

Maxcal-C (a polyherbal formulation) prevents ovariectomy-induced osteoporosis in rats

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ABSTRACT

Objectives: The aim of the present study was to investigate the anti-osteoporotic activity of Maxcal-C in ovariectomy (OVX)-induced osteoporosis in rats.

Materials and Methods: Sham-operated control rats were designated as Group I; Group II animals served as OVX control; Group III OVX control rats treated with Calcium Sandoz (50 mg/kg, p.o.); Group IV and V OVX control rats treated with Maxcal-C (250 and 500 mg/kg, p.o.), respectively. All the aforementioned treatments were given for four weeks after the development of osteoporosis. At the end of the treatment, serum biochemical parameters such as serum calcium and alkaline phosphate were measured. After sacrificing the animals, femoral bone parameters with histology, body weight, and bone breaking strength of 5th lumbar vertebra were measured.

Results: The treatment with Maxcal-C showed a significant improvement in serum biochemical, femoral bone parameters, and bone breaking strength of 5th lumbar vertebra with histopathological changes.

Conclusion: The finding of the present study indicates that Maxcal-C showed a potential anti-osteoporotic activity. These results support the traditional use of Maxcal-C in the treatment of osteoporosis.

KEY WORDS: Bone breaking strength, femoral bone parameters, Maxcal-C, ovariectomy rats, serum calcium

Introduction

Osteoporosis is a universal problem that affects harmfully to the postmenopausal women and elderly men as well.^[1] It is characterized by a reduction in bone density and strength to the degree that fractures occur after minimal trauma.^[2] At present, conventional therapy is used for the treatment of osteoporosis such as supplementation of estrogen, calcitonin, progestin, bisphosphonates, etc.^[3] However, these agents have several drawback, and available data suggested that use of estrogen replacement therapy for long-time may cause severe side effects such as uterus and breast cancer.^[4] Moreover, selective estrogen receptor modulators (raloxifene, lasofoxifene, femarelle, etc.) and various types of bisphosphonates are

used for the treatment of osteoporosis. These drugs are also responsible for undesirable adverse effects.^[5] The management of osteoporosis is still a challenge. Therefore, researchers are looking for a good alternative drug from herbal origin with little side effects worldwide.

At present, there are various polyherbal formulations available in the market for the treatment of osteoporosis. Maxcal-C is one of the polyherbal formulations (Vasu Research Centre, Vadodara) that consist of several herbal extracts as shown in Table 1.^[6-10] So far, there is little work has been done on this herbal drug for its anti-osteoporotic action. Therefore, in the present study, we try to investigate anti-osteoporotic effect of Maxcal-C in ovariectomy (OVX)-induced osteoporosis in rats.

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Table 1:**Composition of Maxcal-C (a polyherbal formulation)**

Ingredients	Botanical name/ english name	Part used	Quantity (mg)
Extract of			
Amalaki	<i>Embllica officinalis</i>	Fruit	100
Godanti Bhasma	Gypsum	Mineral	225
Shankh Bhasma	Conch shell	Mineral	125
Kapardika Bhasma	Marine shell	Mineral	125
Shukti Bhasma	Oyster	Mineral	100
Kukkutandatvak Bhasma	Egg shell	Mineral	25
Haritaki	<i>Terminalia chebula</i>	Fruit	25

Materials and Methods**Drugs and Chemicals**

Maxcal-C (a polyherbal formulation) was gifted by Vasu Research Center, Vadodara, India. Standard drug Calcium Sandoz (cholecalciferol), ketamine hydrochloride, diazepam, povidone, and ampicillin were purchased from commercial market. All biochemical kits were purchased from Span Diagnostics Limited, Surat, India. All other chemicals and reagents used in the study were of analytical grade.

Experimental Animals

Female albino Wistar rats (200–250 g) were obtained from Zydus Research Centre, Ahmedabad, India. All animals were maintained under standardized condition (12-h light/dark cycle, 24°C ± 2°C and humidity 35–60%), and they were allowed free access to low calcium diet and water *ad libitum*. The rats were left for 48 h for adaptation prior to the beginning of the experiment. The study was approved by Institutional Animal Ethics Committee and carried out in accordance with Committee for the Purpose of Control and Supervision of Experiment on Animal guidelines.

Acute Toxicity Study

On the basis of OECD guideline no. 423, the acute oral toxicity was carried out in albino Wistar rats weighing 200–250 g.^[11] Maxcal-C was given (at the dose of 100, 200, 500, 1000, 2000, and 2500 mg/kg, p.o.) for three animals and the signs and symptoms were observed after 0, 30, 60, 120, 180, and 240 min and then once a day for next 14 days.

Ovariectomy of Rats

One-week after acclimatization, all rats were randomly subjected to OVX. To conduct OVX, operating table and surgical instruments were sterilized with alcohol. The rats were anesthetized with injection of ketamine hydrochloride (40 mg/kg, i.p.) and diazepam (5 mg/kg, i.m.). Bilateral dorsal incisions were made on the back, both the ovaries were identified. The ovarian blood vessels were clamped, and the ovaries were removed. The muscle layer was tied, and skin incision was sutured. In the sham operation, the ovaries were exposed as above and manipulated gently but not excised. The animals were given ampicillin sodium (25 mg/kg, i.p.) for 3 days, and povidone-iodine powder applied locally. All rats were untreated for 28 days after surgery to allow for the development of osteoporosis.^[12,13]

Confirmation of Osteoporosis

After 4 weeks, urine was collected from each rat. Urine calcium and creatinine were measured using commercial kits and ultraviolet-visible spectrophotometer (Shimadzu 1800).^[14]

Treatment Schedule

After confirmation of osteoporosis, all rats were divided randomly into four groups ($n = 6$ in each group). Sham-operated control group ($n = 6$) was kept different from the OVX groups.

- Group I: Sham-operated control (1 ml/kg of 1% of sodium carboxymethyl cellulose [CMC], p.o.)
- Group II: OVX Control (1 ml/kg of 1% of sodium CMC, p.o.)
- Group III: OVX + Calcium Sandoz (50 mg/kg, p.o.)
- Group IV: OVX + Maxcal-C (250 mg/kg, p.o.)
- Group V: OVX + Maxcal-C (500 mg/kg, p.o.).

All the treatments were administered orally to the respective groups for 4 weeks after the development of osteoporosis.

At the end of the experiments, blood samples were collected from the retro orbital plexus of rats under light ether anesthesia using glass capillaries. For separation of serum, blood was allowed to clot for 15 min, and then it was centrifuged at 5000 rpm for 20 min. The serum was stored at –20°C until further biochemical estimation. Serum was used for analysis of various biochemical parameters (serum calcium and alkaline phosphate). Body weights of all animals were recorded daily.

Measurement of Length, Diameter, Volume, Density, and Weight of Femur Bone

After collection of blood for biochemical analyses, the rats were sacrificed. The right femur was removed and freed of soft tissue using small scissors, tweezers, and cotton gauge. The bone was dried. Length and diameter of femur were measured using vernier caliper. Bone was weighted, and volume was measured by fluid replacement. Bone density was calculated.^[15,16]

Bone Breaking Strength (Compression of 5th Lumbar Vertebra)

The bone breaking strength was estimated using compression test. A compression force was applied to the specimen by the hardness tester, and the breaking point was considered as a fracture point.^[17]

Histopathological of Femur Bone

Femur bone was removed from each rat. It was kept in 10% phosphate buffered formalin for 24 h. The bone was dehydrated by placing it three times in toluene: Xylene (50:50) (1 h each) and then dehydrated in ascending grades of alcohol at 70%, 90%, and 100% strength (each for 2 h). The infiltration and impregnation were carried out by treating with paraffin wax in toluene twice; each for 1 h. Paraffin wax was used to embed the tissue. Specimens were cut into sections of 5–15 µm thickness on a leitz microtome in horizontal plane and mounted on glass slide with the help of egg albumin in glycerin solution (50% v/v). They were stained with 10% hematoxylin for 3–5 min and the staining was intensified by placing in running water. The hematoxylin stained sections were stained with 10% eosin for 2 min and was quickly passed through ascending grades of alcohol and finally treated with xylene followed by mounting in DPX. The sections were observed and a desired area was photographed in an olympus photomicroscope.^[18]

Statistical Analysis

All the values were expressed as mean \pm standard error of the mean. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test as appropriate using computer based fitting program (Prism, GraphPad version 5, GraphPad Software, Inc). Differences were considered to be statistically significant when $P < 0.05$.

Results

Acute Toxicity Study

The oral administration of Maxcal-C in female Wistar rats up to the dose of 2500 mg/kg did not show any sign of toxicity and no mortality for 14 days. It was shown that Maxcal-C was safe up to oral dose of 2500 mg/kg of body weight. The experimental protocol was carried out using 1/10th (250 mg/kg) and 1/5th (500 mg/kg) dose based on toxicity study.

Confirmation of Osteoporosis

At day 28, osteoporosis development was confirmed in all the female albino rats (Groups B-E) through urinary calcium and creatinine levels as compared with Group A, that is, sham-operated control rats. Urine calcium and creatinine levels were significantly increased in Groups B-E as compared to sham-operated control rats (Group A) [Table 2].

Effect of Maxcal-C on Body Weight and Femur Bone Weight

At the end of the treatments, the body weight was significantly ($P < 0.001$) decreased in OVX control rats as compared to sham-operated control rats. However, the OVX control rats treated with Maxcal-C (250 and 500 mg/kg) were restored the body weight as compared to OVX control rats.

In OVX-control rats, there was a significant ($P < 0.001$) reduction in femur bone weight as compared to sham-operated control rats. In contrast, the treatment with Maxcal-C at the dose of 500 mg/kg or Calcium Sandoz showed a significant ($P < 0.001$) increase in femur bone weight as compared to OVX control rats while Maxcal-C (250 mg/kg) treated rats did not show any significant difference in femur bone weight as compared with OVX control group [Table 3].

Effect of Maxcal-C on Femur Length and Diameter

There was a significant reduction in femur length ($P < 0.001$) and diameter ($P < 0.01$) of OVX control rats as compared to sham-operated control rats. However, the treatment with Maxcal-C (500 mg/kg) or Calcium Sandoz showed a significant ($P < 0.01$) increase in femur length as compared to OVX control rats. In contrast, animals treated with 250 mg/kg did not show any significant change in femur length as compared to OVX control rats. Furthermore, the treatment with Maxcal-C (250 and 500 mg/kg) showed a significant ($P < 0.05$; $P < 0.001$) increase in femur diameter as compared to OVX control rats [Table 4].

Effect of Maxcal-C on Femur Volume and Density

Femur volume in OVX control group did not show any significant alteration as compared to sham-operated control rats. Moreover, the treatment with Maxcal-C at the dose of 500 mg/kg or Calcium Sandoz showed a significant ($P < 0.01$; $P < 0.001$) decrease in femur volume as compared to OVX control rats while animals treated with 250 mg/kg did not show any

Table 2:

Confirmation of osteoporosis in different groups of female albino Wistar rats

Groups (n=6)	Treatment	Urine creatinine (g/24 h)	Urine calcium (mg/24 h)
A	Sham-operated control	0.36 \pm 0.01	0.13 \pm 0.01
B	OVX control	0.55 \pm 0.01***	0.37 \pm 0.01***
C	Calcium Sandoz	0.54 \pm 0.004***	0.36 \pm 0.02***
D	Maxcal-C (250 mg/kg)	0.56 \pm 0.01***	0.34 \pm 0.01***
E	Maxcal-C (500 mg/kg)	0.55 \pm 0.004***	0.37 \pm 0.01***

Values are expressed as mean \pm SEM (n=6). Where, *** $P < 0.001$ as compared to sham-operated control. SEM=Standard error of mean, OVX=Ovariectomy

Table 3:

Effect of Maxcal-C on body weight and femur bone weight in OVX-induced osteoporosis in rats

Groups (n=6)	Treatment	Initial Body weight (g)	Final Body weight (g)	Femur bone Weight (g)
I	Sham-operated control	242.5 \pm 3.22	245.0 \pm 4.56	0.61 \pm 0.02
II	OVX control	236.3 \pm 4.27	215.7 \pm 2.24***	0.46 \pm 0.01***
III	Calcium Sandoz	227.0 \pm 1.94	246.6 \pm 1.90***	0.68 \pm 0.01***
IV	Maxcal-C (250 mg/kg)	224.8 \pm 2.18	235.0 \pm 2.37**	0.53 \pm 0.02
V	Maxcal-C (500 mg/kg)	232.6 \pm 3.19	249.6 \pm 4.70***	0.67 \pm 0.01***

Values are expressed as mean \pm SEM (n=6). Where, *** $P < 0.001$ as compared to sham-operated control, ** $P < 0.01$, *** $P < 0.001$ as compared to OVX control. SEM=Standard error of mean, OVX=Ovariectomy

significant difference in femur volume as compared with OVX control group.

There was a significant ($P < 0.05$) decrease of femur density in OVX control group as compared to sham-operated control rats. Moreover, the treatment with Maxcal-C (500 mg/kg) or Calcium Sandoz showed a significant ($P < 0.001$) increase in femur density when compared with OVX control rats while Maxcal-C (250 mg/kg) treated rats did not show any significant variation in femur density than OVX control group [Table 4].

Effect of Maxcal-C on 5th Lumbar Vertebrae Bone Breaking Strength

In OVX control rats, the 5th lumbar vertebrae bone breaking strength was significantly ($P < 0.001$) decreased when compared with sham-operated control group. In contrast, the treatment with Maxcal-C (250 and 500 mg/kg) showed a significant ($P < 0.001$) increase in bone breaking strength as compared to OVX control rats [Table 4].

Effect of Maxcal-C on Serum Calcium and ALP

In OVX control rats, there was a significant decrease in serum calcium ($P < 0.01$) and an increase in ALP ($P < 0.001$) levels as compared to sham-operated control rats. In contrast, the treatment with Maxcal-C (250 and 500 mg/kg) showed a significant ($P < 0.01$; $P < 0.001$) increase in serum calcium and a decrease in ALP levels as compared to OVX control rats [Table 5].

Histopathology

Histology of the femur of sham-operated control rat revealed normal size, shape, and number of osteoblasts. It also appeared to have normal micro-architecture of the

Figure 1: Histopathology of femur bone. (a) Sham-operated control, (b) ovariectomy control, (c) ovariectomy control rats treated with Calcium Sandoz (d) ovariectomy control rats treated with Maxcal-C (250 mg/kg, p.o.), and (e) ovariectomy control rats treated with Maxcal-C (500 mg/kg, p.o.)

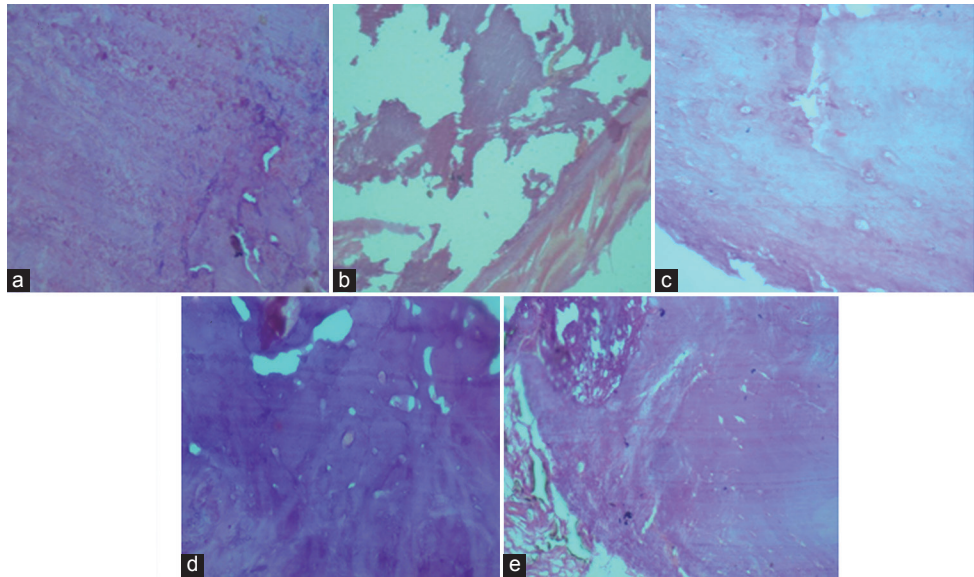


Table 4:
Effect of Maxcal-C on femur bone length, volume, density, diameter, and 5th lumbar vertebrae bone breaking strength in OVX-induced osteoporosis in rats

Groups (n=6)	Treatment	Length (cm)	Volume (ml)	Density (g/ml)	Diameter (mm)	5 th lumbar vertebrae bone breaking strength (kg/cm ²)
I	Sham-operated control	3.36±0.05	0.55±0.02	1.21±0.05	5.05±0.15	7.38±0.07
II	OVX control	2.94±0.02***	0.55±0.02	0.82±0.03*	4.32±0.12**	4.14±0.19***
III	Calcium Sandoz	3.22±0.02##	0.37±0.01###	1.44±0.08###	5.40±0.04###	7.36±0.14###
IV	Maxcal-C (250 mg/kg)	3.12±0.03	0.46±0.02	1.16±0.12	4.87±0.14#	5.38±0.10###
V	Maxcal-C (500 mg/kg)	3.17±0.04##	0.42±0.02##	1.61±0.08###	5.40±0.04###	6.45±0.05###

Values are expressed as mean±SEM (n=6). Where, *P<0.05, **P<0.01, ***P<0.001 as compared to sham-operated control, #P<0.05, ##P<0.01, ###P<0.001 as compared to OVX control. SEM=Standard error of mean, OVX=Ovariectomy

Table 5:
Effect of Maxcal-C on serum calcium and ALP in OVX-induced osteoporosis in rats

Groups (n=6)	Treatment	Calcium	ALP
I	Sham-operated control	9.15±0.38	100.4±3.79
II	OVX control	6.44±0.23**	186.8±4.22***
III	Calcium Sandoz	10.4±0.49###	156.7±2.56###
IV	Maxcal-C (250mg/kg)	9.48±0.52##	164.6±1.50##
V	Maxcal-C (500mg/kg)	10.7±0.50###	158.2±3.15###

Values are expressed as mean±SEM (n=6). Where, **P<0.01, ***P<0.001 as compared to sham-operated control, ##P<0.01, ###P<0.001 as compared to OVX control. SEM=Standard error of mean, OVX=Ovariectomy, APL=Alkaline phosphate

bone. Epiphysis region in OVX rats were sparse, thinning of trabeculae with tendency of disappearing, loss of connectivity, and widening of intertrabecular spaces, and less number of small size of damaged osteoblasts as compare to sham-operated. The treatment with Maxcal-C (500 mg/kg) showed a significant restorative changes with normal size and shape of osteoblasts, but the treatment with Maxcal-C

(250 mg/kg) showed moderately thick elongated trabeculae and narrowed intertrabecular spaces and some damaged osteoblasts and having disturbed micro-architecture [Figure 1].

Discussion

In the current study, osteoporosis model in female albino Wistar rats by bilateral OVX is similar to the earlier established reported.^[12,13] Osteoporosis was confirmed by the increased level of urinary calcium and creatinine.^[14] In the present study, there was a significant decrease in levels of serum calcium and an increase in levels of alkaline phosphate during osteoporotic condition which are in accordance with earlier studies.^[14,15] However, serum calcium and alkaline phosphate were significantly restored after the treatment of Maxcal-C (250 and 500 mg/kg) as compared to sham-operated control animals. The body weight of the OVX control rats was significantly reduced when compared to sham-operated control group while OVX control animals treated with Maxcal-C showed a significant improvement in body weight which might be due to ameliorating effects on bone. Results obtained in the present study indicate that OVX control rats showed decreased bone length, bone weight, bone density, and bone diameter as compared to sham-operated

control rats which are in comparable with previous reports.^[15,19] The treatment with Maxcal-C (500 mg/kg) showed a significant increase in femur length as compared to OVX control rats, but animals treated with 250 mg/kg did not show any significant alteration in femur length. Furthermore, the treatment with Maxcal-C (250 and 500 mg/kg) showed a significant increase in femur diameter as compared to OVX control rats. Femur bone weight and density were significantly decreased in OVX control rats as compared to sham-operated control rats. In contrast, Femur bone weight and density were significantly restored after the treatment of Maxcal-C (500 mg/kg) as compared to OVX control rats while Maxcal-C (250 mg/kg) treated rats did not show any significant variation in femur bone weight and density.

Bone breaking strength of the 5th lumbar vertebra is one of the direct methods for the measurement of bone strength. In this study, bone breaking strength was significantly decreased in OVX control animals, and Maxcal-C treated rats showed a significant improvement in bone breaking strength. This is in accordance with previous report.^[20] In current study, a comparative histopathological study of the femur bone from various treatments further supported the anti-osteoporotic activity.

Conclusion

These results showed that Maxcal-C showed a significant protection in dose-dependent manner against OVX-induced osteoporosis in rats. Finally, it was concluded that Maxcal-C has a significant anti-osteoporotic effect. Our present investigation supports the traditional use of Maxcal-C in the treatment of osteoporosis.

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Conflicts of Interest

There are no conflicts of interest.

References

- Melton L. Bring back the acetils – A novel anticancer movement. *Lancet Oncol* 2003;4:710.
- Raisz LG, Rodan GA. Pathogenesis of osteoporosis. *Endocrinol Metab Clin North Am* 2003;32:15-24.
- Mundy GR. Osteoporosis into the year 2010. *Br J Obstet Gynaecol* 1996;103 Suppl 13:32-7.
- Stepán JJ, Musilová J, Pacovský V. Bone demineralization, biochemical indices of bone remodeling, and estrogen replacement therapy in adults with Turner's syndrome. *J Bone Miner Res* 1989;4:193-8.
- Gorman C, Park A. The truth about hormones. *Time* 2002;160:32-9.
- Rao SS, Pai KS, Bhat KM. Preventive role of *Emblia officinalis* and *Cissus quadrangularis* on bone loss in osteoporosis. *Int J Pharm Pharm Sci* 2013;5:465-70.
- Ahmed I, Kumar L, Kulkarni KS, Kumar V. Efficacy of OST-6, a polyherbal formulation in the management of osteoporosis in postmenopausal women. *Orthop Today* 2002;4:241-4.
- Sen B, Banerjee SK, De Mazumder N, Sarkar N, Roychowdhury A, Majumdar P. Studies on effects of Rupalaya in osteoarthritis. *Indian Med J* 1980;74:151.
- Palbag S, Pal K, Saha D, Nandi MK, De BK, Gautam DN. Pharmaceuticals, ethnopharmacology, chemistry and pharmacology of ayurvedic marine drugs: A review. *Int J Res Ayurveda Pharm* 2013;4:437-42.
- Bahmani S, Ramesh H, Iqbal MM, Rao KS. Anti-osteoporotic activity of ostinu, a herbo-mineral preparation in ovariectomized rats. *Biosci Biotechnol Res* 2010;7:321-6.
- OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23 March, 1996. In: Eleventh Addendum to the OECD Guidelines for the Testing of Chemicals Organisation for Economical Co-Operation and Development, Paris; June, 2000.
- Zhang Y, Yu L, Ao M, Jin W. Effect of ethanol extract of *Lepidium meyenii* Walp. on osteoporosis in ovariectomized rat. *J Ethnopharmacol* 2006;105:274-9.
- Zhang G, Qin L, Hung WY, Shi YY, Leung PC, Yeung HY, et al. Flavonoids derived from herbal *Epimedium brevicornum* Maxim prevent OVX-induced osteoporosis in rats independent of its enhancement in intestinal calcium absorption. *Bone* 2006;38:818-25.
- Gomes A, Haldar S, Giri B, Mishra R, Saha A, Dasgupta S, et al. Experimental osteoporosis induced in female albino rats and its antagonism by Indian black scorpion (*Heterometrus bengalensis* C.L.Koch) venom. *Toxicon* 2009;53:60-8.
- Aswar UM, Mohan V, Bodhankar SL. Antiosteoporotic activity of phytoestrogen-rich fraction separated from ethanol extract of aerial parts of *Cissus quadrangularis* in ovariectomized rats. *Indian J Pharmacol* 2012;44:345-50.
- Chitme HR, Muchandi IS, Burli SC. Effect of *Asparagus racemosus* Willd root extract on ovariectomized rats. *Open Nat Prod J* 2009;2:16-23.
- Notomi T, Okazaki Y, Okimoto N, Tanaka Y, Nakamura T, Suzuki M. Effects of tower climbing exercise on bone mass, strength, and turnover in orchidectomized growing rats. *J Appl Physiol* 2002;93:1152-8.
- Salman S, Kumbasar S, Hacimuftuoglu A, Ozturk B, Seven B, Polat B, et al. The effect of metyrosine/prednisolone combination to oophorectomy-induced osteoporosis. *Iran J Reprod Med* 2012;10:363-72.
- Kalu DN. The ovariectomized rat model of postmenopausal bone loss. *Bone Miner* 1991;15:175-91.
- Soni HK, Kandachia JM, Jani DK, Patel GR. Pharmacological investigation of Bonton Capsule for anti-osteoporotic activity in ovariectomized rats. *Int J Pharm Phytopharmacol Res* 2013;3:52-6.