World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.com/
Research Article

Evaluation of bonton active granules for anti-osteoporotic activity in ovariectomized rat

Soni Hardik[1*], Kandachia Jalak[2], Jani Deepti[2], Patel Ghanashyam[1]

[1]Vasu Research Centre, A Division of Vasu Healthcare Pvt. Ltd., 896/A, G.I.D.C., Makarpura, Vadodara – 390 010, Gujarat, India
[2]Department of Pharmacology, Babaria Institute of Pharmacy, BITS Education campus, Vadodara-Mumbai NH#8, Varnama, Vadodara – 391 240, Gujarat, India

Received: 09-01-2014 / Revised: 19-01-2014 / Accepted: 25-01-2014

ABSTRACT

Osteoporosis is a multi-factorial disease characterized by reduced bone mass and impaired micro-architecture, leading to an increased susceptibility to fractures. The objective of present study was to evaluate anti-osteoporotic activity of Bonton Active Granules in ovariectomized rat model at two different dose levels i.e. Therapeutic Effective Dose (TED) 0.9 g/kg/day and double the dose (TED×2) 1.8 g/kg/day. 24 healthy female wistar rats were divided into 4 groups where each group was containing 6 animals. Group-1 was considered as a normal control group fed with 1% Carboxy Methyl Cellulose (CMC) suspension. Group-2, a Disease control group was containing ovariectomized (OVX) rates fed with 1% CMC suspension. Group 3 and 4 were orally treated with test drug at TED (0.9 g/kg/day) and TED×2 (1.8 g/kg/day) respectively. Present study included parameters like serum alkaline phosphatase (ALP), serum calcium, femoral bone parameters, bone breaking strength, body weight and histopathological study of bone. Treatment with Bonton Active Granules at both dose levels showed significant decrease in serum ALP and significant increase in serum calcium level. It also showed significant changes in femoral parameters and histopathology of bone. Hence, it can be inferred that Bonton Active Granules at both experimented therapeutic doses provides good anti-osteoporotic activity against ovariectomized rat.

Keywords: Bonton Active Granules, ovariectomized rat, anti-osteoporotic activity

INTRODUCTION

Osteoporosis is a complex, multi-factorial disease characterized by reduced bone mass and impaired micro-architectural structure, leading to an increased susceptibility to fractures. Although most of the bone strength (including bone mass and quality) is genetically determined, many other factors (nutritional, environmental and life-style) also influence bone.[1] Osteoporosis is an age-related disease that affects women more often than men. The hypothesis is that osteoporosis is a consequence of estrogen deficiency.[2] The significance of the disorder lies solely in its structural effects, i.e., bone fragility.[3] The clinical significance of osteoporosis lies in the fractures that arise, with their attendant morbidity and mortality. Low bone mass is an important component of the risk fracture, but other skeletal abnormality contributed to bone fragility.[4] Ultimately, osteoporosis leads to bone with less tensile strength and significantly more susceptibility to fracture with less force. At some point, the amount of bone available for mechanical support falls below a certain threshold (the "fracture threshold") and the patient may sustain a fracture.

There is no absolute fracture threshold for a population of patients; rather, it is different for each individual. The bone loss affects cortical and trabecular bone, with trabecular bone loss more predominant in typical postmenopausal osteoporosis.[5] Increased bone remodeling rates are associated with increased skeletal fragility independent of bone mass, partially accounting for the age-related increase in fracture risk in women that is independent of bone loss. Bone remodeling rates double at menopause, triple 13 years later, and remain elevated in osteoporosis. This change

***Corresponding Author Address:** Soni Hardik, Asst. Manager, R&D, Vasu Research Centre, (A Division of Vasu Healthcare Pvt. Ltd.), 896/A, G.I.D.C., Makarpura, Vadodara-390010, Gujarat, India; E-mail: hsoni@vasuresearch.com; hardik_ayurved@yahoo.com*

contributes to increases in age-related skeletal fragility in women.[6] As defined by the World Health Organization (WHO), in 25% of women at age of 65 and in 70% of that above age of 80 suffering from osteoporosis and related problems.[7] Being a natural, Herbal products are widely perceived as safe.[8] Due to their long historical clinical use and reliable therapeutic efficacy, Traditional Indian System of Medicine is getting global attention. Many pharmaceutical companies are using traditional medicine as an excellent pool for discovering novel natural bioactive compounds.[9]

Bonton Active Granules contains extract of *Cissus quadrangularis* (Hadjod) Stem[10-12], *Asparagus racemosus* (Shatavari) Root[13], *Withania somnifera* (Ashwagandha) Root[14], *Vitex negundo* (Nirgundi) Leaves[15], *Terminalia arjuna* (Arjun) Bark[16], *Mucuna pruriens* (Kapikachhu) Seed[17] and *Commiphora mukul* (Guggulu) Gum resin[18].

Bonton Active Granules is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara. Majority of ingredients of Bonton Active Granules are well reported in Ayurvedic texts and scientific research publications for anti-osteoporotic, anti-inflammatory and anti-oxidant activity. However, no such evidence was found which proves the efficacy of such a combination. In the present study, an attempt was made to investigate anti-osteoporotic activity of Bonton Active Granules in ovariectomized rat.

MATERIALS AND METHODS

Preparation of test drug: Bonton Active Granules was mixed with 1% Carboxy Methyl Cellulose (CMC) for the preparation of test drug suspension.

Experimental animals: Wistar albino female rats of 250-300 g were used and acclimatized to the experimental room having ambient temperature (23±2°C), controlled humidity (55±5%) conditions, and 12 hours light and dark cycle. Animals were caged in polypropylene cages with maximum of three animals per cage. The rats were fed with standard food pellets and water *ad libitum*. Study was conducted after obtaining approval by Institutional Animal Ethical Committee (IAEC) (Babaria Institute of Pharmacy, M.Pharm Sem-IV/12-13/03) as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental Design: The experimental animals were divided into four groups, containing six animals in each group. Group 1 was considered as Normal control group and was fed 1% Carboxy Methyl Cellulose (CMC) suspension. Group 2 was considered as Disease control group which was ovariectomized (OVX) and was fed with 1% CMC suspension. Group 3 and 4 were ovariectomized and treated with Bonton Active Granules (0.9 g/kg/day) i.e. Therapeutic Effective Dose (TED) and (1.8 g/kg/day) i.e. Double of Therapeutic Effective Dose (TED×2) respectively. After the 8th day of ovariectomy, test drug was given to group 3 and 4 till eight weeks.

After 8 weeks, animals from all groups were anesthetized for collection of blood samples for estimation of biochemical parameters. Then, animals of all groups were sacrificed and their femurs were removed for femoral parameters and histopathological study of bone.

Induction of osteoporosis by ovariectomized (OVX) method: The operating table area was sterilized with alcohol. Sterile surgical instruments were used for operation. For general anesthesia 75 mg/kg ketamine and 10 mg/kg xylazine intra-peritoneally were administered. After anesthesia, animal was placed in lateral position. The left flank of the rats was shaved and furs removed completely (Figure 1A).The shaved area was washed with 70% alcohol. Then rat was transferred to the operation table. 2 cm incision was made on dorso-lateral area from the second either to fifth lumbar vertebrae or middle part of abdomen (Figure 1B). Incision was of minimum length to allow the extrusion of ovaries. Entrance to the peritoneal cavity was made by dissecting the muscle, which revealed the adipose tissue surrounding the ovary (Figure 1C & 1D). The periovarian fat attached with the ovary was gently pulled away (Figure 1E) from the incision site to prevent detachment of a small piece of ovary. After identifying the ovary and uterine horn the ovarian tissue was removed completely in one action (Figure 1F & 1G). The horn was returned to the abdominal cavity and the muscle and skin were sutured (Figure 1H, 1I, 1J). The procedure was repeated for the right ovary same as the left one. High degree of aseptic procedure was maintained throughout the operation (Figure 1K, 1L). After surgery, the rats were housed individually in polypropylene boxes for a period of one week to allow recovery and then re-grouped in their home cages. There was also some concern regarding site of incision as in case of the ventral approach in rodents, the wound remains almost constant direct contact with the paddy husk bedding, which may result in more frequent wound breakdowns. Hence, it was avoided.[19]

Evaluation parameters

Body weight: Body weight of every group of rats was recorded weekly and mean was considered to evaluate the effect of treatment.[13]

Serum biochemical parameters: The levels of serum calcium[13] and serum alkaline phosphatase (ALP)[13] were determined by colorimetric method and auto analyzer respectively.

Measurement of femoral parameters

a. **Femur length:** The femur length, defined as the distance between the greater trochanter and the medial condyle. At the end of the treatment, rats of all groups were sacrificed and the right femur was isolated. The femur length was measured by using vernier caliper.[13]
b. **Femur weight:** The isolated right femurs were kept for drying. After that the femur of all groups were weighed by digital weighing balance.
c. **Femur diameter:** The external diameter was measured at the femoral mid shaft using vernier caliper.[19]
d. **Femur volume and density:** Bone volume was measured by fluid replacement. Bone volume and density were measured by Archimedes's principle. Each bone was placed in un-stopper vial filled with deionized water, and the vial was put in a desiccator connected to a vacuum for 90 min. The desiccator was agitated periodically to ensure that all trapped air diffused out of the bone, at which time the bone was removed from the vial, blotted with tissue paper, weighed, and returned to the vial containing deionized water. The bone was reweighed in a boat suspended but completely immersed in water previously equilibrated to room temperature, and the density was calculated (grams/volume).[13]

Compression of 5th Lumbar Vertebra (Bone breaking strength): The fifth lumbar vertebrae (L5) were used to measure the mechanical strength by the compression test. A craniocaudal compression force was applied to the specimen by the hardness tester and the breaking point was considered as a fracture point.[20]

Histopathology of femur bone: The left femur was fixed in 10% formalin solution for 48 hours, decalcified in 5% nitric acid for 48hours. After fixation and decalcification, samples were put in paraffin blocks. 5 µm wide sections were taken from paraffin blocks for histopathological examination. After de-paraffinization and rehydration, sections were stained with hematoxylin and eosin stain. After staining the sections were observed under 100X magnification of trinocular microscope. Number of stained osteoblasts and micro-architecture of femur bone were observed.[21]

Statistical Analysis: Data were analyzed by one way ANOVA followed by *Tukey-Kramer* Multiple Comparison Test. All the values were expressed as Mean ± SEM and $P<0.05$ was considered as statistically significant.

RESULTS

Effect of Bonton Active Granules on body weight: The overall body weight analysis revealed that OVX group showed significant decrease ($P<0.001$) in body weight as compared to the normal control group. Significant increase ($P<0.01$) and ($P<0.001$) were observed at TED and TED×2 dose level in comparison to OVX group respectively. (Table 1)

Effect of Bonton Active Granules on serum biochemical parameters: A significant decrease ($P<0.001$) was observed in serum calcium level in the OVX group as compared to the normal control group. Serum calcium level was significantly increased ($P<0.05$) in TED group and ($P<0.001$) TED×2 group when compared to the OVX group (Table 1). Significant increase ($P<0.001$) was found in serum alkaline phosphatase (ALP) level in OVX group as compared to the normal control group. Significant effect ($P<0.001$) was observed at both dose levels of Bonton Active Granules i.e. TED and TED×2 as compared to the OVX group. (Table 1)

Effect of Bonton Active Granules on femoral parameters: Femur length and weight of OVX group were significantly decreased ($P<0.001$) as compared to the normal control group. TED and TED×2group showed significant increase ($P<0.001$) in femur length and weight as compared to OVX group (Table 2). Femur diameter, volume and density of OVX group were significantly decreased ($P<0.001$) as compared to the normal control group. The TED group showed significant increase ($P<0.001$) in femur diameter, volume and ($P<0.01$) in femur density as compared to OVX group. TED×2 group showed significant increase ($P<0.001$) in femur diameter, volume and density as compared to OVX group. (Table 2)

Effect of Bonton Active Granules on bone breaking strength: There was significant decrease ($P<0.001$) in breaking strength of 5th lumbar vertebra of OVX group as compared to normal

control. TED and TED×2 group showed significant increase (*P<0.001*) in bone breaking strength as compared to OVX group. (Table 1)

Effect of Bonton Active Granules on Histopathology of femur bone: Under the microscope, histology of the femur of normal control rat revealed normal size, shape and number of osteoblasts. It also appeared having normal micro-architecture of the bone. OVX group section exhibited sparse, disrupt, spacing enlarged, less number of small size of damaged osteoblasts. The micro-architecture of OVX femur was found disturbed. The Bonton Active Granules at TED (0.9 g/kg/day) and TED×2 (1.8 g/kg/day) treated OVX rats showed significant restorative changes with normal size and shape of osteoblasts. (Figure 2)

DISCUSSION

Human bone is composed of a mineralized organic matrix and bone cells. Osteoblasts are bone cells that synthesize the organic matrix and regulate the mineralization process whereas Osteoclasts causes bone resorption.[20] Decrease in number of osteoblasts causes decrease in bone mineralization and formation process thus causes osteoporosis. The most common type of osteoporosis is the bone loss associated with ovarian hormone deficiency during and after menopause.[21] The approach of the study was to evaluate the osteoprotective activity of Bonton Active Granules. In the present study, evaluation was done using parameters such as loss of mechanical strength of bone, reduced serum calcium and increased serum ALP associated with estrogen deficiency in OVX animals. The ovariectomized rat exhibits most of the characteristics of human postmenopausal osteoporosis by developing the deficiency of estrogen.[22] Estrogen deficiency is a well-known causative factor in the pathogenesis of osteoporosis.[23]

The body weight of the ovariectomized rats was comparatively decreased in comparison with the normal control animals. The OVX rats treated with the Bonton Active Granules restored the body weight at both dose levels which may be due to ameliorating effect on bones.

Regarding bone metabolic marker like serum ALP which is associated with bone formation increases in osteoporosis and other bone metabolic disorders. Similar changes were observed in the present study. ALP is an early indicator of bone formation because it is a byproduct of osteoblasts. Serum ALP levels may double in the post-menopause term, depending on the increase in bone formation cycle.[24] The decrease in calcium level in the ovariectomized rats was created similar condition like postmenopausal women.[25] The treatment of Bonton Active Granules at TED (0.9 g/kg/day) and TED×2 (1.8 g/kg/day) showed significant decrease in serum ALP and significant increase serum calcium level which may be due to enhancement of osteoblastic activity and reduction of osteoclastic activity.

The biomechanical parameters including, compression test of 5th lumbar vertebra is one of the direct measures of bone strength.[26] In this study the 5th lumbar vertebra breaking strength was found decreased in ovariectomized rats and it was restored by treatment of both the dose levels of the Bonton Active Granules however TED×2 (1.8 g/kg/day) was found more significant.

The femur diameter, femur volume, femur weight, femur length and femur density were reduced in OVX group which may be due to increase in fragility followed by loss of minerals. This may be due to small stimulatory effect of growth hormone on longitudinal growth.[27] The treatment of Bonton Active Granules at both dose levels showed significant increase in femur diameter, femur volume, femur weight, femur length and femur density.

Histopathology revealed that normal control possesses more number of osteoblasts as compared to the OVX group. OVX group showed disturbed micro-architecture of the bone as compared to the normal control. The histology of TED (0.9 g/kg/day) and TED×2 (1.8 g/kg/day) group showed almost similarity in osteoblasts as compared to the normal control. This may indicate significant effect of test drug at both dose levels on osteoporotic bone.

CONCLUSION

On basis of available data, it can be concluded that Bonton Active Granules at both dose levels i.e. TED (0.9 g/kg/day) and TED×2 (1.8 g/kg/day) was found statistically highly significant. Hence, it can be inferred that Bonton Active Granules at both experimented therapeutic dose levels provides good anti-osteoporotic activity in ovariectomized rat model.

ACKNOWLEDGEMENT

Authors are sincerely thankful of the management of Vasu Healthcare for providing product sample and also thankful of Babaria Institute of Pharmacy, Vadodara for providing the necessary facilities and technical support for conducting the study.

CONFLICT OF INTEREST: The author(s) have no competing interests for financial support, publication of this research, patents and royalties through this collaborative research. All authors were equally involved in discussed research work. There is no financial conflict with the subject matter discussed in the manuscript.

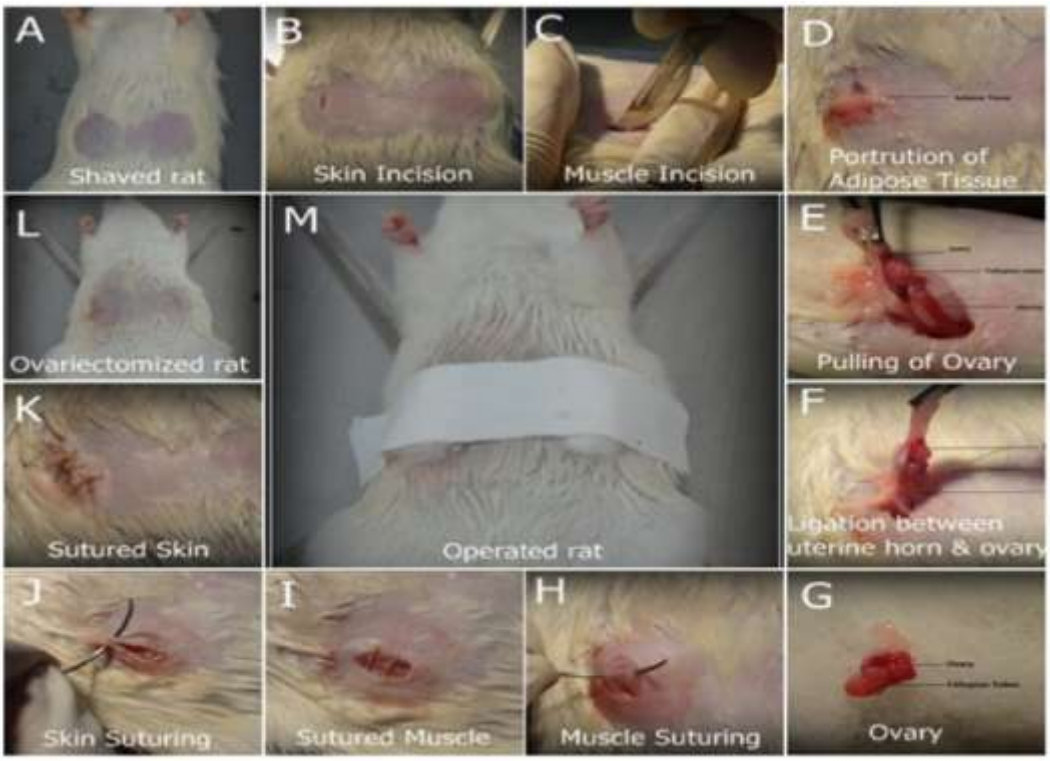

Figure 1: Induction of osteoporosis by ovariectomized (OVX) method

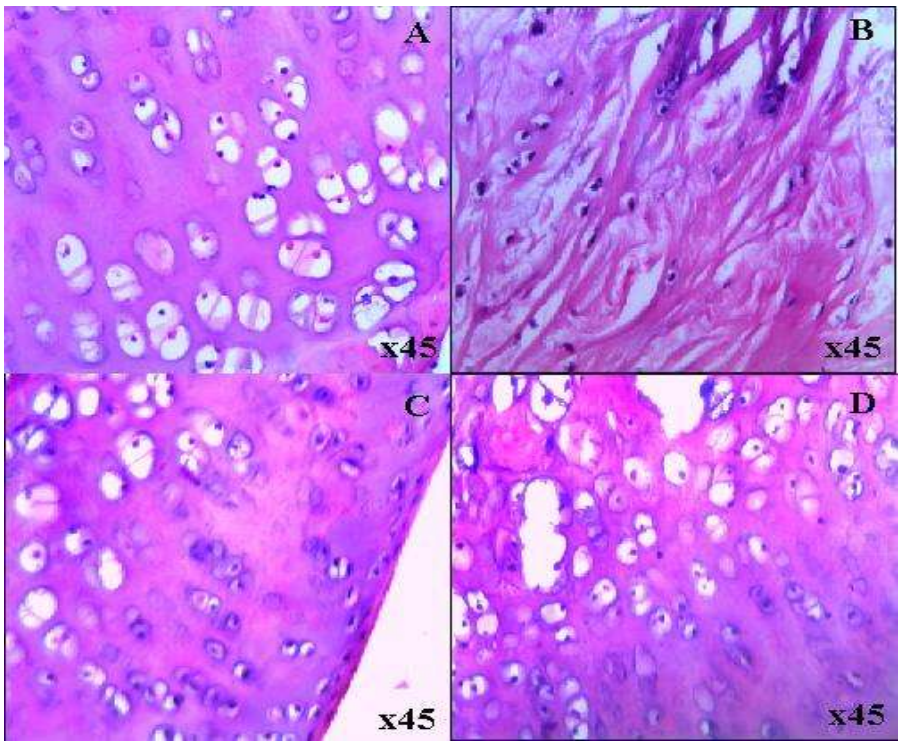

Figure 2: Histopathology of femur bone (A) Normal control group, (B) OVX group, (C) OVX + Bonton Active Granules (TED), (D) OVX + Bonton Active Granules (TEDx2)

Table 1: Effect of Bonton Active Granules on body weight, serum biochemical parameters and bone breaking strength

Groups	Body weight (g)	Serum Calcium (mg/dL)	Serum ALP (IU)	Bone breaking strength (gm/cc^2)
Normal control	239.27 ± 3.75	11.38 ± 0.20	98.50 ± 2.73	7.66 ± 0.33
OVX group	206.10 ± 6.32$^{###}$	10.13 ± 0.13$^{###}$	197.96 ± 7.95$^{###}$	4.00 ± 0.25$^{###}$
OVX + Bonton Active Granules (TED)	235.20 ± 3.17**	10.86 ± 0.13*	100.61 ± 2.73***	8.01 ± 0.36***
OVX + Bonton Active Granules (TED×2)	239.58± 5.12***	11.75 ± 0.09***	108.59 ± 6.77***	7.83 ± 0.30***

All the values are expressed as mean ± SEM (n=6) in each group. Where, $^{*}P<0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$when compared to OVX group. While, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ when compared to normal control group.

Table 2: Effect of Bonton Active Granules on femoral parameters

Groups	Femur length (cm)	Femur weight (g)	Femur diameter (mm)	Femur volume (mL)	Femur Density (gm/mL)
Normal control	3.37 ± 0.01	0.66 ± 0.01	4.38 ± 0.02	0.65 ± 0.01	1.01 ± 0.01
OVX group	2.85 ± 0.01$^{###}$	0.47 ± 0.01$^{###}$	4.17 ± 0.01$^{###}$	0.57 ± 0.01$^{###}$	0.82 ± 0.01$^{###}$
OVX + Bonton Active Granules (TED)	3.28 ± 0.02***	0.59 ± 0.01***	4.37 ± 0.03***	0.64 ± 0.01***	0.91 ± 0.01**
OVX + Bonton Active Granules (TED×2)	3.41 ± 0.02***	0.66 ± 0.01***	4.42 ± 0.01***	0.65 ± 0.01***	1.01 ± 0.01***

All the values are expressed as mean ± SEM (n=6) in each group. Where, $^{*}P<0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$when compared to OVX group. While, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ when compared to normal control group.

REFERENCES

1. Rangrez A et al. Osteoprotective effect three anti inflammatory plants in ovariectomized wistar rats. Pharmacologyonline 2011;1:675-84.
2. Sipos W et al. Pathophysiology of osteoporosis. Wien Med Wochenschr 2009;159:230-4.
3. Heaney R. Estrogens and Postmenopausal Osteoporosis. Clinical Obstetrics and Gynecology 1976;19(4):791-804.
4. Lee S. Osteoporosis. Rheumatic Disease Clinics of North America 2007;33(1): 149-76.
5. Recker R et al. Bone remodeling increases substantially in the years after menopause and remains increased in older osteoporosis patients. Journal of Bone and Mineral Research 2004;19(10):1628-33.
6. Sankaran SK. Osteoporosis prevention and treatment. Pharmacological management and treatment implications. Drugs Aging 1996;9(6):472-7.
7. Janet E, Lynnette K. Handbook of Diagnosis Endocrinology. 2003;ch.13:257.
8. Eisenberg DM et al. Unconventional medicine in the united states - prevalence, costs, and patterns of use. The New England Journal of Medicine 1993;328:246-52.
9. Patra KC et al. Traditional approaches towards standardization of herbal medicines -A review. Journal of Pharmaceutical Science and Technology 2010;2(11):372-9.
10. Potu BK et al. Evidence-based assessment of antiosteoporotic activity of petroleum-ether extract of Cissus quadrangularis Linn. on ovariectomy-induced osteoporosis. Upsala Journal of Medical Sciences 2009;114(3):140-8.
11. Shirwaikar A et al. Anti-osteoporotic effect of ethanol extract of Cissus quadrangularis Linn. on ovariectomized rat. Journal of Ethnopharmacology 2003;89(3):245-50.
12. Mishra G et al. Pharmacological and therapeutic activity of Cissus quadrangularis: An overview. International Journal of Pharmtech Research 2010;2(2):1298-1310.

13. Chitme HR et al. Effect of Asparagus racemosus Willd root extract on ovariectomized rats. The Open Natural Products Journal 2009;2:16-23.
14. Nagareddy PR, Lakshmana M. Withania somnifera improves bone calcification in calcium-deficient ovariectomized rats. Journal of Pharmacy and Pharmacology 2006;58(4):513-9.
15. Dharmasiri MG et al. Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. Journal of Ethnopharmacology 2003;87(2):199-206.
16. Giri RR et al. Effect of Terminalia arjuna in accelerating healing process of experimentally fractured tibia of rats: A preliminary study. Research Journal of Pharmaceutical Biological and chemical sciences 2012;3(4):417-25.
17. Dhanasekaran M et al. Anti-parkinson drug - Mucuna pruriens shows antioxidant and metal chelating activity. Phytotherapy Research 2008;22(1):6-11.
18. Singh BB et al. The effectiveness of Commiphora mukul for osteoarthritis of the knee: an outcomes study. Alternative Therapies in Health and Medicine 2003;9(3):74-9.
19. Zhang Y et al. Effect of ethanol extract of Lepidium meyenii Walp. on osteoporosis in ovariectomized rat. Journal of Ethnopharmacology 2006;105(1):274-9.
20. Notomi T et al. Effect of tower climbing exercise on bone mass, strength and turnover in orchidectomized growing rats. Journal of Applied Physiology 2002;93(3):1152-8.
21. Suleyman S et al. The effect of metyrosine/prednisolone combination to oophorectomy- induced osteoporosis. Iranian Journal of Reproductive Medicines 2012;10(4):363-72.
22. Policegoudra RS, Aradhya SM. Biochemical changes and antioxidant activity of mango ginger (Curcuma amada Roxb.) rhizomes during postharvest storage at different temperatures. Postharvest Biology and Technology 2007;46(2):189-94.
23. Lageunesse D, Martel-Pelletier J. Osteoporosis and osteoarthritis: bone is the common battleground. Medicographia 2010;32(4):391-8.
24. Cooper C, Melton J. Epidemiology of osteoporosis. Trends in Endocrinology & Metabolism 1992;3(6):224-9.
25. Arjmandi BH et al. Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. Journal of Nutrition 1996;126(1):161-7.
26. Lohita P et al. Study of analgesic activity of Litsea glutinosa (L.) ethanolic extract on swiss albino mice. International Journal of Pharmaceutical Sciences and Research 2010;1(9):93-7.
27. Tsuang Y et al. Isoflavones prevent bone loss following ovariectomy in young adult rats. Journal of orthopedic Surgery and Research 2008;3(12):1-9.