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EVALUATION OF ANTI-OSTEOPOROTIC ACTIVITY OF ASTHIPOSHAK TABLETS IN OVARIECTOMIZED RATS

Mrinal Sanaye [*1], Bhavna Bora [1], Mukesh Chawda [2] and Viplav Kshirsagar [2]

Department of Pharmacology [1], Prin. K. M. Kundnani College of Pharmacy, Mumbai - 400005, Maharashtra, India.
Shree Dhootapapeshwar Limited [2], 135 Nanubhai Desai Road, Khetwadi, Mumbai - 400004, Maharashtra, India.

Keywords:

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Correspondence to Author:
Dr. Mrinal Sanaye

Associate Professor in Pharmacology, Prin. K. M. Kundnani College of Pharmacy, Mumbai - 400005, Maharashtra, India.

E-mail: msmrinalms@gmail.com

ABSTRACT: Osteoporosis is a condition that makes the bones porous, fragile, and prone to fractures. Although it is very prevalent in elderly people, it is more common in women, especially after menopause. The present study aimed to evaluate the effect of Asthiposhak Tablets on their anti-osteoporotic activity in ovariectomized (OVX) rats. Thirty-two female albino Wistar rats were randomly divided into four groups (n=8). Group 1 served as sham-operated control. Group 2 rats were ovariectomized (OVX) and served as a negative control. Group 3 received raloxifene (5.4 mg/kg i.p.) and served as the standard control, and Group 4 received Asthiposhak (405 mg/kg p.o.) and served as treatment control. After 60 days of ovariectomy, animals were treated with Asthiposhak for the next 45 days. At the end of the study, femur bone length, weight, bone ash calcium level, and bone mineral density (BMD) were estimated. The levels of serum alkaline phosphatase (ALP), calcium, and phosphorous, and bone histopathology were also evaluated. OVX-induced increased serum ALP, calcium, and phosphorous levels were significantly attenuated in Asthiposhak-treated rats. Asthiposhak treatment significantly prevented an OVX-induced increase in body weight. The calcium content in bone ash was significantly increased on Asthiposhak treatment indicating remineralization of bones. OVX-induced decrease in BMD was significantly reversed in Asthiposhak-treated animals. Femur bone histopathology revealed increased trabecular thickness and decreased osteoclast formation in Asthiposhak-treated animals. Asthiposhak exhibited a significant anti-osteoporotic effect in the experimental model of OVX-induced osteoporosis in rats. These results indicate Asthiposhak can be beneficial in postmenopausal osteoporosis.

INTRODUCTION: Osteoporosis is a systemic bone disease characterized by the reduction in the bone-tissue content and the alteration of bone microstructure [1].

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It increases bone fragility and reduces bone strength, thereby tending to increase the risk of fracture, morbidity, and mortality [2].

There are two types of primary osteoporosis: postmenopausal osteoporosis (primary type 1 osteoporosis) and senile osteoporosis (primary type 2 osteoporosis). Postmenopausal osteoporosis, which is the most common type in women, has become a serious public health issue resulting in increased morbidity, mortality, and high healthcare cost [3].

Postmenopausal osteoporosis is associated with estrogen deficiency, which induces osteoclast formation, thereby resulting in an imbalance between osteoblasts and osteoclasts [4]. Various therapeutic agents like calcium selective estrogen receptor modulators such as raloxifene and droloxifene, estrogen, bis-phosphonates, fluoride, and calcitonin have been used for the clinical treatment of postmenopausal osteoporosis [5, 6].

However, these agents have been reported to have adverse effects limiting their use [7]. Thus, an increasing number of studies have been focused on the use of natural remedies for osteoporosis management owing to their safety profile and various bioactivities.

The traditional medical system of Ayurveda has been an integral part of medical practice in India. Ayurvedic system prescribes various herbs to speed fracture healing. Phytopharmacotherapy for bone and fracture healing is expected to be safer when compared with synthetic drugs in terms of the safety profile [7].

To overcome the wide range of side effects faced by these synthetic drugs, there is an increasing demand for "green medicines" that are thought to be healthier and safer for the treatment of osteoporosis.

The present study was undertaken to investigate the usefulness of an Ayurvedic herbomineral preparation, Asthiposhak, in the management of perimenopausal osteoporosis. Asthiposhak is composed of Kukkutandatvak Bhasma (Processed Hen Egg shell), Shodhit Laksha (Processed Lacciferalacca), Shodhit Guggul (Processed *Commiphora wightii*), Asthisamhruta (*Cissus quadrangularis*), Arjuna (*Terminalia arjuna*), Amalaki (*Emblica officinalis*), Ashvagandha (*Withania somnifera*), Guduchi (*Tinospora cordifolia*), Bala (*Sida cordifolia*) and decoction of Baboola (*Acacia arabica*).

The complete composition of Asthiposhak Tablets is given in **Table 1**. Some of these ingredients are reported for osteo-protective and phytoestrogenic activities and are also reported to prevent further degeneration of bones [8]. Thus, this study aimed at evaluating the anti-osteoporotic activity of Asthiposhak Tabletsin an ovariectomy-induced model in rats.

TABLE 1: COMPOSITION OF ASTHIPOSHAK TABLETS

Each coated tablet contains:	
Powder of:	
KukkutandatvakBhasma (Processed Hen-Egg shell)	100 mg
Shodhit Laksha (processed *Laccifera lacca*)	50 mg
Shodhit Guggulu (processed *Commiphora wightii*)	50 mg
Choorna of:	
Asthisamhruta (*Cissus quadrangularis*)	100 mg
Arjuna (*Terminalia arjuna*)	50 mg
Amalaki (*Emblica officinalis*)	50 mg
Ashvagandha (*Withania somnifera*)	50 mg
Guduchi (*Tinospora cordifolia*)	50 mg
Bala (*Sida cordifolia*)	50 mg
Processed in decoction of:	
Babboola (*Acacia arabica*)	q.s.

MATERIALS AND METHODS:

Chemicals: Raloxifene hydrochloride tablets (Cipla Pvt. Ltd. Goa, India) were purchased from a local retailer and Asthiposhak was procured from Om Pharmaceuticals Limited, Bengaluru, India. Commercial reagent kits for estimation of calcium, ALP, and phosphorous were procured from Erba Transasia Biomedicals, India. Other chemicals and reagents were purchased from SD Fine-Chemicals Pvt. Ltd, Mumbai, India.

Experimental Animals: Thirty-two female albino Wistar rats weighing 180-220 g were procured from Shree Dhootapapeshwar Ayurved Research Foundation (SDARF), Panvel, India. The animals were maintained in the institutional animal house in clean polypropylene cages containing husk bedding.

The animals were fed with a standard pellet diet and water *ad libitum* and were allowed to acclimatize to the laboratory conditions for 8-10 days before the start of the experiment. Standard laboratory conditions (temperature – 24 ºC ± 2 ºC, relative humidity – 50% ± 5%, and 12 h light/12 h dark cycle) were maintained throughout the experiment.

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) before the experimentation (protocol number: KMKCP/IAEC/06/2017). All the procedures were performed according to NIH guidelines for animal handling.

Experimental Design: Six months old female albino Wistar rats weighing 180-220 g were used in the study. All ovariectomized rats were divided into four groups (n=8) and treated for 45 days. Group 1 was sham-operated, Group 2 was negative (OVX) control, Group 3 was OVX + Raloxifene (5.4 mg/kg i.p.) and served as the standard control, and Group 4 was OVX + Asthiposhak (405 mg/kg p.o.). The dose of Raloxifene was based on previous literature [9] and the dose of Asthiposhak was calculated from its human dose.

Induction of Osteoporosis by OVX Surgical Procedure: After acclimatization, all groups were anesthetized before surgery with the combination of ketamine (80 mg/kg i.p.) and xylazine (10 mg/kg i.p.). Group 1 was sham-operated, and the other three groups were ovariectomized. The surgical equipment used was aseptically cleaned with rectified spirit. The operation was carried out by placing each animal on its ventral surface. The fur on the rat abdomen was cleaned with the help of a rectified spirit, and then the fur was completely removed. Ovariectomy was preceded by the midline ventral skin incision (bilateral), 3 cm long, approximately halfway between the midline of the body and the base of the tail.

After the peritoneal cavity was accessed, the adipose tissue was pulled away until the fallopian tube and the ovary surrounded by the variable amount of fat was identified. Ligation was performed around the area of the distal uterine horn with an absorbable suture to avoid bleeding before removal of the ovary. The connection between the uterine horn and each fallopian tube was cut, and the ovary was excised. In sham-operated rats, the ovaries were exposed but not removed. In all the ovariectomized groups, the uterine horn was returned to the peritoneal cavity after removal of ovaries. The muscle incision was sutured with absorbable suture, and skin wounds were closed bilaterally with absorbable catgut. Operated animals were given a combination of amoxicillin and clavulanic acid (Augmentin injection) (25 mg/kg, i.p.) For 5 days. Povidone-iodine solution (Cipladine) was applied topically, and animals were monitored carefully. After two days of surgery, two rats were housed singly in a polyurethane cage for one week to allow recovery and at the same time, the animals were also observed for coprophagy. They were also observed for normal behavior and then re-grouped in their home cages [10, 11].

After ensuring induction of osteoporosis on 60th day the treatment was administered for 45 days. On the 45th day of treatment, blood was withdrawn from the retro-orbital plexus and centrifuged (Eltek refrigerated centrifuge RC 4100 D) at 3000 rpm for 10 min at 4°C to obtain clear serum. Levels of ALP, calcium and inorganic phosphorous in serum were estimated using commercially available diagnostic kits (Erba, Trasnasia Biomedicals, India). At the end of the study, the animals were sacrificed by carbon dioxide asphyxiation, and two femur bones each from left and right limbs were removed and were cleaned with the help of papain juice and sent for histopathology. The estimation of bone ash calcium level was performed on six right femur bones of each group, and histopathology studies were performed on the remaining two right femur bones of each group. Bone mineral density (BMD) was evaluated using six left femur bones of each group.

Evaluation Parameters:

Measurement of the Weight of Femur Bone: At the end of the study, all the rats were euthanized by using carbon dioxide asphyxiation, and femur bones were excised. The bones were then kept in an oven and dried at 100 °C, and weights of the dried bones were determined by using a digital weighing balance (Metler).

Analysis of BMD: BMD studies were performed at Tata Memorial Centre Advanced Centre of Treatment, Research and Education in Cancer (ACTREC) Mumbai, India. Femur bones were scanned with μ-CT (Tri-Foil imaging) at a resolution of 21 μM by applying the following settings: X-ray voltage 60 kV and electric current 130 μA. BMD, bone mineral content, trabecular thickness, and trabecular space were analyzed using MicroView v. 2.0 Software

Determination of Femoral Ash Weight, Ash Percent and Calcium Content: The soft tissues were cleaned from the femur bones that were then broken into small fragments, placed into a container of ethanol and soaked overnight before being extracted with ethanol in a Soxhlet extractor

for 24 h and further extracted with anhydrous ether. After the second extraction, bones were dried at room temperature for 24 h and then placed in tarred fused silica crucibles, kept in a muffle furnace, ashed at 600 °C for 24 h, then ash weight and percentage ash were determined. Further, the ash was used for the calcium assay by a titrimetric method using AOAC standard procedures. The estimation was done based on previously published data [12-14].

Serum Biochemical Markers: The levels of serum calcium, phosphorous, and ALP were estimated using Erba diagnostic kits. The procedure was followed as per the manufacturer's protocol.

Histopathology: Tissue specimens were collected from animals belonging to different treatment groups. After collection, the tissue samples were immediately preserved in the 10% neutral buffered formalin for fixation. Tissues were decalcified using 5% formic acid for 12 days and were subsequently processed by routine method for histopathological observation. The processed tissue was then sectioned (at 5 µm) and taken on clean glass slides and stained by hematoxylin and eosin (H&E) and observed under microscopes at different magnifications and was examined microscopically to check the presence of alterations having pathological significance.

Statistical Analysis: All the values are expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out by one-way ANOVA followed by Tukey Kramer post hoc multiple comparison test using GraphPad Prism version 5.0, San Diego, California, USA.

RESULTS AND DISCUSSION:

Femur Bone Weight: The results of femur weight in animals of different groups are illustrated in **Fig. 1**. The absolute weight was found to be significantly (###P<0.001) decreased in the OVX group as compared to the sham control. Treatment with Asthiposhak (***P<0.001) and raloxifene (***P<0.001) showed a significant increase in femur weight as compared to the OVX group.

Osteoporosis is a metabolic bone disease characterized by loss of bone mass, thus, making the bone more susceptible to fractures [15]. Osteoporosis is characterized by a decrease in bone density that results in decreased bone strength, thus making bones fragile [12]. Bones are living tissues that constantly undergo remodeling; if the rate of replacement of bone mass loss is not matched with the rate of formation of new bone mass, it results in osteoporosis [16].

Bone remodeling is carried out through the coordinated actions of bone-removing cells called osteoclasts and bone-forming cells called osteoblasts [17]. Bone remodeling is initiated by the activation of resting osteoclast precursors, which is a complex process involving the interaction of osteoblast and osteoclast precursors and is regulated by several systemic hormones and locally produced factors.

The ovariectomy model in female rats simulates many common characteristics of postmenopausal osteoporosis occurring in humans, such as increased bone turnover, bone resorption exceeding bone remodeling resulting in micro-architectural deterioration of bone mass [11, 18]. These similarities thus provide a strong basis for selecting it as a suitable animal model to study the efficacy of anti-osteoporotic agents for postmenopausal osteoporosis. Thus, this model was used in the present study to evaluate the efficacy of Asthiposhak in OVX-induced osteoporosis.

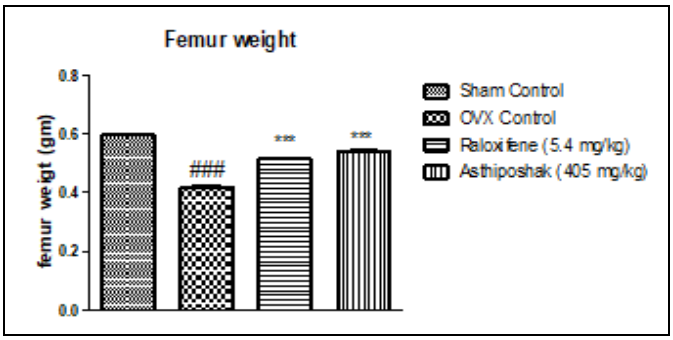

FIG. 1: EFFECT OF ASTHIPOSHAK ON FEMUR BONE WEIGHT Values are expressed as MEAN ± SEM (n=8). Statistical analysis was carried out by one-way ANOVA followed by Tukey Kramer post hoc multiple comparison test. ###P<0.001 *vs.* Sham Control Group; ***P<0.001 *vs.* OVX Control Group. OVX=Ovariectomy

Bone Mineral Density: BMD in the OVX control was found to be significantly (###P<0.001) lowered when compared to the sham control. Whereas, in Asthiposhak (***P<0.001) and raloxifene (***P<0.001) treated groups, BMD of the femur bone was observed to be significantly improved. The results are in **Fig. 2**.

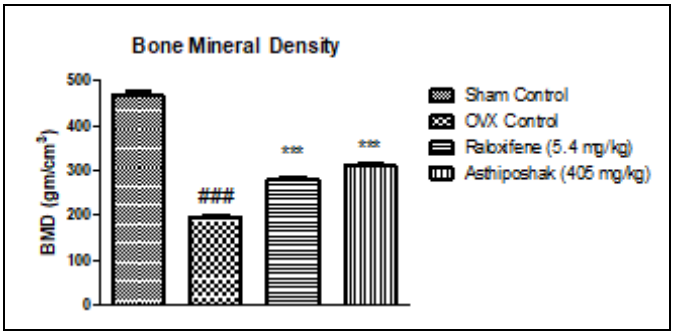

FIG. 2: EFFECT OF ASTHIPOSHAK ON BONE MINERAL DENSITY Values are expressed as MEAN ± SEM (n=8). Statistical analysis was carried out by one-way ANOVA followed by Tukey Kramer post hoc multiple comparison test. ###P<0.001 *vs.* Sham Control Group;***P<0.001 *vs.* OVX Control Group. OVX = Ovariectomy

The precise mechanism by which estrogen deficiency increases bone loss in postmenopausal osteoporosis is not fully understood though several hypotheses have been proposed. Estrogen regulates bone remodeling by suppressing the production of bone-resorbing cytokines, including IL-1, IL-6, tumor necrosis factor α (TNF-α), and macrophage colony-stimulating factor [2, 19, 20]. These factors increase bone resorption mainly by increasing osteoclast formation and differentiation. Asthiposhak is a herbomineral formulation widely prescribed by Ayurvedic practitioners for bone and joint care. Asthiposhak is indicated in the treatment of osteoporosis, healing of fractures, and calcium deficiency states. Among these ingredients, *Terminalia arjuna* stem bark is reported for its estrogenic activity [21]. Whereas *Emblica officinalis*, *Withania somnifera*, and *Tinospora cordifolia* are all found to prevent further degeneration of bones [22-24].

Serum ALP, Calcium and Phosphorous: The effect of Asthiposhak on serum ALP, calcium, and phosphorous is illustrated in **Fig. 3A**, **3B,** and **3C,** respectively. The activity of serum ALP was significantly (###P<0.001) increased in the OVX control when compared with the sham control. Groups treated with Asthiposhak (**P<0.01) and raloxifene (*P<0.05) significantly suppressed the rise in serum ALP levels. Serum calcium levels were also found to be significantly increased (##P<0.01) in the OVX control as compared to the sham control. Asthiposhak (**P<0.01) and raloxifene (*P<0.05) treatment significantly attenuated serum calcium levels as compared to the OVX control. Serum phosphorous levels were also found to be significantly increased (##P<0.01) in the OVX control as compared to the sham control. However, Asthiposhak (*P<0.05) and Raloxifene (*P<0.05) treatment significantly attenuated serum phosphorous levels when compared with the OVX control.

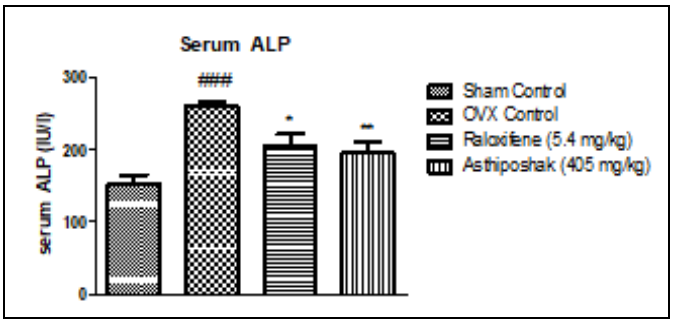

FIG. 3A: EFFECT OF ASTHIPOSHAK ON SERUM ALP Values are expressed as MEAN ± SEM (n=8). Statistical analysis was carried out by one-way ANOVA followed by Tukey Kramer post hoc multiple comparison test. ###P<0.001 *vs.* Sham Control Group;*P<0.05, **P<0.01 *vs.* OVX Control Group. OVX = Ovariectomy

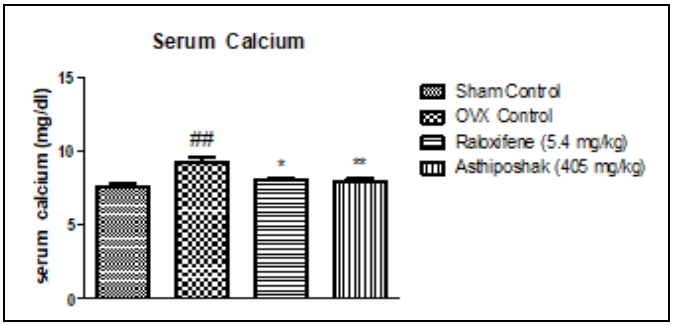

FIG. 3B: EFFECT OF ASTHIPOSHAK ON SERUM CALCIUM Values are expressed as MEAN ± SEM (n=8). Statistical analysis was carried out by one-way ANOVA followed by Tukey Kramer post hoc multiple comparison test. ##P<0.01 *vs.* Sham Control Group;*P<0.05, **P<0.01 *vs.* OVX Control Group. OVX = Ovariectomy

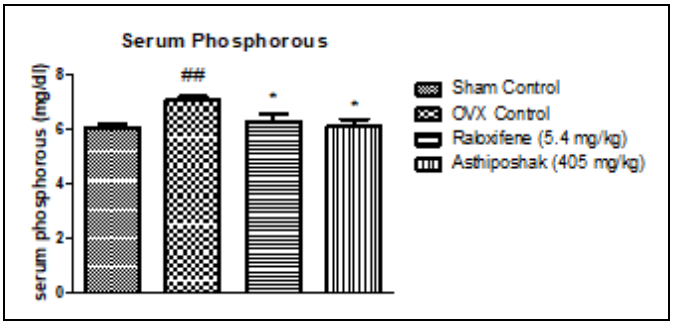

FIG. 3C: EFFECT OF ASTHIPOSHAK ON SERUM PHOSPHOROUS Values are expressed as mean ± SEM (n=8). Statistical analysis was carried out by one-way ANOVA followed by Tukey Kramer post hoc multiple comparison test. ##P<0.01 *vs.* Sham Control Group;*P<0.05 *vs.* OVX Control Group. OVX = Ovariectomy

The estimation of serum levels of ALP, calcium, and phosphorous provides a valuable tool for evaluating the alterations in bone metabolism and accelerated risk of bone loss in the postmenopausal state [25, 26]. In our study, the ovariectomized animals demonstrated a rise in the levels of serum ALP, calcium, and phosphorous, thus indicating the bone resorption. Serum ALP is one of the most widely used biomarkers for osteoporosis. It is synthesized by osteoblasts to provide a high phosphate concentration hence ALP activity is found to be increased during bone demineralization [27]. It is found to be elevated in osteoporosis due to its subsequent release in the blood [28] as observed in the OVX-control group. However, a significant decrease in serum ALP levels was observed in the Asthiposhak- and raloxifene-treated rats, indicating decreased bone turnover and increased osteoblastic activity supporting bone mass formation. *Withania somnifera* in Asthiposhak improves bone calcification in calcium-deficient ovariectomized rats [23]. *Tinospora cordifolia* is one of the prime Rasayana (immunomodulating) herbs in Ayurveda, and its extract is documented to influence osteogenesis and therefore plays a vital role in osteoporosis management [24, 29].

Bone is the main repository of calcium and its active resorption in osteoporosis results in increased serum calcium [30]. Our study supports these observations as 15 days after ovariectomy; serum calcium levels were found to be elevated in a multifold manner in OVX. The homeostatic mechanisms governed by calcitonin and parathyroid hormone regulate serum calcium concentrations by increasing its excretion in the kidneys [31, 32], as was observed on the 45th and 60th-day post ovariectomy, although the levels of ALP remained elevated, indicating the progression of bone de-mineralization. Serum calcium levels on treatment with Asthiposhak were found to be significantly lower than the OVX group, thus indicating decreased bone resorption. Phosphorus is another major mineral constituting bone mass. Phosphorus in the body is present in the form of phosphates [30]. Both calcium and phosphate are deposited in the bone and are also resorbed together. The maintenance of phosphate levels in the body is also closely related to that of calcium. Asthiposhak treatment prevented the increase in serum calcium and phosphorous levels, strongly suggesting their accelerated utilization for bone formation.

Femur Ash Calcium Level, Ash Weight and Ash Percent: As shown in **Fig. 4A**, the bone ash calcium content of the femur was significantly reduced (###P<0.001) in OVX control rats as compared to the sham control. The administration of Asthiposhak (***P<0.001) and Raloxifene (***P<0.001) significantly increased the calcium content as compared to the OVX control.

The total ash weight (shown in **Fig. 4B**) and ash percent (shown in **Fig. 4C**) were significantly (###P<0.001) reduced in the OVX control as compared to the sham-operated group. There was a significant (***P<0.001) increase in the total ash weight and ash percent on Asthiposhak and raloxifene administration as compared to the OVX control.

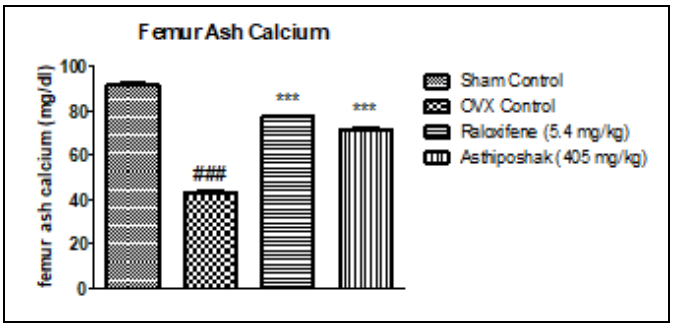

FIG. 4A: EFFECT OF ASTHIPOSHAK ON FEMUR ASH CALCIUM Values are expressed as MEAN ± SEM (n=8). Statistical analysis was carried out by one-way ANOVA followed by Tukey Kramer post hoc multiple comparison test. ###P<0.001 *vs.* Sham Control Group;***P<0.001 *vs.* OVX Control Group. OVX = Ovariectomy

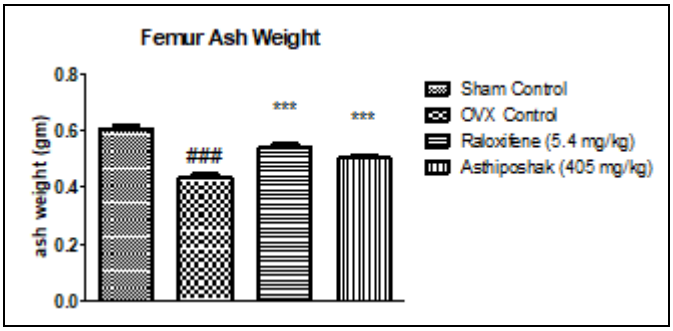

FIG. 4B: EFFECT OF ASTHIPOSHAK ON FEMUR ASH WEIGHT Values are expressed as MEAN ± SEM (n=8). Statistical analysis was carried out by one-way ANOVA followed by Tukey Kramer post hoc multiple comparison test. ###P<0.001 *vs.* Sham Control Group;***P<0.001 *vs.* OVX Control Group. OVX = Ovariectomy

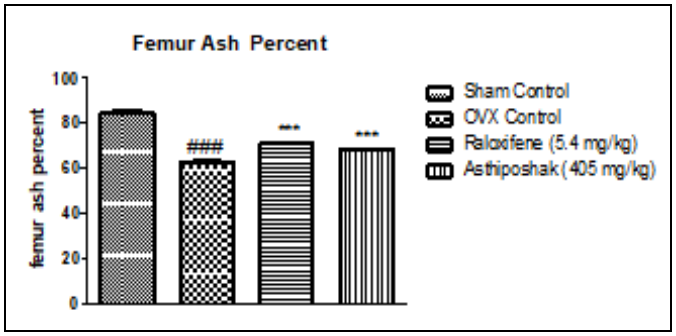

FIG. 4C: EFFECT OF ASTHIPOSHAK ON FEMUR ASH PERCENT Values are expressed as MEAN ± SEM (n=8). Statistical analysis was carried out by one-way ANOVA followed by Tukey Kramer post hoc multiple comparison test. ###P<0.001 *vs.* Sham Control Group;***P<0.001 *vs.* OVX Control Group. OVX = Ovariectomy

The hardness and rigidity of a bone is due to the presence of mineral salts in the osteoid matrix, which is the crystalline complex of calcium and phosphate (hydroxyapatite) [30, 31] in the OVX group, the loss of mineral in the osteoid matrix due to osteoporosis induction was marked by decreased total ash calcium levels [27, 31], whereas the prevention of bone loss and restructuring of bone with Asthiposhak treatment was evident by increased ash calcium content and ash weight. BMD has been described as a surrogate measure of bone strength and a primary contributor to bone quality [32] and was observed to be markedly decreased in the OVX group due to increased bone turnover.

A significant increase in BMD on treatment with Asthiposhak, on the other hand, confirmed the remodeling of bones and the prevention of osteoporosis. This can be attributed to the individual ingredients of Asthiposhak tablets. Reportedly, hen eggshell calcium is more effective in increasing bone mass in postmenopausal women [32].

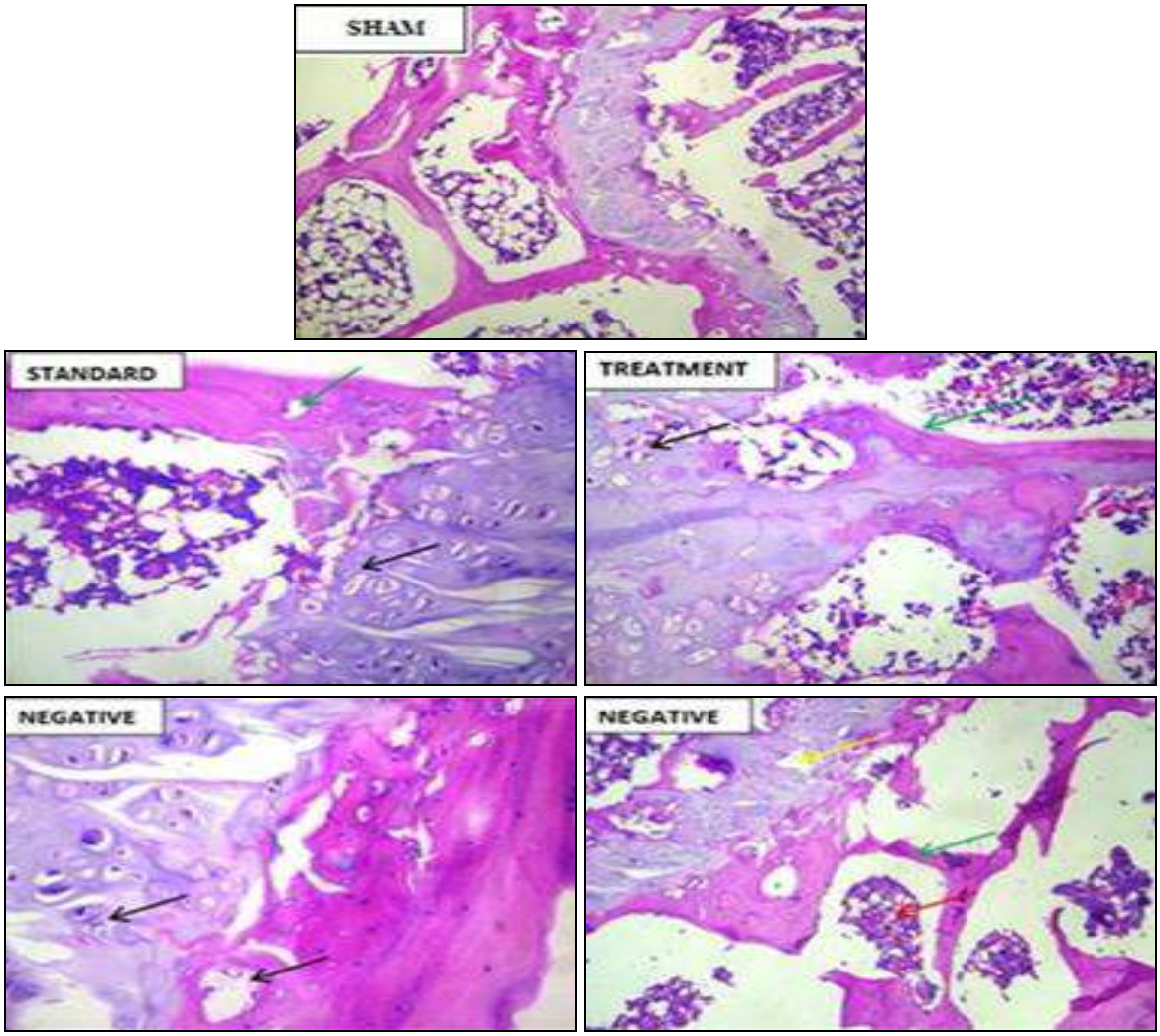

FIG. 5: HISTOPATHOLOGICAL EVALUATION Red Arrow: Bone marrow; Green arrows: Bone Trabeculae; Black arrow: Osteoclasts formation and Yellow Arrow: Bone Resorption

Histopathological Estimation: The histo-pathological sections studied from the femur bone revealed depleted bone marrow in the sham-operated group. Relatively increased osteoclast formation was observed in the OVX control rats with increased bone resorption, decreased trabecular thickness, and depleted bone marrow. The standard group exhibited decreased bone resorption with increased trabecular thickness. The asthiposhak-treated exhibited increased trabecular thickness with relatively decreased osteoclast formation (shown in **Fig. 5**).

Effect on Architecture of Femur Bone: Conventionally, as osteoporosis advances, a reduction in trabecular thickness is observed as a result of continuous bone resorption along with increased osteoclast formation [31, 33], as evidenced in the histopathological evaluation of the OVX bone. Reduced trabecular thickness, absence of bone resorption, and reduced osteoclast formation as observed on histopathology of bones of Asthiposhak-treated rats indicated restructuring of bones (**Fig. 6A** and **6B**). Thus, observed restoration of bone architecture on treatment with Asthiposhak confirmed the prevention of osteoporosis in ovariectomized rats.

Commiphora wightii extract is documented for its anti-resorptive action [34]. It is also widely used in Ayurveda to reduce pain and inflammation [35]. *Cissus quadrangularis* is a widely used herb for fracture healing [36]. Phytoestrogenic steroids present in it have been shown to influence early regeneration and quick mineralization of bone fracture healing process [37]. Due to their beneficial role in fracture healing [38, 39]. *Emblica officinalis* is another herb that is reported to effectively reduce bone loss and increase bone strength in the experimental model of osteoporosis [40].

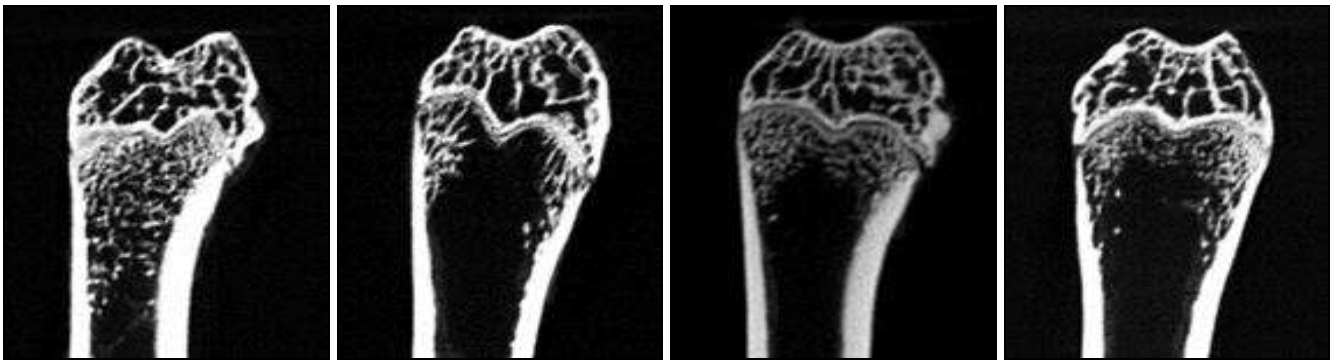

FIG. 6A: CORONAL VIEW OF FEMUR BONE A = Sham Group, B = OVX Group, C = Raloxifene Group, D = Asthiposhak Group

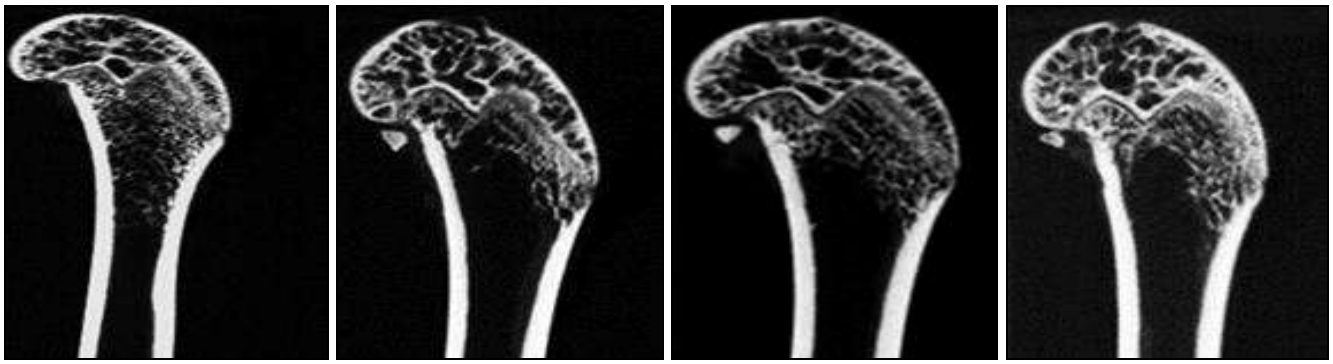

FIG. 6B: SAGITTAL VIEW OF FEMUR BONE A = Sham Group, B = OVX Group, C = Raloxifene Group, D = Asthiposhak Group

CONCLUSION: Treatment with Asthiposhak exhibited significant prevention of bone demineralization due to ovariectomy as indicated by the averting rise in serum ALP, calcium and phosphorous levels, and calcium content in the femur bone ash. Re-mineralization of bone was evident with an increase in BMD and decreased osteoclast formation observed in the bone architecture of the group treated with Asthiposhak in histopathological studies.

Thus, it can be concluded that Asthiposhak has a therapeutic potential to inhibit bone resorption by

promoting bone mineralization in ovariectomized rats. Asthiposhak possesses a promising anti-resorptive effect against estrogen deficiency-induced osteoporosis. The anti-osteoporotic effect exhibited by Asthiposhak is equivalent to that observed on treatment with standard drug Raloxifene Thus, Asthiposhak can be used in the management of postmenopausal osteoporosis.

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AUTHOR CONTRIBUTIONS: Dr. Mrinal Sanaye and Dr. Mukesh Chawda were involved in the conceptualization of the research and interpretation of the data. They were also actively involved in reviewing the manuscript. Dr. Mrinal Sanaye is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work. Miss Bhavna Bora carried out animal studies and substantially contributed to the acquisition, analysis, and interpretation of the data for the research. Viplav Kshirsagar participated in drafting the manuscript and critically revised it. All the authors approved the final version of the manuscript to be published.

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