad Prism software (Microsoft, San Diego, CA, USA). One-way ANOVA was first performed to test for any significant difference among the groups. When significant, a post test, Dunnet multiple comparison test was applied to determine the specific difference between the groups. The level of significance was $p < 0.05$ for all tests.

RESULTS

Effect of OST-6 on body weight and food consumption

The effect of OST-6 on the body weights of all the groups is presented in Table 2. There was no significant difference between the SHAM, the OVX and OST-6 treated group at any time during the experimental period. With respect to food consumption the animals in the OVX group and OST-6 group had similar but slightly less consumption compared with the SHAM group feeding on normal pelleted diet. This decrease in

Table 2. Effect of OST-6 on body weight changes

Group	Initial body weight (g)	Week 4 (g)	Week 8 (g)	Week 12 (g)	Week 16 (g)
SHAM	255.5 ± 5.61	259.4 ± 5.16	255.8 ± 5.97	258.3 ± 5.3	245 ± 4.03
OVX	254.4 ± 7.64	254.3 ± 4.50	255.4 ± 4.83	255.4 ± 4.41	257 ± 4.56
OST-6	255.2 ± 6.06	252.6 ± 7.2	251.5 ± 4.26	246.4 ± 3.7	254.3 ± 3.27

All values are expressed as mean ± SEM.

Table 3. Effect of OST-6 on femoral length, weight, volume and density

Parameter	SHAM	OVX	OST-6
Length (mm)	34.33 ± 0.22	33.6 ± 0.25	33.77 ± 0.26
Weight (g)	0.3796 ± 0.0098	0.3232 ± 0.0049[a]	0.3589 ± 0.0057[b]
Volume (mL)	0.1815 ± 0.0027	0.1751 ± 0.0023	0.1743 ± 0.0036
Density (g/mL)	2.16 ± 0.0060	1.78 ± 0.028[a]	2.06 ± 0.0568[b]

All values are expressed as mean ± SEM.
[a] $p < 0.001$ compared with SHAM group.
[b] $p < 0.001$ compared with OVX group.

Table 4. Effect of OST-6 on biomechanical strength of tibia as determined by impact test

Parameter	SHAM	OVX	OST-6
Impact energy (J)	68.20 ± 0.725	66.94 ± 0.361	66.32 ± 0.425
Impact velocity (m/s)	2.437 ± 0.020	2.423 ± 0.0071	2.417 ± 0.0077
Maximum load (kn)	0.045 ± 0.005	0.044 ± 0.002	0.042 ± 0.0025
Energy to max. load (J)	0.0911 ± 0.0068	0.069 ± 0.001[a]	0.0852 ± 0.0032
Time to max. load (ms)	1.035 ± 0.244	1.008 ± 0.009	1.339 ± 0.0910
Defl. at max. load (mm)	2.745 ± 0.1774	2.480 ± 0.053	3.236 ± 0.2166[b]
Total energy (J)	0.1066 ± 0.0014	0.0736 ± 0.005[a]	0.0920 ± 0.0017[b]

All values are expressed as mean ± SEM.
[a] $p < 0.001$ compared with SHAM group.
[b] $p < 0.001$ compared with OVX group.

food consumption was due, in part, to switching the rats from the normal diet to the CD diet. The OST-6 treated animals had less abdominal fat when compared with the SHAM and the OVX group.

Femur parameters

The effect of OST-6 on femoral length, dry weight, volume and density is presented in Table 3. The result shows that there was no significant difference in the femoral length among the groups. Ovariectomy resulted in a significant reduction in the femoral weight and density without a change in volume compared with the SHAM animals. Treatment with OST-6 significantly increased the femoral weight and density.

Impact test

The results of the impact test (Table 4) indicate that the total energy required to break the tibias was significantly lower in the OVX animals compared with the SHAM animals. Treatment with OST-6 increased the biomechanical strength of the tibias as evident from the greater fracture energy required to break them. However, there was no significant difference in the other applied parameters such as impact energy, impact velocity and maximum load among the different groups.

Histological examination

The sections of the femur in the region proximal to the epiphyseal growth plate were examined for any histological changes. The animals of the SHAM group showed normal compactness of the diaphysis and competent trabeculae (Fig. 1a). The OVX animals showed sparse, uniform thinning of the trabeculae resulting in widened intertrabecular spaces (Fig. 1b). Cartilaginous proliferates in the area of softened plates of focal to restricted islets were also observed. The OST-6 treated animals showed a minimum number of thin trabeculae and less frequent cartilaginous proliferation (Fig. 1c). Observation for reddish TRAP positive cells (Fig. 2a, 2b, 2c) showed an increased number in the OVX animals compared with the SHAM and the OST-6 treated animals.

DISCUSSION

The objective of this study was to evaluate further the efficacy of OST-6 in the prevention of bone loss under calcium and oestrogen deficient conditions. The results from this experiment combined with earlier studies (Mitra *et al.*, 2001) indicate that OST-6 prevents the progress of bone loss even in conditions of severe calcium and oestrogen deficiency. The present study creating an experimental model of the menopause by OVX and concurrent CD indicated that the model provided the needs of the study.

Although not significant, the animals in the OVX group had a greater body weight gain compared with the other groups. This weight gain due to ovariectomy despite similar food consumption has been well documented (Kalu *et al.*, 1991). Treatment with OST-6 significantly reduced abdominal fat compared with the SHAM and the OVX animals, inspite of the equal final body weights, thus suggesting a tissue specific effect. This finding is of particular significance because it may be due to the stimulatory effect of OST-6 on the synthesis of growth hormone (GH), which is known to

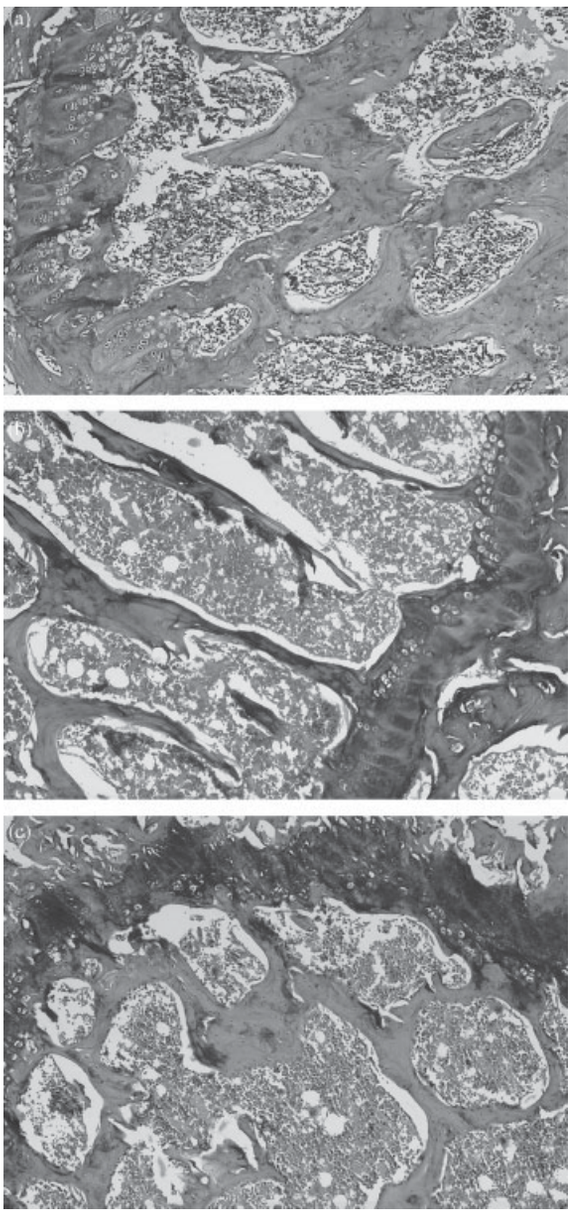

Figure 1. (a) Epiphyseal region showing normal compact trabeculae with intertrabecular spaces in a SHAM rat (H&E, × 250). (b) Epiphyseal region showing sparse, thinning of trabeculae with tendency for disappearance, loss of connectivity and widening of intertrabecular spaces in an OVX rat (H&E, × 250). (c) Epiphyseal region showing moderately thick elongated trabeculae and narrowed intertrabecular spaces in an OST-6 treated rat (H&E, × 250).

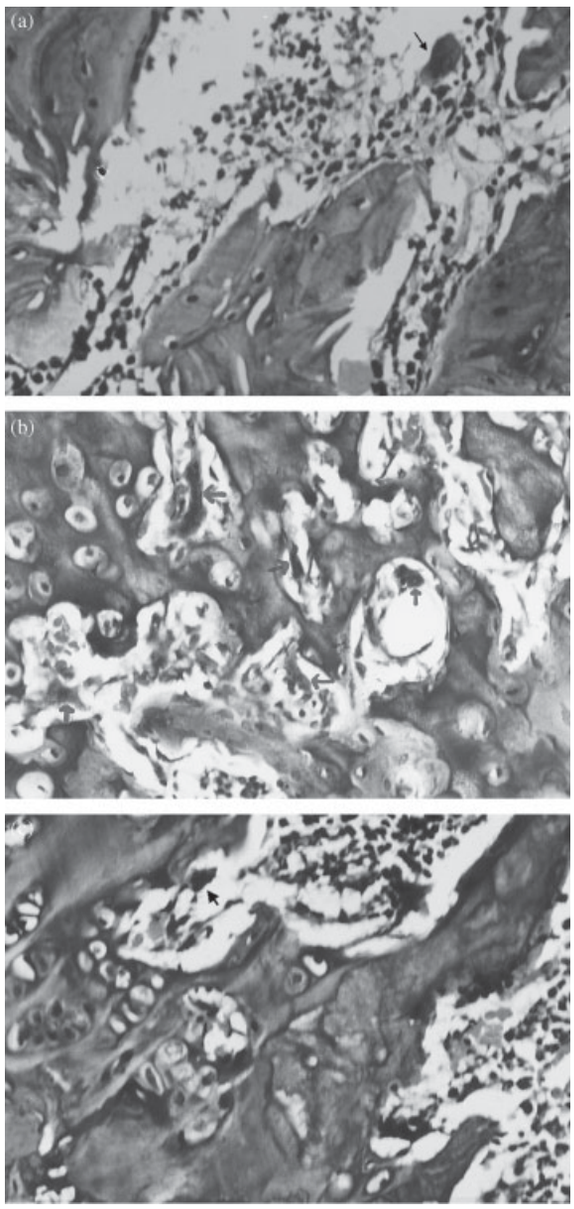

Figure 2(a, b, c). Femur sections showing reddish stained TRAP positive osteoclasts (↑) (TRAP, × 1000). Note the increased number of osteoclasts in (b).

decrease the adipose tissue mass (Rudman *et al.*, 1990) and to increase the bone mass (Arjmandi *et al.*, 1994).

Our findings suggest that CD-OVX rats develop bone changes similar to those seen in oestrogen deficient osteoporotic women, most notably a decrease in bone density (Donahue *et al.*, 1988) and femur weight. Treatment with OST-6 significantly improved the femur weight and density. Although not significant, a slight increase in femoral length could be observed in the OST-6 treated group compared with the OVX group indicating a small stimulatory effect of GH on the longitudinal growth (Ohlsson *et al.*, 1998).

The results of the impact test indicate a requirement of lesser energy to break the tibia in the OVX group compared with the SHAM group. Treatment with OST-6 had greatly strengthened the biomechanical properties as evident from the higher fracture energy required to break them. The rationale for selection of the impact test was to incorporate and simulate the conditions very similar to that seen in falls, impacts or collisions. Previous studies have shown that ovariectomy results in decreased mechanical properties in the long bones and the same was observed in the present study. Bone strength is related to bone density, architecture, connectivity and mineralization (Einhorn, 1992). It has also been reported that cortical bone contributes an important role to skeleton strength (Rockoff *et al.*, 1969) and linear correlations were observed between the bone density and impact strength (Pantaliov *et al.*, 1999). Our study report is also consistent with the above findings in that ovariectomy resulted in decreased bone density as well as in reduced biomechanical strength.

Histological examination also revealed the antiosteoporotic property of OST-6 as demonstrated by the

restoration of trabecular bone with less TRAP positive cells in OST-6 treated group compared with the OVX group. The beneficial effects of OST-6 could be in part due to *Praval bhasma* that is a rich and natural source of calcium obtained from oyster shell and due to appropriate ayurvedic processing has the advantage of easy absorption from the intestine. In addition, *Terminalia arjuna* also contains significant amounts of calcium. At the same time the presence of phytoesterols in *Withania somnifera* cannot be neglected. *Withania somnifera* is found to contain a number of phytosterols, alkaloids, 18 fatty acids, beta-sitosterol and polyphenols (Elsakka *et al.*, 1990). The recent discoveries of phytosterols/phytoestrogens especially the isoflavones of soybean, possessing antiosteoporotic activity further confirms the antiosteoporotic effects of *Withania somnifera* (Ren *et al.*, 2001).

In conclusion, OST-6 treatment in calcium deficient ovariectomized rats further affirmed the beneficial effects and thus indicates its potential in preventing osteoporosis in a natural way through herbal resources.

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