

Anti-osteoporotic activity of ostinu, a herbo-mineral preparation in ovariectomized rats

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ABSTRACT

Ostinu is a herbomineral formulation consisting of *Terminalia arjuna*, *Phyllanthus embellica*, *Cissus quadrangularis*, *Commiphora mukul*, *Withania somnifera*, Shanka bhasma, Pravala bhasma, Godanti bhasma and Kapardika bhasma. Aim of this study was to evaluate effect of Ostinu on bone loss induced by ovariectomy in wistar rats. Female Wistar rats weighing 170-200gm were divided into five groups of eight animals in each group. Normal and control groups were administered vehicle. Standard group received Alendronate (ALN) sodium 1mg/kg.b.w. Test groups were treated with Ostinu 80mg/kg (Ostinu-1) and 160mg/kg (Ostinu-2). All drugs were administered orally daily. To perform ovariectomy animal was anesthetized with ketamine (60mg/kg) and diazepam (5mg/kg). Bilaterally ovariectomy was performed in all groups except normal group, which were sham operated. After 12 week of treatment all the animals were sacrificed with overdose of ether anesthesia. Blood was collected from sacrificed animals and serum calcium and phosphorus was estimated. Femur and tibia were collected and evaluated to obtain weights, ash weight, ash calcium & phosphorus content and femur volume & density. Scanning electron microscopy on femur is carried out to determine bone resorption. Ostinu 160mg/kg and Alendronate very significantly ($p < 0.01$) prevented decrease in femur volume & density, femur ash calcium & phosphorus and significantly ($p < 0.05$) prevented decrease in tibia weight, serum calcium and phosphorus compared to control. Alendronate has very significant ($p < 0.01$) effect on femur weight and femur ash weight but Ostinu 80mg/kg has only significant ($p < 0.05$) effect compared to control. The results show that Ostinu prevented bone loss in post-menopausal osteoporosis in Wistar rats.

Key words: Ostinu, Osteoporosis, Ovariectomy, Herbal formulation.

INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue leading to enhanced bone fragility and a consequent increase in fracture risk¹. The disease is common in postmenopausal women, elderly men and in case of prolonged steroid therapy. In osteoporosis, bones become so fragile that they can break almost spontaneously. Ayurveda, an ancient system of Indian medicine has mentioned several herbs that are useful in correction of bone disorder. Many plant drugs have been proved for their beneficial effects

in fracture healing and bone calcification.^{2, 3} the constituents of Ostinu were powdered and mixed in appropriate proportion (Table 1).

MATERIAL AND METHODS

Animals

Female Wistar rats weighing 170-200g were obtained from Drug Testing Laboratory Bangalore. They were housed in animal house of Government College of Pharmacy. Animals were maintained and handled as per CPCSEA guidelines. The experimental protocols were approved by Institutional Animal Ethics Committee of

Government College of Pharmacy, Bangalore. Animal house was well maintained under standard hygienic conditions, at a temperature ($22\pm 1^{\circ}\text{C}$), room humidity ($60\%\pm 10\%$). Animals were provided with rat food pellets (Lipton, India) and purified water *ad libitum*.

Drugs

Ostinu samples were provided by Srushti herbal Pharma, Bangalore. Cipla limited Mumbai provided gift samples of standard drug alendronate sodium trihydrate. Anesthetics ketamine (Anket) and diazepam (valium) were purchased from a pharmacy store.

EXPERIMENTAL

Protocol

Acute oral toxicity study was performed according to OECD (Organization for Economic Co-operation and Development) guidelines 425.⁴

Each animal was anesthetized with ketamine (60mg/ kg) and diazepam (5mg/ kg). Doses of anesthetics were chosen from "Experimental and surgical techniques in rat" and adjusted suitably.⁵ The anesthetized animal was laid on its ventral surface. A small incision was made half way between middle of back and the base of tail to gain access to peritoneal cavity. Ovaries surrounded by fat are found underneath. The ovary was pulled out grasping the fat. With pointed scissor ovary was served with single cut and uterine horn and fat returned to abdominal cavity. Bilaterally ovariectomy was performed.^{6,7} The muscle incision was sutured with catgut. Then skin incision was sutured and antiseptic powder was applied. In sham operated group ovaries were touched with forceps and returned back to abdominal cavity. The operated rats were housed singly in cage for one-week and allowed to recover. Then animals were randomized into five groups of eight animals each and housed in pairs. (Table 2)

The drugs are prepared for administration as suspension using 0.5% sodium CMC as suspending agent in distilled water. Standard group was treated with alendronate 1mg/Kg, test groups were treated with Ostinu 80mg/ Kg and Ostinu 160mg/ Kg. Normal and control group-received

vehicle only. Body weights of all animals were recorded at the beginning and at weekly interval through out 12-week experiment.

After 12 week of treatment all the animals were weighed and sacrificed with overdose of ether anesthesia. Blood was collected and serum separated for estimation of calcium, phosphorus and alkaline phosphate using Auto span diagnostic kits in Qualigens "Semi auto analyzer". Femurs and tibias were removed, cleaned and evaluated to obtain weight, ash weight, calcium and phosphorus content of ash, bone volume and density and bone resorption.

Evaluation

Measurement of bone length, width and weights⁸

Femurs and tibias were cleaned from surrounding tissue. They were then kept in oven and dried at 110°C for 20 minutes. Femur length, defined as the distance between greater trochanter and medial condyl, was measured using digital slide caliper (Japan). Just below the femur neck two widths were measured one sagittal another medial. Weight of the dried bones were determined using digital weighing balance.

Bone volume and density measurement⁹

Volume and density of right femurs were measured by Archimedes principle. Femur was placed in an unstoppered vial filled with deionized water, and the vial was put in a desiccator connected to a vacuum for 90min. The desiccator was agitated periodically to ensure that all trapped air diffused out of bone. The femur was removed from vial blotted with tissue paper, weighed and returned to vial containing deionized water. The femur was reweighed in a boat suspended, but completely immersed in water.

Femur volume was calculated using formula.

$$\text{Volume} = (\text{mass in air} - \text{mass in water}) / \text{Density of water}$$

Femur density (g/cc bone volume) was then calculated,

$$\text{Density} = \text{mass in air (mg)} / \text{Volume in ml}$$

Ash weights and mineral content of bone¹⁰

For ashing the samples, femurs were

placed in tared silica crucibles weighed dried at 110°C and ashed for 12 hrs at 900°C. The ash weights were determined. Then ash was dissolved in 1ml conc. HCl, and diluted with deionized water. The assay for calcium and phosphorus content was carried out using auto analyzer. Since femur density correlates well with bone calcium content, the femur calcium was expressed in mg/ cc bone volume.

Scanning electron microscopy¹¹

Femur of each group that were frozen were trimmed on the frontal plate to expose the growth plate and cancellous bone using a rotating diamond saw, and then bones were dehydrated in ethanol and dried. Bones were examined on a JSM- 840 scanning electron microscope. Scanning electron micrograph of the metaphyseal region of the distal femur was taken at 100X in order to see the resorption pits. All the samples were examined uniformly at specific position to minimize the error (Figure no.1).

Serum analysis

Serum calcium and phosphorus estimation was done using Auto Span diagnostic kits in 'Qualigen's 704' semi automatic analyzer.

At alkaline PH calcium binds with orthocresolphthalein complexone (OCPC) to form a bluish-purple complex. The intensity of the colour so formed is proportional to calcium concentration and is measured at 578 nm. Interference from magnesium is overcome by the presence of 8-hydroxyquinoline in reagent-I, which binds free

magnesium ions concentration in the sample.

Phosphorus reacts with molybdate to form phosphomolybdate, increase in absorbance due to formation of this complex is measured at 340nm. The absorbance is proportional to concentration of phosphorus in the sample.

Statistical analysis

Data is expressed, as mean \pm SEM. Comparison among groups was statistically processed by one-way analysis of variance (ANOVA) with Tukey's post hoc analysis in "Graph Pad InStat" software. P value less than 0.05 is considered as significant and P value less than 0.01 is considered very significant.

RESULTS

In safety studies Ostinu was found to be safe up to a dose of 5000mg/kg body weight, as on gross observation no untoward effects were seen.

Compared with normal animals ovariectomy very significantly reduced ash weight, ash calcium & phosphorus, femur & tibia weight, femur volume & density, serum calcium and phosphorus in control animals. Ostinu-1, very significantly prevented decrease in femur density and significantly prevented decrease in femur & tibia weight, ash weight, ash calcium and phosphorus, serum calcium & phosphorus and femur density compared to control. Ostinu-2, very significantly prevented decrease in femur volume & density,

Table 1: Composition of Ostinu

Ingredients	Botanical name	Parts Used	Quantity per gm (in mg)
Amalaki	<i>Phyllanthus embelica</i>	Fruit	24
Arjuna	<i>Terminalia arjuna</i>	Bark	141
Guggulu	<i>Commiphora mukul</i>	Gum resin	118
Ashwagandha	<i>Withania somnifera</i>	Stem & Root	47
Asthishrunkala	<i>Cissus quardangularis</i>	Stem & leaves	141
Shanka bhasma	Incinerated counc	Counc shell	188
Pravala bhasma	Incinerated coral	Coral	188
Godanti bhasma	Incinerated godanti	Godanti	129
Kapardika bhasma	Incinerated kapardika	Kapardika	24

Table 2: Effect of Ostinu on Ovariectomized rats

S. No.	Parameters (Sham operated)	Normal (Ovariectomized)	Control 80mg/Kg	Ostinu 1 160mg/Kg	Ostinu 2 (Alendronate)	Standard
1.	Body Weight gain/loss (gm)	43.375 ± 2.666	57.625 ± 2.009 [#]	58.25 ± 3.544 [#]	59.500 ± 4.044 [#]	59.125 ± 3.131 [#]
2.	Femur Weight (mg)	548.662 ± 10.331 ^{**}	502.950 ± 4.462	536.60 ± 7.640 [*]	542.175 ± 7.009 [*]	544.612 ± 8.81 ^{**}
3.	Tibia Weight (mg)	421.425 ± 4.945 ^{**}	380.350 ± 10.238	414.34 ± 5.939 [*]	413.712 ± 6.668 [*]	416.075 ± 7.052 [*]
4.	Femur Length (mm)	42.451 ± 1.513	36.981 ± 1.644	40.39 ± 1.627	41.623 ± 1.366	41.755 ± 1.528
5.	Femur Width (mm)	4.6472 ± 0.1312	4.2345 ± 0.1062	4.6376 ± 0.1271	4.6376 ± 0.1271	4.71475 ± 0.1418
6.	Femur Volume (ml)	434.098 ± 5.534 ^{**}	408.007 ± 5.683	428.98 ± 3.859 [*]	431.433 ± 3.035 ^{**}	432.06 ± 4.321 ^{**}
7.	Femur Density (g/cc)	1.4922 ± 0.06229 ^{**}	1.2257 ± 0.03065	1.4431 ± 0.04497 ^{**}	1.4820 ± 0.03449 ^{**}	1.4710 ± 0.05082 ^{**}
8.	Serum Calcium	9.6393 ± 0.3457 ^{**}	8.1411 ± 0.2216	9.219 ± 0.2383 [*]	9.282 ± 0.2344 [*]	9.324 ± 0.2281 [*]
9.	Serum Phosphorus	6.1372 ± 0.2303 ^{**}	4.8786 ± 0.1772	5.787 ± 0.2614 [*]	5.839 ± 0.1616 [*]	5.822 ± 0.2656 [*]
10.	Femur Ash Weight (mg)	262.30 ± 3.855 ^{**}	240.76 ± 3.936	256.39 ± 11.663 [*]	258.8125 ± 3.289 [*]	259.875 ± 2.839 ^{**}
11.	Femur Ash Calcium (mg)	116.4062 ± 6.450 ^{**}	85.34875 ± 3.176	108.552 ± 6.859 [*]	114.085 ± 4.843 ^{**}	113.64 ± 5.306 ^{**}
12.	Femur Ash Phosphor (mg)	60.470 ± 2.745 ^{**}	43.3075 ± 1.818	55.7325 ± 3.316 [*]	58.506 ± 3.271 ^{**}	60.638 ± 3.700 ^{**}

One-way ANOVA followed by Tukey multiple comparison post test. * p < 0.05, ** p < 0.01 Vs control. [#] p < 0.05 Vs Normal

femur ash calcium & phosphorus and significantly prevented decrease in femur & tibia weight, ash weight, serum calcium & phosphorus compared to control. Standard drug alendronate very significantly prevented decrease in femur volume & density, femur ash weight, ash calcium & phosphorus, femur weight and significantly prevented decrease in tibia weight, Serum calcium & phosphorus compared to control (Table No. 2).

DISCUSSION

Ostinu is evaluated for its anti-osteoporotic activity using various parameters in clinically

important ovariectomized rat model of osteoporosis. We aimed at clinical application of Ostinu as a preventive, thus treatment with test drugs was started during induction of osteoporosis. Equivalent doses of Ostinu were extrapolated from intended clinical human dose based on body weight and surface area. The conversion factor used for dose conversion is seven.^{12,13} Alendronate is used as standard, as it is widely used treatment of osteoporosis. Protective effect of treatments on loss in bone volume, density and weight can be ascribed primarily to effect on bone mass. Ostinu prevented loss in bone calcium and phosphorus thus protecting against demineralization of bones due to

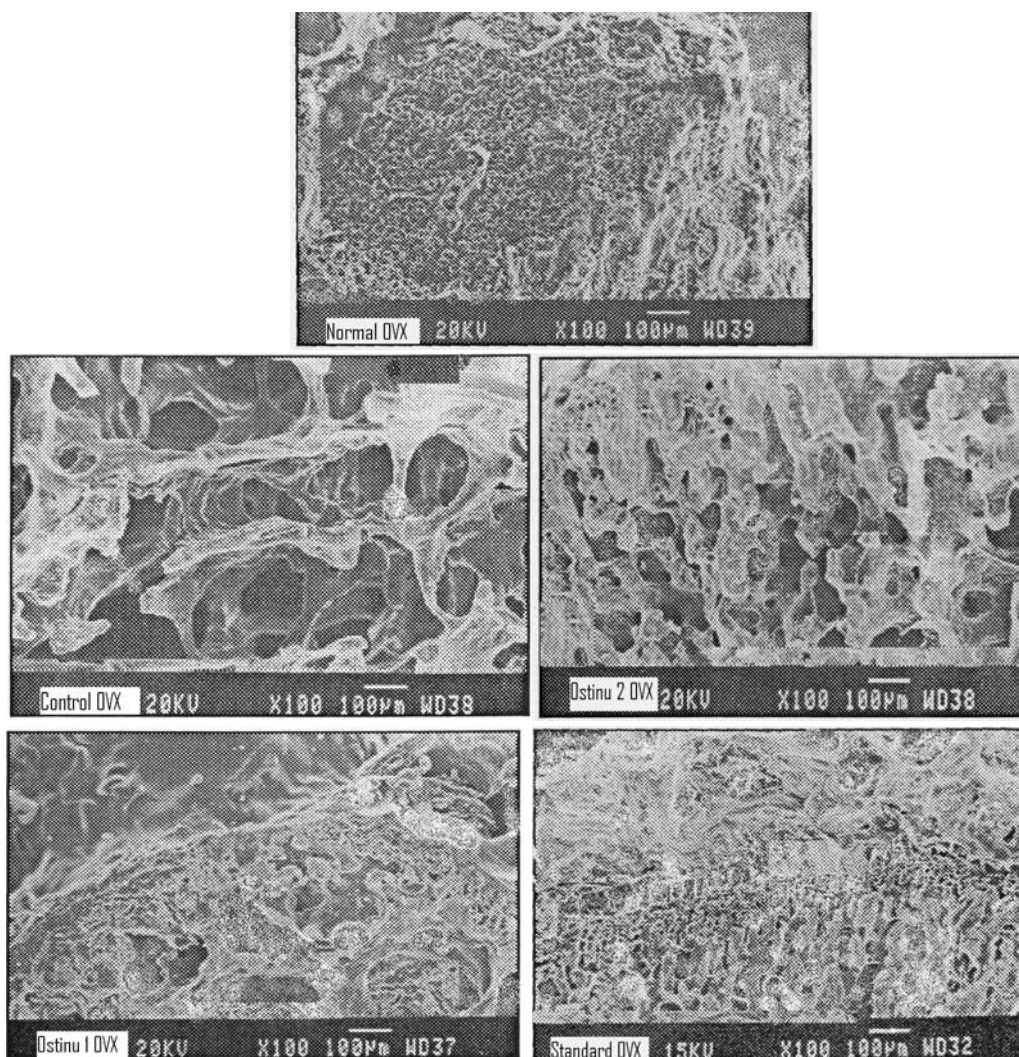


Fig. 1: The Scanning Electron Photomicrographs of Femurs at 100X

Table 2: Treatment groups

Group	Group Name	Treatment
I	Normal (Sham operated)	Vehicle (0.5% Sodium CMC in distilled water)
II	Control (Ovariectomized)	Vehicle (0.5% Sodium CMC in distilled water)
III	Ostinu 1	Ostinu 80mg/ Kg
IV	Ostinu 2	Ostinu 160mg/ Kg
V	Standard	Alendronate 1mg/ Kg

osteoporosis. Herbs and minerals in Ostinu may be acting synergistically to correct bone metabolic disorders in post menopausal osteoporosis. These findings suggest that Ostinu could be useful for managing post menopausal osteoporosis. This has a potential implication in further development of herbs and mineral formulations as anti osteoporotic agents.

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