Methanolic Extract of *Cuminum cyminum* Inhibits Ovariectomy-Induced Bone Loss in Rats

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Several animal and clinical studies have shown that phytoestrogens, plant-derived estrogenic compounds, can be useful in treating postmenopausal osteoporosis. Phytoestrogens and phytoestrogen-containing plants are currently under active investigation for their role in estrogen-related disorders. The present study deals with anti-osteoporotic evaluation of phytoestrogen-rich plant *Cuminum cyminum*, commonly known as cumin. Adult Sprague-Dawley rats were bilaterally ovariecto-mized (OVX) and randomly assigned to 3 groups (10 rats/group). Additional 10 animals were sham operated. OVX and sham control groups were orally administered with vehicle while the other two OVX groups were administered 0.15 mg/kg estradiol and 1 g/kg of methanolic extract of *Cuminum cyminum* fruits (MCC) in two divided doses for 10 weeks. At the end of the study blood, bones and uteri of the animals were collected. Serum was evaluated for calcium, phosphorus, alkaline phosphatase and tartarate resistant acid phosphatase. Bone density, ash density, mineral content and mechanical strength of bones were evaluated. Scanning electron microscopic (SEM) analysis of bones (tibia) was performed. Results were analyzed using ANOVA and Tukeys multiple comparison test. MCC (1 g/kg, p.o.) significantly reduced urinary calcium excretion and significantly increased calcium content and mechanical strength of bones in comparison to OVX control. It showed greater bone and ash densities and improved microarchitecture of bones in SEM analysis. Unlike estradiol it did not affect body weight gain and weight of atrophic uterus in OVX animals. MCC prevented ovariectomy-induced bone loss in rats with no anabolic effect on atrophic uterus. The osteoprotective effect was comparable with estradiol. Exp Biol Med 233:1403–1410, 2008

Key words: *Cuminum cyminum;* postmenopausal osteoporosis; phytoestrogen; luteolin; apigenin

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Introduction

Osteoporosis, although a major public health problem in aging communities, has received relatively little attention until recently. It is a systemic disease characterized by low bone mass and microarchitectural deterioration of the skeleton; leading to enhanced bone fragility and increased fracture risk (1). National Osteoporosis Foundation reported 10 million cases in the USA in 2004. Over 1.5 million fractures are caused by osteoporosis annually in the USA. According to the International Osteoporosis Foundation, osteoporosis affects an estimated 75 million people in Europe, USA and Japan. In most countries the incidence of osteoporosis is about 2–4 times higher in women than men because of postmenopausal hormonal changes. A sharp decrease in ovarian estrogen production is the predominant cause of rapid bone loss during the first decade after menopause.

Conventional therapies for postmenopausal osteoporosis include anti-resorptive agents like estrogen, calcitonin, bisphosphonates, etc. These agents, although well suited for the osteoporosis treatment, are not the ideal treatment. They do not change the bone mass or replenish the already lost bone. Estrogen is used for prevention and treatment for postmenopausal osteoporosis but its use is associated with high risk of endometrial or breast cancer and noncompliance of the patients. Use of bisphosphonates is associated with gastrointestinal side effects. There is a timely need to develop new drugs of natural or synthetic origin which will possess less undesirable side effects and can substitute or reduce the need for currently used drugs.

Increasing number of epidemiological, experimental and clinical studies has suggested that the consumption of phytoestrogen rich diet may have protective effects on estrogen-related conditions such as menopausal symptoms and estrogen-related diseases such as prostate and breast cancers, osteoporosis and cardiovascular diseases (2–5). Phytoestrogens are polyphenolic, non-steroidal plant-derived compounds with estrogen-like activity. Soy foods, which are reported to contain a high amount of phytoestrogens, have received considerable attention for their potential role in preventing osteopenia induced by ovar-

iectomy in rats or by menopause in women (2, 6). *Cuminum cyminum* (Apiaceae) (CC), commonly known as cumin, is a phytoestrogen-rich plant containing estrogenic components like β-sitosterol, stigmasterol and flavonoids luteolin and apigenin (7, 8). The plant has been evaluated earlier for ovicidal (9) and estrogenic action (10). Owing to its estrogenic nature it is possible that the plant may be useful in estrogen-related disorders like postmenopausal osteoporosis. The present study was undertaken to evaluate the effect of methanolic extract of *Cuminum cyminum* (MCC) on bone loss induced by ovariectomy in young adult rats. The objective of the study was to assess the effect of cumin extract on estrogen-deficiency induced bone loss and not the bone loss induced due to combined effect of age and hormonal deficiency as in the case of aged ovariectomized (OVX) rats.

Materials and Methods

Plant Material and Extraction. Fruits of CC were procured from an ayurvedic clinician in Mumbai, India and authenticated at Blatter Herbarium, St. Xavier's College, Mumbai, India. MCC was obtained by soxhlet extraction of CC fruits with methanol for 48 h and evaporation of methanol to get the semi-solid extract.

Phytochemical Analysis. Qualitative phytochemical evaluation of MCC was performed according to Khandelwal (11). Quantification of total flavonoidal content was done spectrophotometrically by aluminium chloride method (12) and total flavonoidal glycosidal content was estimated by high performance thin layer chromatographic (HPTLC) analysis using luteolin glycoside as reference compound.

Animals. The animal experiment protocol was approved by Institutional Animal Ethics Committee, Bombay College of Pharmacy, Mumbai, India and was performed in accordance with guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Forty adult virgin female Sprague-Dawley rats (12 weeks old, 200–250 g) were randomized in four treatment groups of ten animals each. Animals were housed at 23 ± 2°C and relative humidity 60 ± 5% with 12-h light-dark cycle. Food (standard rodent pellet diet) and water was provided *ad libitum*. Three groups were bilaterally ovariectomized while one was subjected to sham operation. Ovariectomy was performed under ketamine + xylazine anaesthesia (80 mg/kg + 10 mg/kg; i.p.) according to the method described by Wanforth *et al*. (13). Animals were housed individually for a week after the surgery and later on in a group of five. Body weight of the animals was recorded weekly.

Study Design. Sham (SH) control and OVX control animals were administered orally 0.5% w/v sodium carboxy methyl cellulose (sodium CMC) (sdfine, India) which was used as vehicle. The other two OVX groups were administered β-Estradiol 3-benzoate (Sigma) equivalent to 0.15 mg/kg of β-estradiol and 1 g/kg of MCC. Dose of estradiol was selected on the basis of doses used by earlier researchers for the same activity (14, 15). Dose of MCC was decided based on the results of the preliminary study performed in our laboratory (at 0.5 and 1 g/kg, administered orally for 6 weeks; data not shown). Suspensions of β-estradiol benzoate and MCC were prepared in 0.5% w/v of sodium CMC. Drug treatments were started a day after ovariectomy and were given in two divided doses (at 10.00 h and 18.00 h) with 2 ml/kg dosing volume and continued for 10 weeks.

On the last day of treatment urine was collected by micturation induced by manual pressure (16) from overnight fasted animals and preserved at –20°C till further analysis. At necropsy, on day 71, blood was collected from dorsal aorta under ether anesthesia. After, centrifugation serum was harvested and kept at –20°C until analysis. Femur and tibia of all animals were separated from adjacent tissue, cleaned and preserved in 70% ethanol at 4°C till further analysis. Uteri were isolated, and absolute weight of uterine tissue was recorded and normalized with body weight (relative weight of uterus, i.e., weight of uterus per 100 g of body weight) of animals.

Serum Estradiol Assay. Serum estradiol levels were determined by ELISA (DRG Instruments GmBH, Germany).

Biochemical Analysis. Serum alkaline phosphatase (ALP) and tartarate resistant acid phosphatase (TRAP) activity was determined by nitrophenol based method as described by Bessy *et al*. (17) and Godkar (18) respectively. Serum calcium (Ca) and phosphorus (P) were measured by using commercially available Ca estimation kit (Accurex, India), based on o-cresophthalein complexon method (19) and ammonium molybdate method (20), respectively. Serum total proteins (TP) were measured by Folin-Lowry method (21). Calcium content of urine was measured by flame photometry (22) (Systronics, Mumbai, India) after suitable dilution with double distilled water.

Bone Density, Ash Density and Mineral Content. Bone volume and density were measured by the method based on Archimedes' principle (23, 24). For ashing, one tibia (the one which was used for volume and density measurement) of each animal was weighed, kept in silica crucible and ashed at 700°C for 7 h. The total ash weights were recorded and ash density (ash weight/bone volume) was calculated.

For bone Ca and P estimation, ash was dissolved in 6 M hydrochloric acid (1 ml of acid for 50 mg of ash). After neutralization with sodium hydroxide and suitable dilution, Ca was measured using the Ca estimation kit. Phosphorus was measured by the method mentioned previously. Bone Ca and P contents were normalized with bone weight.

Bone Mechanical Strength. Mechanical strength of bones was measured by three point bending test (TPBT) (25) for tibia and femoral neck strength (FNS) (26) using

Table 1. Effect of Estradiol (0.15 mg/kg, p.o., 10 Weeks) and MCC (1 g/kg, p.o., 10 Weeks) on Serum Biochemical Parameters and Urinary Calcium Excretion in Ovariectomized Rats ($n = 10$/Group)[a]

	Serum ALP (mM)	Serum TRAP (uM)	Serum Ca (meq/L)	Serum P (mg/dl)	Serum TP (mg/dl)	Urinary Ca (mg/dl)
Sham control	2.63 ± 0.28	0.16 ± 0.02	5.78 ± 0.29	36.29 ± 1.35	10.47 ± 0.27	51.54 ± 6.42
OVX control	3.19 ± 0.15	0.21 ± 0.03	5.51 ± 0.24	35.67 ± 1.71	10.75 ± 0.29	109.91[a] ± 14.30
Estradiol	2.62[b] ± 0.18	0.19 ± 0.03	5.75 ± 0.28	33.99 ± 1.66	10.66 ± 0.28	72.97 ± 3.85
MCC	2.28[b] ± 0.25	0.26 ± 0.01	5.07 ± 0.07	32.60 ± 1.28	11.57 ± 0.26	45.82[b] ± 6.37

[a] Values are mean ± SEM.
[a] $P < 0.05$ vs. SH control; [b] $P < 0.05$ vs. OVX control.

Instron material testing machine. For TPBT, tibia was placed on 2 supports 13 mm apart. Load was applied to midshaft, with a cross-head speed of 10 mm/minute. The cross head compressed the middle of the tibial shaft with linearly increasing force, until fracture occurred. The load at which bone broke was recorded.

For FNS, the proximal one-half portion of femora was embedded into a block made with white cement. Linearly increasing force with cross head speed 20 mm/minute was applied to femoral head and the force causing femoral neck fracture was measured. Mechanical strength values in TPBT and FNS both were normalized for bone weight.

Scanning Electron Microscopy (SEM). After mechanical strength tests, fractured surfaces of tibia were examined by SEM at magnification of ×600 for qualitative estimation of bone resorption. SEM examinations were carried out for bone resorption pits. The resorption pits and irregularities on the bone surface were distinguished by careful observation of secondary and back scattered SEM photomicrographs simultaneously. Representative SEM photomicrographs were analyzed for number of resorption pits using image analysis software (Dewinter Biowizard 4.1, Dewinter Software Inc.).

Statistical Methods. Group means were compared by ANOVA with GraphPad Prism (GraphPad Software, Inc.). Multiple comparison tests were performed with Tukey for significant differences at $P < 0.05$.

Results

Phytochemical Analysis. Qualitative phytochemical analysis of MCC attested presence of steroids, flavonoids and saponins. MCC was found to contain 1.05% w/w of flavonoids by aluminium chloride method. HPTLC analysis showed presence of luteolin glycoside, an estrogenic flavonoid along with some other closely related flavonoidal glycosides. Total flavonoidal glycoside content was found to be 45.20% w/w (data not shown).

Serum Estradiol Assay. Significantly diminished levels of serum estradiol in all OVX animals except the animals treated with estradiol, confirmed unerring ovariectomy (data not shown).

Biochemical Analysis. Serum ALP and TRAP levels in OVX control animals were raised in comparison with SH control animals. Estradiol as well as MCC caused significant decrease in ALP levels but no change in TRAP levels was observed. Serum Ca and P levels of OVX control, estradiol and MCC treated animals were similar to SH control animals. Significant increase in urinary Ca excretion was observed in OVX control. MCC significantly decreased urinary Ca excretion while estradiol showed a reduction, though not significant (Table 1).

Bone Density, Ash Density and Mineral Content. Bone and ash densities of OVX control animals were found to be less than SH control animals. Estradiol and MCC improved bone densities and significantly increased ash densities in OVX animals. Ovariectomy caused significant decrease bone Ca (content per unit mass of bone). Bone P (content per unit mass of bone) was reduced but not significantly in OVX animals as compared to SH control. Significant rise in bone Ca was observed with estradiol and MCC. Bone P content was improved but not significantly by estradiol and was unaffected by MCC treatment (Table 2).

Bone Mechanical Strength. Maximal load values for FNS were significantly reduced in OVX control animals while values for TPBT were unaffected. Mechanical strength in FNS was significantly increased by treatment of estradiol and MCC (Fig. 1).

Scanning Electron Microscopy. Representative photomicrographs (secondary and back scattered images) of each treatment group are given in Figure 2. SEM analysis and image analysis of SEM photomicrographs revealed that resorption sites are more in number in OVX control animals than in SH control animals. Treatment with estradiol and MCC both improved the microarchitecture of bones.

Body Weight and Uterine Weights. The mean body weight gain of OVX control animals over a 10-week period of study was more as compared to SH control. MCC did not bring down the increased body weight gain of OVX animals while estradiol caused a small reduction. Absolute and relative uterine weight was significantly lower in OVX rats than in SH rats. Significant increase was observed with estradiol whereas MCC did not show any increase in weight of atrophic uterus (Table 3).

Table 2. Effect of Estradiol (0.15 mg/kg, p.o., 10 Weeks) and MCC (1 g/kg, p.o., 10 Weeks) on Bone Density and Mineral Content in Ovariectomized Rats ($n = 10$/Group)[a]

	Bone density (g/cc)	Ash density (g/cc)	Bone Ca/bone weight (mg)	Bone P/bone weight (mg)
Sham control	11.30 ± 1.20	3.45 ± 0.48	107.41 ± 9.13	421.21 ± 39.21
OVX control	8.72 ± 0.95	2.90 ± 0.32	65.11[a] ± 4.98	352.88 ± 33.12
Estradiol	12.27 ± 1.66	4.16[b] ± 0.28	79.72[b] ± 6.54	421.70 ± 40.11
MCC	13.21 ± 1.70	4.67[b] ± 0.63	138.91[b] ± 11.81	365.65 ± 38.02

[a] Bone volume and density of tibia samples was determined by Archimedes' principle. Bones (tibia) were ashed at 700°C for 7 h; ash density was calculated as ash weight/bone volume. Ca and P content were determined by dissolving ash in 6 M hydrochloric acid. Mineral content values were normalized with bone weight. Values are mean ± SEM.
[a] $P < 0.05$ vs. SH control; [b] $P < 0.05$ vs. OVX control.

Discussion

The present study is a comprehensive investigation of anti-osteoporotic potential of *Cuminum cyminum* in ovariectomized rats. *Cuminum cyminum* is a phytoestrogen-rich plant reported to contain estrogenic flavonoids and some sterols with estrogenic activity. Our phytochemical analysis confirmed the presence of luteolin glycoside in MCC and the content of total flavonoidal glycosides was found to be 45.20% (data of phytochemical analysis is not presented since it is beyond the scope of paper). Owing to the estrogenic nature and the content of flavonoids in plant, it was decided to evaluate its anti-osteoporotic potential in estrogen-deficiency induced osteoporosis.

Ovariectomized rat model is a well established model for estrogen-deficiency induced bone loss and has been used by a large number of researchers previously (27–29). Young adult rats of 12 weeks of age (30) have been used in the study. Rats in this age group are skeletally mature and do not feature fast growing bone (as in case of young rats) or age-related bone loss (as in aged rats). The experimental model differs from aged-OVX rats wherein the osteoporosis is induced only by estrogen deficiency and not by a combination of natural bone loss due to age and ovarian hormone deficiency.

Bone loss after ovariectomy is associated with high bone turnover where bone resorption exceeds the bone formation. In the present study bone turnover was assessed by serum ALP and TRAP, commonly used bone remodeling markers. OVX control animals showed increased bone turnover, as demonstrated by increased ALP and TRAP activity, than SH control rats. Estradiol was better than MCC in controlling the accelerated bone turnover. The homeostasis of serum Ca and P was maintained even after ovariectomy and was not disturbed by either estradiol or MCC. The measurement of Ca and P was associated with serum total protein estimation in order to ensure that serum protein levels are normal and there is no excessive binding of serum Ca to proteins.

Archimedes' principle, used to measure bone density, is the standard method for determination of density (g/cm^3) of bones of small animals and yields results statistically comparable with dual-energy X-ray absorptiometry (DEXA) (31). In the present study OVX animals showed decrease in bone and ash densities and significant reduction in bone Ca compared to SH control animals. Ash density and bone Ca were significantly increased by MCC as well as estradiol. It is noteworthy that the values obtained for bone Ca in case of MCC treated animals were much higher than the corresponding values of SH control.

Previous studies in rats have shown inconsistent results in that urinary Ca excretion has been shown to increase (28, 32) or decrease (29, 33, 34) after ovariectomy. In the present study significant reduction in urinary excretion of Ca was observed in OVX control animals as compared to SH control. Several findings indicate that the fall in serum calcitonin may be secondary to bone loss due to ovariectomy (23). Calcitonin inhibits renal calcium excretion at physiological concentrations. Thus the increase in urinary excretion of Ca observed in OVX animals can be due to decreased levels of calcitonin secondary to ovariectomy. Decrease in renal excretion of calcium by estradiol can be attributed to its ability to increase renal reabsorption (28) or restoration of level of serum calcitonin, which is found to be decreased after ovariectomy. At this point we are unaware whether MCC also possess any such effects. Significant decrease in bone Ca and no change in serum Ca and concomitant significant increase in urinary Ca excretion in OVX control rats indicated accelerated bone turnover, where bone resorption is exceeding bone formation, which was underlined by serum ALP and TRAP

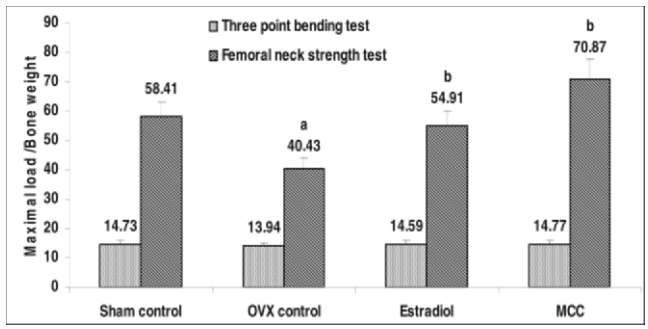

Figure 1. Effect of estradiol (0.15 mg/kg, p.o., 10 weeks) and MCC (1 g/kg, p.o., 10 weeks) on mechanical strength of bones ($n = 10$/group). Values are mean ± SEM. [a] $P < 0.05$ vs. SH control; [b] $P < 0.05$ vs. OVX control.

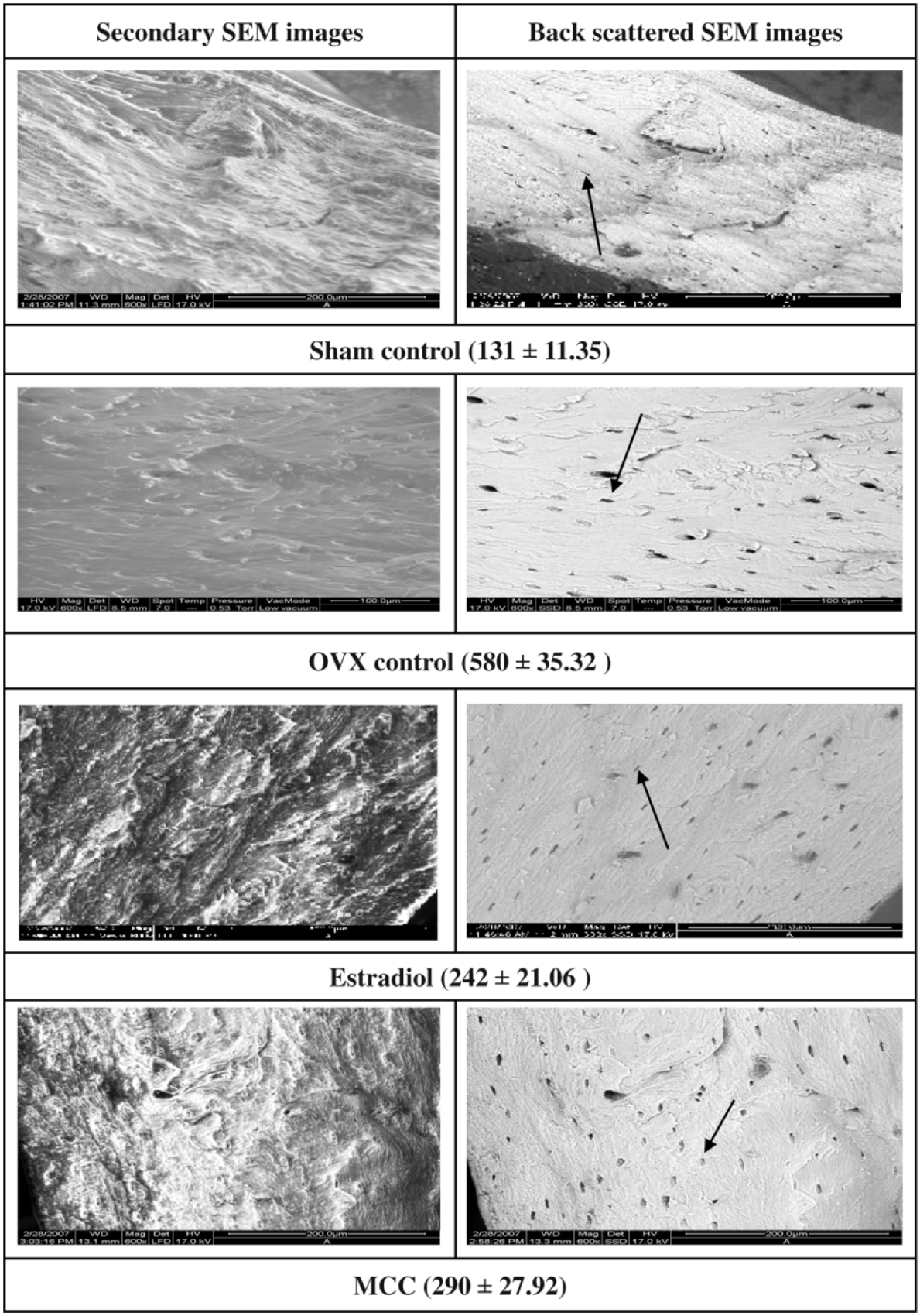

Figure 2. Representative SEM photomicrographs of fractured surfaces of tibia. Bone resorption sites are shown by arrows. Resorption sites and irregularities on the bone surface are differentiated by careful observation of secondary and back scattered images simultaneously. Values in parenthesis denote mean ± SEM of number of resorption pits counted with the help of image analysis software; $n = 6$. MCC and estradiol decreased the number of resorption sites as compared to OVX control.

Table 3. Effect of Estradiol (0.15 mg/kg, p.o., 10 Weeks) and MCC (1 g/kg, p.o., 10 Weeks) on Body Weight Gain and Weight of Uterus ($n = 10$/Group)[a]

	Body weight gain (g)	Absolute weight of uterus (mg)	Relative weight of uterus (mg)
Sham control	54.11 ± 4.33	338.10 ± 21.11	162.52 ± 8.53
OVX control	84.90[a] ± 7.20	77.84[a] ± 3.13	31.23[a] ± 9.48
Estradiol	79.80 ± 6.82	169.49[b] ± 29.25	57.11[b] ± 3.01
MCC	92.60 ± 9.68	76.43 ± 2.50	30.30 ± 1.46

[a] Relative weight of uterus is the weight of uterus normalized with body weight (mg per 100 g of body weight). Values are mean ± SEM.
[a] $P < 0.05$ vs. SH control; [b] $P < 0.05$ vs. OVX control.

levels. Treatment with MCC significantly decreased urinary excretion of Ca, which compliments other observations like significant increase in bone Ca and ash density and increase in bone density.

Mechanical strength of bones is the most important parameter related to fracture risk and is a direct measure of the same. The bone composition for long bones at midshaft, proximal and distal region differs. The midshaft region is dominated by >95% cortical bone whereas the proximal femur is dominated by cancellous bone (27). The effect of menopause or ovariectomy is more pronounced on cancellous than cortical bone and estrogen has been known to have little effect on reducing cortical bone loss (23, 35). Our results are in agreement with these findings as maximal load values in TPBT were unaffected by ovariectomy, while there was significant decrease in maximal load values for FNS indicating the significant loss of cancellous bone. Estradiol as well as MCC significantly improved the mechanical strength of cancellous bone.

Microarchitectural observation of fractured surfaces of tibia (midshaft region) was performed with SEM. Results of SEM analysis showed poor microarchitecture of bone (tibia) with increase in number of resorption pits (as seen by image analysis of SEM photomicrographs) in OVX control animals as against SH control. As mentioned earlier midshaft of tibia consisted primarily of cortical bone which is reported to be less affected by ovariectomy. The findings that the inferior microarchitecture seen in SEM analysis of tibia of OVX animals and no effect on mechanical strength of tibia as indicated by bone mechanical testing seems to be contradictory. It can be speculated that resorption activity increases in OVX animals even in cortical bone but not to the extent which affects the mechanical strength of bone or at least not within 10 weeks of ovariectomy (duration of the study). Treatment with MCC and estradiol improved the microarchitecture and supported the findings of bone mechanical strength. Mechanical strength is a product of bone mass and quality (36). Increase in bone mass is evident from increased bone and ash densities in estradiol and MCC treated animals. The parallel increase in mechanical strength of bones in FNS and improved microarchitecture in SEM analysis signifies the bone preserving effect of MCC and estradiol in terms of bone mass as well as the quality.

Estradiol significantly increased weights of atrophic uteri and also decreased the body weight gain in OVX animals while MCC did not exhibit any such effects. Estradiol and MCC both significantly prevented estrogen deficiency induced bone loss in animals but differed in effect on uterus, a primary target organ for estrogen. It is possible that they differ in the mechanism of action. As mentioned earlier and confirmed by the phytochemical analysis, MCC is rich in estrogenic isoflavonoid, luteolin. It has been demonstrated earlier that luteolin has an anabolic effect on an osteoblastic MC3T3-E1 cells and it decreased the 3-morpholinosydnonimine-induced production of nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in osteoblasts (37). Role of oxidative stress (38) and TNF-α (39) in osteoclastic bone resorption is well documented. The discrepancy between the bone and uterine effects of MCC can be explained by the tissue selective effects of phytoestrogens, similar to the selective estrogen receptor modulation by tamoxifen, raloxifene. Expression of ERβ in osteoblasts is greatly increased during bone mineralization and is primarily responsible for osteoprotective effect of phytoestrogens which show much higher affinity for ERβ than for ERα. Apigenin, the second major isoflavonoide of cumin, has been shown to be more ERβ active (40).

MCC was found to be osteoprotective at 1 g/kg in OVX rats where the treatment was started immediately after ovariectomy. From the study design it is difficult to extrapolate the optimum dose of MCC and whether it will be effective in established osteoporosis. To know the effectiveness of MCC in osteoporosis induced in human by menopause and aging, an additional study in aged OVX rats is being undertaken in our laboratory. The dose of MCC is too high to be translated into human therapeutic dose, therefore the active efforts are going on to isolate and purify the active principles of the extract which would minimize the dose.

Results of the present investigation indicate that MCC has bone preserving effect comparable with estradiol. Estrogen replacement therapy which is the popular regime for postmenopausal osteoporosis is associated with increased risk of breast, ovarian and endometrial cancer (41). Anti-osteoporotic activity of MCC is associated with no anabolic effect on uterus. This selective action of MCC may

be useful in terms of reduced risk of untoward effects observed with hormone replacement therapy.

In conclusion our results show that *Cumunum cyminum*, a commonly used spice, possesses significant anti-osteoporotic activity in OVX rats. It improved mass, quality and mechanical strength of bones in OVX rats with no uterotrophic activity. The bone sparing action of MCC may be associated with its isoflavonoidal content and the mechanism of action possibly includes activation of ERβ, anabolic action on osteoblasts and inhibitory effect on oxidative stress and inflammatory cytokines. It can help postmenopausal women from losing their bone and seems to be a potential candidate for the development of new herbal approaches for osteoporosis treatment without any serious side effects.

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1. World Health Organization. Guidelines for Preclinical Evaluation and Clinical Trials in Osteoporosis. Geneva, Switzerland, 1998.
2. Setchell KDR, Lydeking-Olsen E. Dietary phytoestrogens and their effects on bone: evidence from in vitro and in vivo, human observational and dietary intervention studies. Am J Clin Nutr 78 (suppl):593S–609S, 2003.
3. Cos P, Bruyne TD, Apers S, Berghe DV, Pieters L, Vlietinck AJ. Phytoestrogens: recent developments. Planta Medica 69:589–599, 2003.
4. Rishi RK. Phytoestrogens in health and illness. Ind J Pharmacol 34: 311–320, 2002.
5. Tham DM, Gardener CD, Haskell WL. Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological and mechanistic evidence. J Clin Endocrinol Metab 83:2223–2235, 1998.
6. Brynin R. Soy and its isoflavones: a review of their effects on bone density. Altern Med Rev 7:317–327, 2002.
7. Dr. Dukes Phytochemical and ethnobotanical databases. Available at: http://www.drdukes.com. Accessed January 24, 2005.
8. *Cuminum* linn. In: Batnagar SS, Chopra RN, Prashad B, Ghosh JC, Saha MN, Lala S, Santapau H, Sastri BN, Eds. The Wealth of India—A dictionary of Indian raw materials and industrial products—Raw materials. Delhi, India: Director Council of Scientific and Industrial Research, Vol. II:pp396–398, 1950.
9. Berger TBM, Erler F, Dagh F. Ovicidal activity of essential oils from five plants against two stored-product insects. J Sto Prod Res 36:161–168, 2000.
10. Malini T, Vanithakumari G. Estrogenic activity of *Cuminum cyminum* in rats. Ind J Exp Biol 25:442–444, 1987.
11. Khandelwal KR. Phytochemical evaluation. In: Practical Pharmacognosy Techniques and Experiments (12th ed.). Pune, India: Nirali Prakashan, pp149–156, 2004.
12. Malencic D, Popovic M, Miladinovic J. Phenolic content and antioxidant properties of soybean (*Glycine max* (L.) Merr.) seeds. Molecules 12:576–581, 2007.
13. Wanforth HB, Flecknell PA. Specific surgical operations. In: Experimental and Surgical Technique in the Rat (2nd ed.). New York: Academic Press, pp203–312, 1992.
14. Yin J, Tezuka Y, Subehan, Shi L, Nobukawa M, Kadota S. *In vivo* anti-osteoporotic activity of isotaxiresinol, a lignan from wood of Taxuz yunnanesis. Phytomedicine 13:37–42, 2006.
15. Ke HZ, Simmons HA, Pirie CM, Crawford DT. Droloxifene, a new estrogen antagonist/agonist, prevents bone loss in ovariectomized rats. Endocrinology 136: 2435–2441, 1995.
16. Weiss J, Taylor GR, Zimmermann F, Nebendahl K. Collection of body fluids. In: Laboratory Rat Book. New York: Academic Press, pp485–510, 2000.
17. Bessy OA, Lowry OH, Brock MJ. A method for the rapid determination of alkaline phosphatase with five cubic millimetres of serum. J Biol Chem 164:321–329, 1946.
18. Godkar P. Enzymes. In: Textbook of Medical Laboratory Techniques, Ed. Mumbai, India: Bhalani Publishing House, pp149–167, 1994.
19. Connerty HV, Briggs AR. Determination of serum calcium by means of o-cresophthalein complexon. Am J Clin Pathol 45:290–296, 1966.
20. Godkar P. Water and mineral metabolism. In: Textbook of Medical Laboratory Techniques, Ed. Mumbai, India: Bhalani Publishing House, pp233–251, 1994.
21. Lowry O, Rosebrough N, Farr AL, Randall R. Protein measurement with the folin Phenol reagent. J Biol Chem 193:265–275, 1951.
22. Changrani NR, Chonkar A, Adeghate E. Effects of streptozotocin-induced type 1 diabetes mellitus on total protein concentrations and cation contents in the isolated pancreas, parotid, submandibular, and lacrimal glands of rats. Ann N Y Acad Sci 1084:503–519, 2006.
23. Kalu DN. Evaluation of the pathogenesis of skeletal changes in ovariectomized rats. Endocrinology 115:507–512, 1984.
24. Lee YB, Lee HJ, Kim KS, Lee JY, Nam SY, Cheon SH, Sohn HS. Evaluation of preventive effect of isoflavone extract on bone loss in ovariectomized rats. Biosci Biotech Biochem 68:1040–1045, 2004.
25. Peng Z, Tuukkanen J, Zhang H. The mechanical strength of bone in different rat models of experimental osteoporosis. Bone 15:523–532, 1994.
26. Pytlik M, Janiec W, Cegiela U, Silwinski L. Influence of α- Eschin on the femoral bone strength in ovariectomized rats. Pol J Pharmacol 51: 511–515, 1999.
27. Kalu DN, Liu CC, Hardin R, Hollis BW. The aged rat model of ovarian hormone deficiency bone loss. Endocrinology 124:7–16, 1989.
28. Cai D J, Zhao Y, Glasier J, Cullen D, Barnes S, Turner CH, Wastney M, Weaver CM. Comparative effect of soy protein, soy isoflavones and 17βestradiol on bone metabolism in adult ovariectomized rats. J Bone Miner Res 20:828–839, 2005.
29. Horcajada-Molteni MN, Crespy V, Coxam V, Davicco MJ, Remesy C, Barlet JP. Rutin inhibits ovariectomy-induced osteopenia in rats. J Bone Min Res 15:2251–2258, 2000.
30. Zhang Y, Lai WP, Leung PC, Wu CF, Wong MS. Short- to mid term effects of ovariectomy on bone turnover, bone mass and bone strength in rats. Biol Pharm Bull 30:898–903, 2007.
31. Keenan MJ, Hegsted M, Jones KL, Delany JP, Kime JC, Melancon LE, Tulley RT, Hong KD. Comparison of bone density measurement techniques: DXA and Archimedes principal. J Bone Miner Res 12: 1903–1907, 1997.
32. Arjmandi BH, Hollis BW, Kalu DN. *In vivo* effect of 17β estradiol on intestinal calcium absorption in rats. Bone Miner 26:181–189, 1994.
33. Reddy PN, Lakshmana M. Prevention of bone loss in calcium deficient ovariectomized rats by OST-6, a herbal preparation. J Ethnopharmacol 84:259–264, 2003.
34. Fanti P, Monier-Faugere MC, Geng Z, Schmid J, Morris PE, Cohen D, Malluche HH. The phytoestrogen genistein reduces bone loss in short-term ovariectomized rats. Osteoporos Int 8:274–281, 1998.
35. Turner RT, Riggs BL, Spelsberg TC. Skeletal effects of estrogen. Endocrin Rev 15:275–300, 1994.
36. Recker RR. Low bone mass may not be the only cause of skeletal fragility in osteoporosis. Proc Soc Exp Biol Med 191:272–274, 1989.

37. Choi EM. Modulatory effects of luteolin on osteoblastic function and inflammatory mediators in osteoblastic MC3T3-E1 cells. Cell Biol Int 31:870–877, 2007.
38. Lean JM, Davis JT, Fuller K, Jagger CJ, Kirstein B, Partington GA, Zoe LU, Chambers TJ. A crucial role for thiol anti-oxidants in estrogen-deficiency induced bone loss. J Clin Invest 112:915–923, 2003.
39. Moffett SP, Zmuda JM, Oakley JI, Beck TJ, Cauley JA, Stone KL, Lui LY, Ensrud KE, Hillier TA, Hochberg MC, Morin P, Peltz G, Greene D, Cummings SR. Tumor necrosis factor-α polymorphism, bone strength phenotypes and the risk of fracture in older women. J Clin Endocrinol Metab 90:3491–3497, 2006.
40. Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S and Gustafsson JA. Comparison of ligand binding specificity and transcription tissue distribution of estrogen receptors α and β. Endocrinology 138:863–870, 1997.
41. Young RL, Kumar NS, Goldzieher JW. Management of menopause when estrogen can not be used. Drugs 40:220–230, 1990.