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Anti-osteoporotic activity of ethanol extract of *Terminalia arjuna* (Roxb.) Wight & Arn. on ovariectomized rats

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Terminalia arjuna (Roxb.) Wight & Arn., a widely growing plant in India, possesses different medicinal properties and its bark is well known for their bone remineralization properties. The objective of present study was to evaluate anti-osteoporotic activity of ethanol extract of *T. arjuna* (EET) on ovariectomized female Sprague Dawley model rats, divided into six groups. Sham operated control group received vehicle, standard group received estrogen (0.0563 mg/kg) and the remaining ovariectomized groups were orally administered with EET at 100, 300 and 500 mg/kg body weight, respectively for 42 days. The findings were assessed by uterine weight, bone loss determined by CT-scanning of femur, ash content, biomechanical, biochemical and histopathological parameters. Ethanol extract showed significant increase in uterine weight and femoral bone length, weight, density and significant increase of ash weight, ash percentage, ash calcium and hardness in lumbar vertebrae as compared to control group. EET treated groups also showed significant decrease level of creatinine, calcium and phosphorus (p<0.01 to p<0.001) as compared to control group. Histopathological studies further supported protective effect of the extract. These findings suggest a potential protective role of EET against estrogen deficient osteoporosis in ovariectomized rats.

Keywords: *Terminalia arjuna*, Estrogen, Postmenopausal osteoporosis, Ash content, Bone mineral density.
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Introduction

Postmenopausal osteoporosis is a frequently occuring major health hazard in recent years, afflicting over 2000 million people worldwide[1]. It is characterized by a rapid loss of mineralized bone tissue and micro architectural deterioration of bone tissues, leading to enhanced bone fragility and consequent increase in fracture risk of sites rich in cancellous bone such as vertebrae, hip and distal forearm[2]. The bone formation is a balanced and continuous process, an imbalance between these results into osteoporosis[3]. The most common type of osteoporosis is the bone loss associated with ovarian hormone deficiency at menopause[4]. Estrogen deficiency is considered as the main determinant for bone loss in postmenopausal women[5]. As the population ages, the incidence of hip fractures and costs for treatment will rise dramatically in the future, unless effective prophylactic measures are taken to avoid damages to bones[6]. Hormone replacement therapy (HRT) has proven efficacy in preventing bone loss and reducing the incidence of skeletal fractures in postmenopausal women. However, long-term HRT increases the high risk of breast cancer, endometrial cancer, thromboembolic events and vaginal bleeding[7,8].

Terminalia arjuna (Roxb.) Wight & Arn. bark is considered to be the most important constituent from medicinal point of view. The ethanol extract contains tannins, a glycoside, a large quantity of carbonates of calcium and smaller amounts of aluminium and magnesium[9,10]. Terpenoids, arjumin, arjunic acid, arjunolic acid, terminic acid and flavonoids, arjunolone, arjunone, gallic acid, quercetin are present in the bark of the plant. Tannins, pyrocatechols, punicallin, punicalagin, terchebulin and casurinin were isolated from bark of *T. arjuna*[11]. The bark powder has been found to improve antioxidant status in the patients of coronary heart disease and these beneficial effects may be due to its high flavonoid content[12]. It is reported to possess anticancer, hypolipidemic and cardiotonic properties[13,14]. The bark of this plant is well known for their bone remineralization properties and

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extensively used to treat osteoporosis and other bone related disorders as it improves the synthesis and secretion of female hormones. This is useful in relieving general debility in postmenopausal women[15]. In the present investigation, we studied the anti-osteoporosis activity of ethanol extract of *T. arjuna* bark on ovariectaomizied rats.

Materials and Methods

Drugs and chemicals

Calcium, phosphorous and alkaline phosphatase kit was obtained from Erba diagnostics, Mallaustr, Germany. Estrogen and Ketamine obtained from Neon Laboratories Ltd. Mumbai, India. Auto analyzer (Star, Davangere, Karnataka, India), Muffle furnace (Growell Instrument, Bangalore, India), Refrigerated centrifuge (MPW 350R, Koria), UV- Spectrophotometer (UV 1601, Shimadzu corporation, Japan), All other chemicals and reagents were of analytical grade.

Plant material and preparation of extract

T. arjuna is widely available in India. The bark was collected during May-July 2011. It was identified and authenticated by Prof S A Kappali, Botanist, Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka. The voucher specimen (No.Bsc/Pharmacy/15/2011) was deposited in Department of Pharmacology, H S K College of Pharmacy, Bagalkot. The fresh and air dried bark were subjected to coarse powdering and passing through #44 mesh sieve to get uniform size. The powder was extracted with petroleum ether for defatting and then by ethanol (40-60 °C) for 24 h by using Soxhlet apparatus, which yielded 0.67 % brownish solid mass. Thus ethanol extract was suspended and prepared using 5 % Tween 80 and anti-osteoporotic activity studies were carried out in ovariectomized rats[16].

Animals

Female Sprague-Dawley rats, (200-300 g), procured from the National Institute of Nutrition, Hyderabad were used for study and they were acclimatized for quarantine for 10 days under standard conditions (Temperature 22±2 °C, Relative humidity 55±5 %) for 12 h dark and 12 h light cycle, respectively and were given standard pellet food (Hindustan lever, Bangalore, Karnataka) and water *ad-libitum* throughout the experimental period. The study was approved by Institutional Animal Ethics Committee, H S K College of Pharmacy, Bagalkot, Karnataka.

Acute toxicity study

Acute toxicity study was carried out as per the OECD guidelines 425. Swiss mice of either sex fasted overnight were selected randomly and fed with ethanol extract of *T. arjuna* (EET) at a limit test dose of 5000 mg/kg body weight. The animals were observed at every 30 min up to a period 6 h and thereafter every day for 14 days[17]. As per OECD guidelines the safest dose was found to be 5000 mg/kg body weight, and hence therapeutic dose for animals were selected between $1/50^{th}$ to $1/10^{th}$ of safest dose.

Anti-osteoporosis activity

The female Sprague Dawley rats were divided in to six groups of 8 rats in each and treated as follows:

Group-I: Sham operated control receive vehicle in distilled water.
Group-II: Overiectomized control receive vehicle (Tween 80 in distilled water).
Group-III: Ethanol extract of *T. arjuna* (100 mg/kg) in ovariectomized rats.
Group-IV: Ethanol extract of *T. arjuna* (300 mg/kg) in ovariectomized rats.
Group-V: Ethanol extract of *T. arjuna* (500 mg/kg) in ovariectomized rats.
Group-VI: Standard estrogen (0.0563 mg/kg) in ovariectomized rats.

After seven days of acclimation, the rats were ovariectomized or Sham operated and then anesthetized with Ketamine HCL (50 mg/kg, i p); the ovaries were removed bilaterally. Sham operation was performed in same manner but only exposing the ovaries. They were administered with prophylactic Gentamycine (10 mg/kg, i p) for 3 days. The treatment of EET oral gavages continued for 42 days[18] and body weight was measured weekly. At the end of the 42^{nd} day, all the rats were deprived of food for one night. On the next day, urine (0-24 h) was collected, then the animals were anaesthetized by ketamine (50 mg/k, i p) and blood were taken from common carotid artery. The blood samples were centrifuged at 2500 rpm for 15 min to separate the serum and preserved (-20 °C) for analysis of calcium, phosphorus and alkaline phosphate[16,19]. Immediately after collecting the urine and blood, uterus was carefully removed and weighed[20]. The lumbar vertebra and femurs were isolated and stored at -80 °C until biochemical, biomechanical and histopathological studies were performed.

Femur physical parameter

Fresh isolated left femurs were weighed using an electronic scale. Femurs length was measured using a digital slide calipers. The length was measured from the proximal tip of the femur head to the distal tip of the medial candyle[21]. Bone volume and density were measured by fluid displacement[22].

Lumbar compression test

The fourth lumbar vertebra was located and then it was isolated. The fresh vertebra was placed in digital hardness tester compress until it gets fractured and the reading was recorded in Newton's (kg/m) N[23].

CT scanning of femur bone

Harvested left femurs were firmly secured on to a platform and placed on the scanning table in specific order. The sample were scanned simultaneously using a CT-scanning machine (Somatom Plus-4, Siemens, Germany) for each bone two axial sections, one at 40 % of the length from distal end in diaphysis (cancellous) were taken. Using an inbuilt image analysis system, each axial section was magnified (x 64) to distinguish the cortex from the medullary canal. Keeping the window centering (c) and window width (w) parameter constant, a minimum of 2 diam. (two each for total width and medullary canal) was drawn on the magnified image to calculate medullary canal width (m) and total width (w). Assuming the cross section at the midshaft region to be circular, combined cortical thickness (CCT) was obtained by subtracting medullary width from total width (W) and cortical area was derived from the formula, $CA= \pi[(W/2)^2-(m/2)^2]$. Periosteal area (PA), medullary area (MA) and ratio of cortical area to periosteal area (CA/PA) were then calculated from these values[24].

Femoral ash weight, ash percentage and ash calcium content

The left femur after measuring bone length was placed in tarred fused silica crucibles and kept in Muffles Furnace, dried to a constant temperature at 600 °C for 24 h. Then ash weight was determined and sample was suitably diluted with deionized water to assay for calcium[25].

Biochemical estimation

The level of serum total calcium, inorganic phosphorus and alkaline phosphates and urine calcium, phosphorus and creatinine were determined by colorimetric method using commercially available test kit (Erba diagnostics, Mallaustr, Germany)[26].

Histopathological observation

The right femur were fixed in 10 % formalin for 12 h at 4 °C, decalcified in 5 % ethyenediamine tetra acetic acid (EDTA) for 7 days and embedded in paraffin. The block was then cut in to 5 µm thickness along the sagittal plane passing through the transversals axis of the femur and section were stained with hematoxylin and eosin, examined for morphology under a light microscope[27].

Results

Effect on uterine weight

The uterine weights of all the groups are presented in Table 1. Significant decrease in uterine weight in control group as compared to Sham operated normal group ($P<0.001$) was observed. There is significant increase in uterine weight in estrogen treated standard group ($P<0.001$) and ethanol extract treated groups at dose 100 mg/kg ($P<0.001$), 300 mg/kg ($P<0.05$), 500 mg/kg ($P<0.001$).

Effect on femur physical parameter

Table 2 reveals the control group showing significant decrease in length ($P<0.001$), weight ($P<0.05$), density ($P<0.001$) and there is not much change in volume of femoral bone compared to Sham operated normal group. There was significant increase in femoral bone length in estrogen treated standard group ($P<0.001$) and EET treated group at dose of 100 mg/kg ($P<0.05$), 300 mg/kg ($P<0.001$) and 500 mg/kg ($P<0.01$). Similarly, increase in femur weight in ethanol extract 100 mg/kg ($P<0.01$), 300 mg/kg ($P<0.01$), 500 mg/kg ($P<0.001$) and significant increased volume and density in extract treated with 100, 300 and 500 mg/kg ($P<0.001$) were observed as

Table 1—Effect of ethanol extract of *T. arjuna* on uterine weight and lumbar hardness in ovariectomized rats

Group	Uterine weight (g)	4th Lumbar hardness (kg/m)N
Sham	0.820±0.042	470.4±20.69
Control	0.469±0.040^{c}	143.9±8.586^{c}
Standard	0.971±0.099***	405.6±18.42***
EET (100 mg/kg)	0.757±0.054***	305.9±26.70***
EET (300 mg/kg)	0.786±0.033*	248.6±31.39*
EET (500 mg/kg)	0.7189±0.046***	343.0±16.93***

All the values are expressed as mean±SEM, n=8, $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs. control group (OVX), and $^{a}P<0.05$, $^{b}P<0.01$ and ^{c} $P<0.001$ vs Sham operated normal [One Way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test]. EET= Ethanol extract of *T. arjuna*.

Table 2—Effect of ethanol extract of *T. arjuna* on femoral length, weight, volume and density in ovariectomized rat

Group	Length(cm)	Weight(g)	Volume(mL)	Density(gm/mL)
Sham	3.18±0.091	0.399±0.027	0.245±0.011	1.85±0.210
Control	2.19±0.211[c]	0.281±0.014[b]	0.222±0.011	0.77±0.060[c]
Standard	3.22±0.085*	0.416±0.015***	0.342±0.012***	1.51±0.168***
EET (100 mg/kg)	2.83±0.144*	0.395±0.024**	0.331±0.015***	1.45±0.171***
EET (300 mg/kg)	3.11±0.161***	0.383±0.016**	0.315±0.016***	1.51±0.109***
EET (500 mg/kg)	3.31±0.179***	0.471±0.022***	0.334±0.021***	1.43±0.090***

All the values are expressed as mean±SEM, n=8, **P*<0.05, ***P*< 0.01 and ****P*< 0.001 vs. control group (OVX), and [a]*P*<0.05, [b]*P*<0.01 and [c] *P*<0.001 vs sham operated normal [One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test]. EET= Ethanol extract of *T. arjuna*.

Table 3—Effect of ethanol extract of *T. arjuna* on CT- scanning of femur bone

Group	Total width (mm)	Medullary width (mm)	Combined cortical thickness (mm)	Periosteal area (mm^2)	Medullary area (mm^2)	Cortical area (mm^2)	CA/PA
Sham	5.100±0.577	2.467±0.088	1.23±0.088	19.72±4.59	3.57±0.303	10.72±2.430	0.606±0.170
Control	4.500±0.173	2.300±0.115	0.70±0.05[b]	15.55±1.25	3.84±0.444	11.77±1.643	0.746±0.043
Standard	4.700±0.057	1.700±0.057***	0.80±0.057	16.21±0.94	1.76±0.202***	15.07±0.518	0.923±0.031**
EET (100 mg/kg)	4.433±0.120	2.400±0.057	0.83±0.033	15.16±0.54	3.98±0.180	11.14±0.575	0.733±0.014
EET (300 mg/kg)	4.200±0.057	2.300±0.057	0.76±0.033	12.99±0.51	3.69±0.174	9.68±0.521	0.743±0.037
EET (500 mg/kg)	4.100±0.057	1.767±0.033**	0.73±0.033	13.00±0.60	2.03±0.0781**	10.96±0.563	0.840±0.005

All the values are expressed as mean±SEM, n=8, **P*<0.05, ***P*< 0.01 and ****P*< 0.001 vs. control group (OVX), and [A]*P*<0.05, [b]*p*<0.01 and [c] *P*<0.001 vs sham operated normal [One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test]. EET= Ethanol extract of *T. arjuna*.

compare to control group indicate protection in the bone loss in overiectomized rats in dose dependent action.

Effect on lumbar compression parameter

The lumbar compression result is presented in Table 1. There was significant decreased hardness of 4th lumbar in control group (*P*<0.001) as compared to Sham operated group and there was significant increase in hardness in lumbar vertebrae in EET in dose of 100 mg/kg, 300 mg/kg (*P*<0.001) and 500 mg/kg (*P*<0.05) in dose dependently and also in estrogen treated standard (*P*<0.001) group as compared to control group.

Effect on femur bone

Cortical bone morphometric indices are presented in Table 3 and Plate 1 & 2. The results shown no significant change in total width, medullary width, periosteal area, cortical area and CA/PA ratio and shown significant increase in combined cortical thickness in Sham operated rats as compared to control. Whereas significant decrease in medullary width in extract treated group in dose of 500 mg/kg (*P*<0.001) and estrogen treated standard group (*P*<0.001) was observed. There is significant decrease in medullary area in dose of 500 mg/kg (*P*<0.01) and

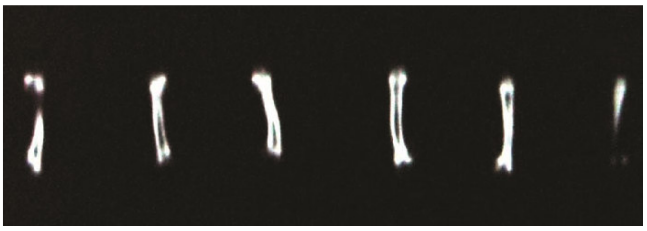

Plate 1—Topographic image of the arrangement of femur bones under the scanner (sagital section)

Plate 2—Un-magnified axial sections of femur bones at 40% of the length from distal end in femur bone

estrogen treated standard (*P*<0.001); also significant increase in CA/PA in estrogen treated standard group (*P*<0.01) as compared to control group.

Effect on femoral ash weight, percentage and calcium

The femoral ash content (Table 4) results indicated significant decrease in ash weight, percentage and calcium in bone of control (*P*<0.001) group as compared to Sham operated group. Similarly significant increase of ash weight, ash percentage and calcium in ethanol extract treated groups at 300

mg/kg (P<0.001) and 500 mg/kg (P<0.001) dose dependent protection has been observed.

Biochemical parameters

Biochemical parameters in serum (Table 5) and urine (Table 6) were estimated and showed significant elevated ALP (P<0.001) in control group and there is a significant reduction in the ALP in estrogen (P<0.001) and ethanol extract at 100 mg/kg (P<0.01), 300 mg/kg (P<0.001) and 500 mg/kg (P<0.001) dose dependently as compared to control group. Similarly the decreased serum calcium and phosphorus and increased creatinine, calcium and phosphorus in control rats were observed. The estrogen and ethanol extract treated group showed significant increase in serum (P<0.01 to P<0.001). But decreased significant level of creatinine, calcium and phosphorus (P<0.01 to P<0.001) was observed as compared to control group.

Histopathological evaluation

The histopathological study indicates (Plate 3) increase in bone turnover and enhanced bone fragility with disruptive and lytic changes in the bone architecture observed in the ovariectomized rats was indicative of the development of osteoporosis in rats due to estrogen deficiency and mimics human postmenopausal osteoporosis. The ethanol extract treated group showed increased ossification, mineralization, calcified cartilaginous deposit and marginal osteoclastic activity, these findings indicate marked restorative and reduced bone resorption and enhanced the bone formation.

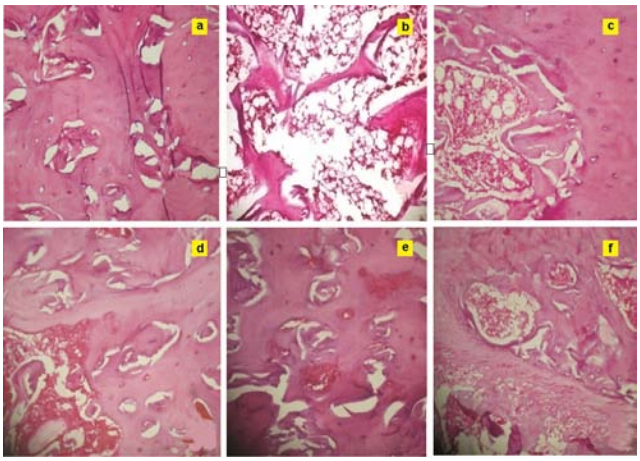

Plate 3—Effect of EET on ovariectomized rats induced osteoporosis. (a) Sham group showing normal, compact and uniform tabacular; (b) photomicrograph of the OVX group showing sparse, thinning of trabeculae with tendency for disappearance, loss of connectivity, and widening of inter tabacular space in OVX rat; (c), (d), (e) 100, 300 and 500 mg/kg EET show trabecular restoration; (f) Standard showing moderately, thick elongated trabecule and narrowed inter trabecular space and restoration of normal architecture along with increasing bone cells [40X]

Table 4—Effect of ethanol extract of *T. arjuna* on ash content of femoral bone in overiectomized rats

Group	Ash weight (gm)	Ash (%)	Calcium (mg/dL)
Sham	0.283±0.01	63.93±2.192	4.571±0.145
Control	0.179±0.01^{c}	44.32±1.837^{c}	1.737±0.152^{c}
Standard	0.302±0.01***	65.80±2.543***	4.774±0.266***
EET (100 mg/kg)	0.224±0.01	62.70±3.760***	4.093±3.418*
EET (300 mg/kg)	0.245±0.01*	64.38±3.198***	4.093±0.400***
EET (500 mg/kg)	0.259±0.11**	65.64±3.164***	4.648±0.437***

All the values are expressed as mean±SEM, n=8, *P<0.05, **P< 0.01 and ***P< 0.001 vs. control group (OVX), and ^{a}P<0.05, ^{b}P<0.01 and ^{c} P<0.001 vs Sham operated normal [One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test]. EET= Ethanol extract of *T. arjuna*.

Table 5—Effect of ethanol extract of *T. arjuna* on serum biochemical marker in ovariectomized rats

Groups	Alkaline phosphatase (IU/L)	Calcium (mg/dL)	Phosphorous (mg/dL)
Sham	150.3±14.84	5.061±0.5762	6.106±0.3529
Control	643.5±64.27^{c}	2.347±0.3945^{c}	1.213±0.1272^{c}
Standard	115.8±12.57***	4.187±0.2780***	5.707±0.1922***
EET (100 mg/kg)	388.4±74.68**	4.276±0.4204**	2.095±0.1124**
EET (300 mg/kg)	304.5±46.95***	1.867±0.1602	1.867±0.1602
EET (500 mg/kg)	327.9±50.31***	5.116±0.3964***	1.957±0.2327*

All the values are expressed as mean ±SEM, n=8, *P<0.05, **P< 0.01 and ***P< 0.001 vs. control group (OVX), and ^{a}P<0.05, ^{b}P<0.01 and ^{c}P<0.001 vs Sham operated normal [One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test]. EET=Ethanol extract of *T. arjuna*.

Table 6—Effect of *T. arjuna* ethanolic extract on urinary biochemical marker in ovariectomized rats

Group	Creatinine (mg/dL)	Calcium(mg/dL)	Phosphorous (mg/dL)
Sham	1.149± 0.117	1.968± 0.2268	4.178 ± 0.4129
Control	0.498± 0.057[c]	4.762± 0.5842[c]	6.531 ± 0.2943[c]
Standard	1.042±0.095***	2.790± 0.3140***	4.784 ± 0.2715***
EET (100 mg/kg)	1.226±0.141***	4.093± 0.2310	5.457 ± 0.2867*
EET (300 mg/kg)	1.113±0.102***	2.982± 0.2187***	4.800 ± 0.3826***
EET (500 mg/kg)	1.250±0.112***	3.200± 0.2447**	5.070 ± 0.2652**

All the values are expressed as mean ±SEM, n=8, **P*<0.05, ***P*< 0.01 and ****P*< 0.001 vs control group (OVX), and [a]*P*<0.05, [b]*P*<0.01 and [c] *P*<0.001 vs Sham operated normal [One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test]. EET= Ethanol extract of *T. arjuna*

Discussion

The current approach of designing the anti-osteoporotic drug is directed with two basic processes of bone remodeling[28]. These agents are aimed to prevent bone resorption (estrogen, calcitonin, bisphosphonates, calcium, vitamin D and raloxifene) or to stimulate bone formation (fluoride, anabolic steroids)[29]. The most common type of osteoporosis is the bone loss associated with ovarian hormone deficiency at menopause. The mechanism by which ovary and hormone deficiency results in bone loss remains uncertain and several hypotheses have been linked to this condition. In human estrogen deficiency, it has been proposed to augment plasma calcium levels as result of increased bone resorption[30]. Thus alternative approaches for managing osteoporosis are needed and in this regard authors evaluated the anti-osteoporotic activity of *T. arjuna* in ovariectomized rats. The ovariectomized rat exhibit most of the characteristics of human postmenopausal osteoporosis[31]. In this study, the histopathology, biochemical markers and biochemical results are similar to the earlier study in ovariectomized rats[32]. The bone loss with respect to body weight was observed throughout the study, along with decrease in the femoral length, weight, volume, hardness and uterine weight in control ovariectomized rats. Radiography such as CT-scanning is being used to evaluate and measure cortical dimension[33], which is also known as radiographic morphometry, involved measurement of cortical bone morphometric indices. No significant change in bone morphometric indices may be due to lack of effect on osteoclastic and osteoblastic bone resorption[34]. In this group the decreased bone mineral content was evidenced by the reduction of total ash weight, percent and calcium content, asserting its role in the prevention of bone loss[35]. Abnormal increase in loss of calcium, phosphorous and creatinine levels in urine are supporting bone loss in the ovariectomized rats[29]. In fact, increase in the level of ALP was observed with respect to decrease in calcium and phosphorus in serum on ovariectomized animals. In our study, the increase in bone turnover and enhanced bone fragility with disruptive and lytic changes in the bone architecture was observed in the histopathological study following ovariectomy, is indicative of the development of osteoporosis in rats due to estrogen deficiency and mimics human postmenopausal osteoporosis[32]. Histopathological examination of the femurs of extract treated group observed ossification, mineralization, calcified cartilaginous deposit and marginal osteoclastic activity, all of which indicated marked restorative effect. The protective action of extract may be due to an increase in bone formation with reduction in bone resorption[31]. The decrease in calcium absorption in the ovariectomized rat report is similar to postmenopausal women[36]. The decrease in serum ovarian hormone deficiency can decrease intestinal calcium absorption without altering vitamin D metabolism. To some extent, we observed significant fall in serum calcium and phosphorus level which may relate in part to lower serum albumin levels and significant decrease in serum ALP in treated OVX (control group) rats may relate in part to lower serum albumin levels despite compensation metabolism and long half-Life[37]. ALP expression, collagen synthesis and osteocalcin synthesis in monolayer bone cell cultures are the stimulation of osteoblast proliferation and activities together leads to the increased bone formation.

Conclusion

It can be concluded that the use of ethanol extract of *T. arjuna* may inhibit osteoclast differentiation and will be beneficial, safe and effective in management

of osteoporosis. Further studies are required to elucidate the molecular mechanism of *T. arjuna* in treatment of osteoporosis.

Acknowledgement

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References

1 Meryl S L, Metabolic Bone Diseases: Text book of Rheumatology, 5th edn, WB Saunders Co., London, 1997, 1563-72.
2 Prabha Shankar B, Osteoporosis-a silent epidemic, *Pharm Times*, 2002, **34**, 21-22.
3 Gruber H E, Ivey J L, Baylink D J, Matthews M, Nelp W B, Sisom K and Chesnut C H 3rd, Long term calcitonin therapy in postmenopausal osteoporosis, *Metabolism*, 1984, **33**, 295-303.
4 Riggs B L and Melton W, *In*: Evolutional osteoporosis, *N Eng J Med*, 1986, **314**, 1676-79.
5 Turner R T, Riggs B L and Spielberg T C, Skeletal effects of estrogen, *Endocr Rev*, 1994, **15**, 275-300.
6 Kannus P, Purkkari J, Sievanen H, Heinonen A, Vuori J and Jarvinen M, Epidemology and hip fractures, *Bone*, 1996, **18**, 57S-63S.
7 Genunt H K, Baylink D J and Gallagher J C, Estrogens in the prevention of osteoporosis in post menopausal women, *Am J Obstet Gynecol*, 1998, **161**, 1841-6.
8 Termine J D and Wang M, Post menopausal women and osteoporosis: available choices for maintenance of skeletal health, *Maturities*, 1998, **30**, 241-245.
9 Nibha V and Manjula V, Effect of *Terminalia arjuna* on antioxidant defense system in cancer, *Mol Biol Rep*, 2009, **36**(1), 159-64.
10 Dwivedi S and Udupa N, *Terminalia arjuna*: Pharmacognosy, phytochemistry, pharmacology and clinical use, A review, *Fitoterpia*, 1989, **60**(5), 413-420.
11 Lin T C, Chien S C, Chen H F and Hsu F L, Tannins and related compounds from Combretaceae plants, *Chin Pharmac J*, 2001, **52**, 1-26.
12 Gauthaman K, Maulik M, Kumari R, Manchanda S C, Dinda A K and Maulik S K, Effect of chronic treatment with bark of *Terminalia arjuna*: a study on the isolated ischemic-reperfused rat heart, *J Ethnopharmacol*, 2001, **75**(2-3), 197-201.
13 Alpana R, Lauria P, Rajeev G, Pradeep K and Sharma V N, Hypercholesterolaemic effects of *Terminalia arjuna* tree bark, *J Ethnophamacol*, 1997, **55**(3), 105-169.
14 Chopra R N and Ghosh S, *Terminalia arjuna*: Its chemistry, pharmacology and therapeutic action, *Indian Med Gaz*, 1929, 64, 70-73.
15 Nadkarni K M, Indian Materia Medica, 3rd edn, Popular Prakashan, Mumbai, 1996, 167.
16 Xie F, Wu C F, Lai W P, Yang X J, Cheung P Y and Yao X S, The osteoprotective effect of *herba epimedii* (HEP) extract *in vivo* and *in vitro*, *Evid Based Complement Altern Med*, 2005, **2**(3), 353-61.
17 Organization for Economic Co-operation and Development, OECD Guidelines for the testing of chemicals, OECD Guideline 425: Acute oral toxicity: Up-and-down procedure, Approved, June 1998.
18 Lasota A and Danowaska K D, Experimental osteoporosis-different methods of ovariectomy in female white rats, *Rocz Akad Med Bialymst*, 2004, **49**(1), 129-31.
19 Shirwaikar A, Khan S and Malini S, Antiosteoporotic effect of ethanol extract of *Cissus quadrangularis* Linn. on ovariectomized rat, *J Ethanopharmacol*, 2003, **89**(2), 245-50.
20 Charles HC, Approach to the elderly patient with Osteopenia or Osteoporosis: Text book of internal medicine, 2nd Edn, Philadelphia: Lippincott Raven publishers, 1997.
21 Yongzhong Z, Longjiang Y, Mingzhang A and Wenwen J, Effect of ethanol extract of *Lepidium meyenii* W. on osteoporosis in ovariectomized rat, *J Ethnopharmcol*, 2006, **105**, 274-9.
22 Asankursekhar D, Dolan D, Maitrayee M, Sandip M and Chandan M, Phytoestrogenic effect of black tea extract (*Camellia sinensis*) in an oophorectomized rat (*Rattus norvegicus*) model of osteoporosis, *Life Sci*, 2005, **77**, 3049-57.
23 Ogey A, Bayraktar F and Sevin G, A comparative study of Raloxifen and estrogen on bone strength and cholesterol levels in ovariectomized rats, *Endocrine Abs*, 2001, **3**, 10.
24 Reddy P N and Lakshmana M, Assessment of experimental osteoporosis using CT-scanning quantitative X-ray analysis and impact test in calcium deficient overictomized rats, *J Pharmacol Toxicol Methods*, 2005, **52**, 350-55.
25 Jiepin W, Fujun S, Qibing M, Jianbo W, Rong Z and Siwang W, NO-donating genistein prodrug alleviates bone loss in overictomizied rats, *Swiss Med Weekly*, 2009, **138**(41-42), 602-7.
26 Burtis C A and Ashwood E R, Teetz Text Book of Clinical Biochemistry, 2nd Edn, Saunders, London, 1986, 1890-91.
27 Bancroft J D and Cook H C, Manual of Histological Techniques, Churchill Livingstone, New York, 1980.
28 Hodgson S F, Watts N B, Bilezikian J P, Clarke B L, Gray T K, Harris D W, Johnston C C Jr, Kleerekoper M, Lindsay R, Luckey M M, McClung M R, Nankin H R, Petak S M, Recker R R and AACE Osteoporosis Task Force, American Association of clinical endocrinologists medical guidelines for clinical practice for the prevention and treatment of postmenopausal osteoporosis: 2001 edition, with selected updates for 2003, *Endocr Pract*, 2003, **9**, 544-64.
29 Olszynski W P, Shawn Davison K, Adachi J D, Brown J P, Cummings S R, Hanley D A, Harris S P, Hodsman A B, Kendler D, McClung M R, Miller P D and Yuen C K, Osteoporosis in men: epidemiology, diagnosis, prevention and treatment, *Clin Ther*, 2004, **26**, 15-28.
30 Hui S L, Slemenda C W and Johnston C C, Age and bone mass as predictors of fracture in a prospective study, *J Clin Invest*, 1988, **81**, 1804-9.
31 Cummings S R, Kelsey J, Nevitt M and O'Dowd K, Epidemiology of osteoporosis and osteoporotic fractures, *Epidemiol Rev*, 1985, **7**, 178-208.
32 Looker A, Johnston C C and Wahner H, Prevalence of low femoral bone mineral density in older U.S. women, *J Bone Miner*, 1995, **10**, 796-802.

33 Reeve J, Future prospects for hormone replacement therapy, *Med Bull*, 1992, **48**, 458-68.

34 Christiansen C, The different routes of administration and the effect of hormone replacement therapy on osteoporosis, *Fertil Steril*, 1994, **62**, 152S-6S.

35 Sagraves R, Estrogen therapy for postmenopausal symptoms and prevention of osteoporosis, *J Clin Pharmacol*, 1995, **35**, 2-10.

36 Colditz G A, Hankinson S E and Hunter D J, The use of estrogens and progestins and the risk of breast cancer in postmenopausal women, *J Med*, 1995, **332**, 1589-93.

37 Dresner-Pollak R, Parker R A, Poku M, Thompson J, Seibel M J and Greenspan S L, Biochemical markers of bone turnover reflects femoral bone loss in elderly women, *Calcif Tissue Int*, 1996, **59**, 328-33.