Original Research Article

Effect of nigella sativa on bone mass density (BMD) in albino rats

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Abstract

Background: Osteoporosis may be diagnosed in postmenopausal women and in men aged 50 years and older if the measurement of bone mineral density in the lumbar spine, total hip or femoral neck is at least 2.5 standard deviations below that of a young control (T score –2.5 or less). Based on current guidelines, a diagnosis of osteoporosis relies on a history of fragility fracture or the result of bone mineral density (BMD) evaluation. *Nigella sativa* seeds (NS) has been used traditionally for various illnesses. The most abundant and active component of NS is thymoquinone (TQ). However paucity of data is available in this regard especially in animal model mimicking postmenopausal osteoporosis. **Materials & Methods:** A total 42 female rats were selected and divided in to seven groups of six in each. Pregnant rats excluded, only non-pregnant rats were used (confirmed by the Veterinary Surgeon attached to the Department). Firstly, the rats were immobilized using ether anaesthesia at a minimal dose following which, a freshly prepared suspension of thiopentone sodium i.v. was given through the dorsal tail vein cannulation at a dose of 10mg/kg. The left hind legs of all the rats were immobilized using plaster casts so as to promote the process of osteoporosis. **Results:** It is evident from measurement of dry bone weight that there is significant bone loss in the ovariectomised rats in comparison to sham rats. Treatment with Nigella sativa extract significantly improved the bone weight, though there is no dose related improvement. There is a significant change in raloxifene treated as well as in combination treated groups in comparison to all the doses of test drug showing the better effectiveness of raloxifene. **Conclusion:** The histopathological studies as well as radiographic finding of bone showing high osteoblastic activity and minimum osteoclastic activity indicating bone formation, also supports its anti-osteoporotic activity. Based upon the results of the present study in animal model, it is quite justified that this indigenous drug may be tried as an adjunct therapy in the treatment as well as prevention of osteoporosis.

Keywords: Osteoporosis, *Nigella sativa (NS)*, raloxifene, albino rats, bone mineral density (BMD), anti-osteoporotic activity, histopathology, radiological changes

Introduction

Osteoporosis is characterized by reduced bone mass and the disruption of bone architecture that results in increased risks of fragility fractures, which are the main consequences of the disease [1]. Osteoporosis-related fractures are associated with substantial pain, suffering, disability, and possibly even death for the affected patients [2]. Studies have shown that bone loss starts from the age of 30–40 years in both men and women. In women, it has been postulated that menopause is followed by an immediate decrease in bone mass and density within a year. This increased rate of bone loss

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reaches equilibrium approximately 10 years after menopause and then merges into a continuous age-related loss [3]. While type 1 or postmenopausal osteoporosis generally occurs before the age of 65 years and affects women, Type 2 osteoporosis is universal after peak bone mass has been attained and is found in both men and women [3, 4]. Experimental research can improve our understanding of pathogenesis and of the activity of pharmaceutical agents in the prevention or treatment of the disease. Although many aspects of the disorder have been revealed, others remain unclear, including the mechanisms involved in calcium homeostasis in the extracellular space and its effect on bone physiology and disease [5] and the cell and molecular pathways triggered after mechanical loading to orchestrate bone renewal [6]. Current research is focused on new therapeutic possibilities targeting the osteolytic enzymes of the osteoclast and the mechanisms activating bone progenitor cells and those controlling apoptosis as new potential treatments [7-9].The use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades. Nigella sativa, a natural herb which belongs to Ranunculaceae family, has long been used as a natural medicine for treatment of many acute, as well as, chronic conditions. It is also known as black cumin or habatus sauda. The seed is the source of the active ingredients of this plant [10, 11]. Nigella sativa seeds (NS) has been used traditionally for various illnesses. The most abundant and active component of NS is thymoquinone (TQ). The main cause of osteoporosis is menopause or estrogen deficiency [11]. Several medicinal plants have been studied using postmenopausal osteoporosis animal model such as soy, blueberry, achyranthes bidentata, and labisia pumila [12-14] Inflammation is mediated by two enzymes, cyclooxygenase and lipoxygenase, which generates prostaglandins and leukotrienes from arachidonic acid, respectively [15]. Therefore, both prostaglandins and leukotrienes are the main mediators of inflammation [16]. TQ was believed to exert anti-inflammatory effects by inhibiting the synthesis of prostaglandins and leukotrienes [17].

It was found to inhibit in a dose-dependent manner the cyclooxygenase and lipoxygenase pathways of rat peritoneal leukocytes that were stimulated with calcium ionophore A23187 [18]. To date, there is no study of NS or TQ on postmenopausal osteoporosis animal model [19]. Recent studies revealed its anti-osteoporotic activity. However paucity of data is available in this regard especially in animal model mimicking postmenopausal osteoporosis.

Materials & methods

The entire work comprises of investigating the effect of the indigenous drug Nigella sativa on bone mass density, studied in Wistar albino rats. The following materials were used for this study. Healthy female albino rats, roughly the same age of 8-12 weeks, weighing between 150-200 gms were selected for this study. The animals were inbred in the departmental animal house. They were kept in polypropylene cages and maintained under controlled conditions of temperature i.e. room temperature of 25±1ºc; relative humidity 45-55% and 12:12 hr light/dark cycle. The animals were given free access to standard rat pellet, with water ad libitum under hygienic conditions. Each group had separate set of animals and care was taken to ensure that animal used in an experiment was not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize non specific stress, if any.Animals in groups were exposed to experimental procedure from time to time. Food was withdrawn 12 hours before and also during the experimental hours. They transferred back to the animal house at the end of each session. The animals were observed everyday for development of any complications or behavioural changes during the whole period of study.

Drugs and Chemicals

The drugs used in the study were obtained from the following sources

Nigella sativa seeds extracts - Indian Herbs Research and Supply Co Ltd, Saharanpur (India)

Raloxifene -Zydus (Cadila) Ahmedabad

Normal saline-Parenteral drugs (India) limited, Indore

Ether-Purchased from s.d fine-chem pvt. Ltd Boisar

Thiopentone sodium- Neon laboratories limited, Mumbai

Ceftriaxone injection-Alkem laboratories limited, Kolkata

Antibiotic ointment-Stedman Pharmaeuticals Pvt Ltd, Tamilnadu

Diclofenac injection-Cadila, Ahmedabad

The doses of the test and control drug were calculated on the basis of body weight according to the available literature and the response to pilot doses.

Methods

A total 42 female rats were selected and divided in to seven groups of six in each. Pregnant rats excluded, only non-pregnant rats were used (confirmed by the Veterinary Surgeon attached to the Department). Firstly, the rats were immobilized using ether anaesthesia at a minimal dose following which, a freshly prepared suspension of thiopentone sodium i.v. was given through the dorsal tail vein cannulation at a dose of 10mg/kg. The left hind legs of all the rats were immobilized using plaster casts so as to promote the process of osteoporosis [13]. The Study protocol was approved by the Institution Animal Ethics Committee is depicted in the following table:

Table 1: Protocol design of animal study

Gropu of Animals	Operative procedure	Treatment received	Remarks
I	SHAM Operation	DW 1ml	Control Group
II	Ovariectomy	DW 1 ml	Do
III	Ovariectomy	NS 400 mg/kg	Test Group
IV	Ovariectomy	NS 800 mg/kg	Do
V	Ovariectomy	NS 1200 mg/kg	Do
VI	Ovariectomy	Raloxifene 5.4mg/kg	Control Group
VII	Ovariectomy	Raloxifene 5.4mg/kg + 800mg/kg NS	Test Group

Surgical Procedure

Sham operation: The rats of group I underwent sham operation. Two dorso-lateral incisions, each of 2.0 cms were made on each side of the back of the rat. The muscles of dorsal abdominal wall were dissected and then stitched again without disturbing the pelvic contents. The stitch was given using mersilk.

Ovariectomy: Ovariectomy was done in rats of the groups II to VII. Two dorso-lateral incisions, each of 2.0 cms were made on each side of the rat [20]. The muscles were separated and the fallopian tubes located. Following the fallopian tubes laterally, the ovaries were identified as small whitish grain like structures. Non absorbable sutures were applied on the distal portions of the fallopian tubes and the ovaries of both the sides were crushed [21]. Ceftriaxone injection (35 mg i.p.) was given one hour prior to the procedure. All the surgical procedures were done under strict aseptic measures and followed by injection of diclofenac 0.2ml i.p. Antiseptic cream was applied locally to the wounds. The wounds were allowed to heal and the diet of the animals was taken care of. All the rats were untreated for 15 days after surgery to allow for the development of osteoporosis.

Experimental Procedures

After 15 days, the rats were administered the test and standard drugs as per the approved protocol. The drugs were administered orally, daily for a period of 90 days. The powder extract of the test drug was added to distilled water at room temperature to prepare a crude suspension of *Nigella Sativa*, a few minutes before giving it to rats by oral gavage. On the morning of day 91, blood samples from all the groups were withdrawn by tail vein method to assess biochemical parameters viz serum calcium, phosphorous and serum alkaline phosphatase. The rats were euthanized using high dose ether anaesthesia. The left femur of all rats were dissected out and taken to the pathology department, where the bones were kept in 10% potassium hydroxide solution for 7 days for curing. After 48 hrs of fixation, the lengthened tibias were removed from the fixative and positioned for high resolution single beam radiography. After this, thin sections of tibia were made, fixed and stained using Haematoxylin and Eosin [22] for histopathological study.The data was analysed using graphpad prism version 7. All data were expressed as mean ± SEM. Statistical significance for data was determined using a one-way analysis of variance (ANOVA) followed by post-hoc Bonferroni multiple comparison test. The level of significance was accepted as $P < 0.05$.

Results

Our study aims to investigate the effect of Nigella sativa on bone mineral density in albino rats against experimentally induced osteoporosis. All the procedures were undertaken after prior clearance from Institutional

Animal Ethics Committee (IAEC). Osteoporosis was induced in the rats by ovariectomy and immobilization of left hind leg. Rats were treated with either normal saline orally (control group), Nigella sativa seed extract (test group) or raloxifene (standard group). Biochemical parameters like serum calcium, phosphorous and alkaline phosphatase were measured and compared with control group. Similarly, dry bone weight was measured and compared. Besides, histopathological and radiological investigations were carried out and compared. The observations of the various experiments are presented here under.

Table 2: Effect of NSSE on serum calcium, phosphorous and serum alkaline phosphatase in ovariectomised rats (IU/Litre)

Groups	Serum Calcium(mg/dl)	Serum Phosphorous(mg/dl)	Serum ALP(IU/L)	Bone weight (gm)
SHAM	9.02 ± 0.002	3.78 ± 0.005	57.88 ± 0.15	0.65 ± 0.0007
OVX	7.86 ± 0.004	4.00 ± 0.002	86.18 ± 0.03	0.58 ± 0.0036
OVX-NS 400	9.08 ± 0.004	4.00 ± 0.036	74.30 ± 0.03	0.68 ± 0.0036
OVX-NS 800	9.02 ± 0.002	4.01 ± 0.074	74.30 ± 0.03	0.68 ± 0.0025
OVX-NS 1200	9.09 ± 0.004	4.00 ± 0.036	74.30 ± 0.03	0.68 ± 0.0021
OVX-R	9.01± 0.003	4.01± 0.070	74.26 ± 0.03	0.68 ± 0.0036
OVX-R-NS	9.01± 0.004	4.00 ± 0.036	74.26 ± 0.03	0.68 ± 0.0036

Data were analysed by one way ANOVA followed by post hoc Bonferroni multiple comparison test. OVX was compared with other groups, n= 6, *="P" value <0.05 [Table 2].

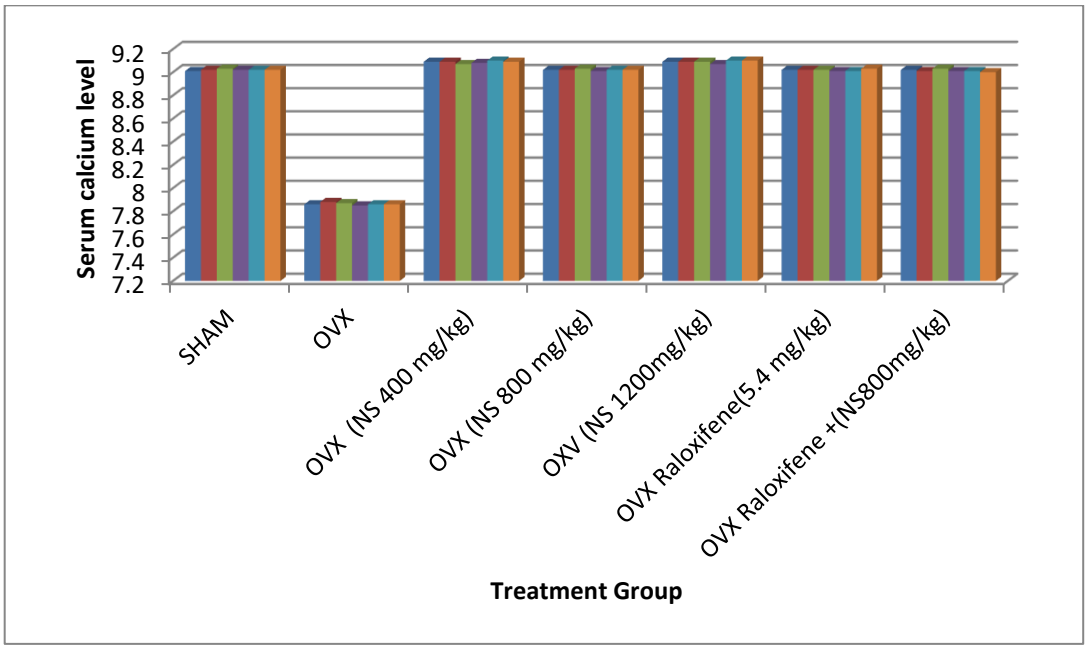

Fig 1*:* Effect of NSSE on serum calcium (mg/dl) in ovariectomised rats

Values are expressed as Mean ± SEM (n = 6), one way *ANOVA (GraphPad Prism) followed by post hoc Bonferroni multiple comparison test. OVX was compared with other groups,* ***** *= "P" value <0.05.*

The result [Table 2/Figure 1] shows a significant difference in serum calcium level between Sham and ovariectomy group indicating hypocalcaemia in ovariectomy group. Significant hypercalcemia was observed in groups treated with Nigella sativa at all doses, groups treated with raloxifene and groups treated with combination of both test and standard drugs in comparison to ovariectomy group. On comparison of

serum calcium levels, there was a significant difference between NS 400 mg/kg and 800 mg/ kg, but no such difference was observed between 800 mg/kg and 1200 mg/kg.NS 800 mg/ kg did not show significant change in calcium level as compared to standard drug raloxifene 5.4mg/kg. The combination showed significant increase in calcium level when compared with individual drugs.

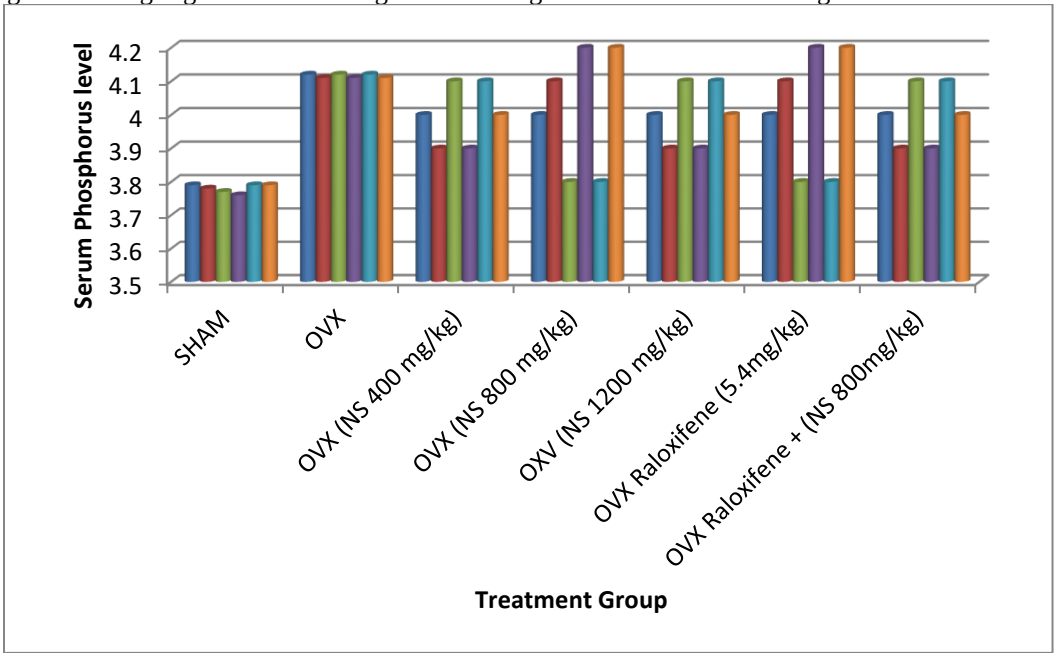

Fig 2: Effect of NSSE on serum phosphorus in ovariectomised rats (mg/dl)

Values are expressed as Mean ± SEM (n = 6), one way *ANOVA (GraphPad Prism) followed by post hoc Bonferroni multiple comparison test. OVX was compared with other groups,* ***** *= "P" value <0.05.*

This figure 2/table 2 shows that there is no significant difference in serum phosphorous level between Sham and ovariectomy group. There is also no significant difference between ovariectomy group and groups treated with Nigella sativa at all doses, groups treated with raloxifene and groups treated with combination of both test and standard drugs. No significant change was observed in serum phosphorous level at all the doses of the test drug. Similar effect was observed with the test drug and the combination of test and standard drug.

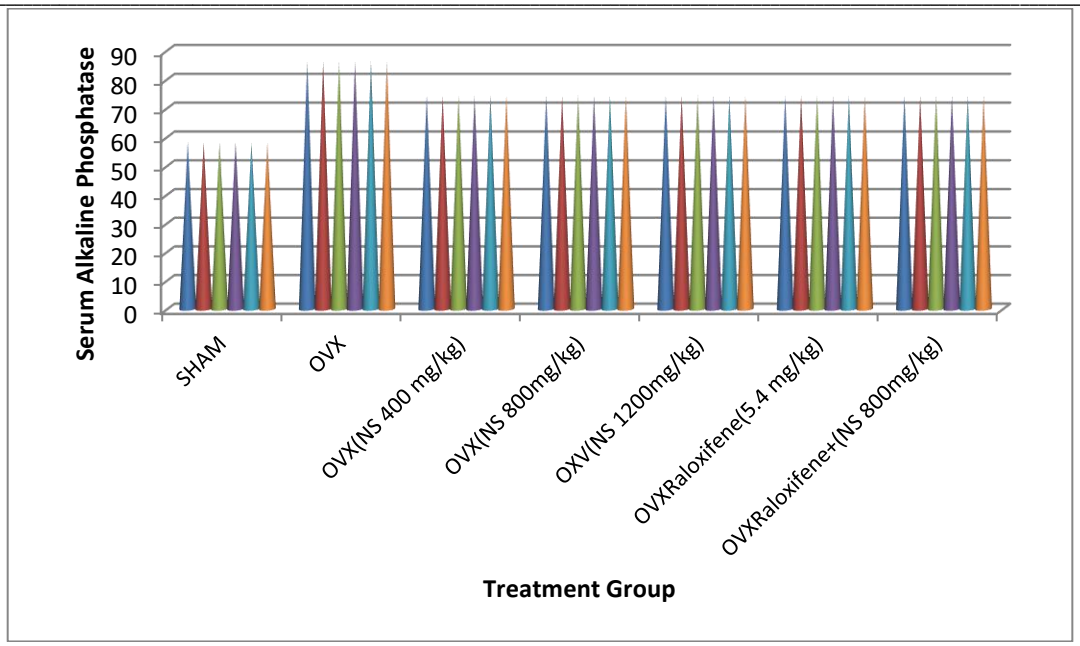

Fig 3: Effect of NSSE on serum alkaline phosphatase in ovariectomised rats (IU/Litre)

Values are expressed as Mean ± SEM (n = 6), one way *ANOVA (GraphPad Prism) followed by post hoc Bonferroni multiple comparison test. OVX was compared with other groups,* ***** *= "P" value <0.05.*

The result (Fig. 3/Table 2) shows a significant difference in serum alkaline phosphatase level between Sham and ovariectomy group indicating bone loss in latter. There was significant difference in alkaline phosphatase level between ovariectomy and all other drug treated groups. It clearly shows that there is significance difference between sham and other treatment groups but there is no difference inthe effect of treatment between groups receiving different doses indicating no dose related effects. NS 800 mg/ kg did not show significant change in alkaline phosphatase level as compared to standard drug. NS supplementation in OVX-NS rats lowered serum alkaline phosphatase levels but still significantly higher compared to sham rats, indicating more stable bone formation. The combination did not show significant increase in the level compared to individual drugs.

Table 3: Effect of NSSE on bone weight (femur) (gm) in ovariectomised rats

Rat No.	SHAM	OVX	OVX-NS (400 mg/kg)	OVX-NS (800 mg/kg)	OXV-NS (1200 mg/kg)	OVX - Raloxifene (5.4 mg/kg)	OVX-Raloxifene (5.4 mg/kg)+ NS (800 mg/kg)
1	0.6587	0.5852	0.6830	0.6860	0.6825	0.6815	0.6821
2	0.6567	0.5852	0.6829	0.6860	0.6826	0.6814	0.6822
3	0.6607	0.5851	0.6831	0.6859	0.6825	0.6816	0.6820
4	0.6567	0.5853	0.6830	0.6861	0.6826	0.6815	0.6821
5	0.6607	0.5851	0.6829	0.6860	0.6825	0.6816	0.6820
6	0.6587	0.5853	0.6831	0.6860	0.6825	0.6814	0.6822
Mean ± SEM	0.65 ± 0.007	0.58 ± 0.003	0.68 ± 0.0036	0.68 ± 0.0025	0.68 ± 0.0021	0.68 ± 0.0036	0.68 ± 0.0036

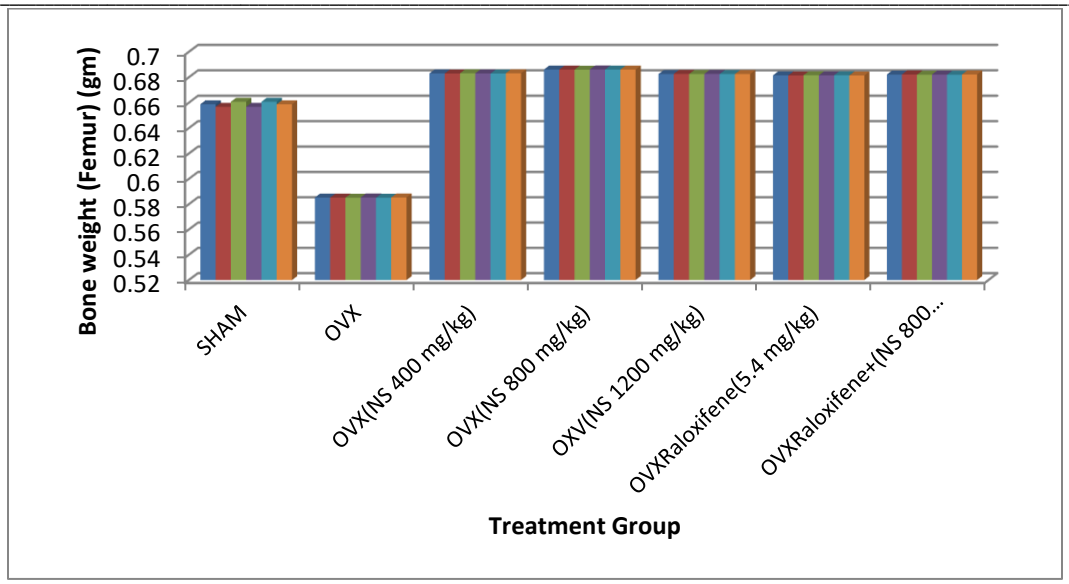

Fig 4: Effect of NSSE on bone weight (femur) (gm) in ovariectomised rats

Values are expressed as Mean ± SEM (n = 6), one way *ANOVA (GraphPad Prism) followed by post hoc Bonferroni multiple comparison test. OVX was compared with other groups,* ***** *= "P" value <0.05.* From this table 3 and figure 4 it was shown that there is a significant bone loss in the ovariectomised rats in comparison to sham rats. There is a significant difference in bone weight between ovariectomised and rest of the groups indicating bone formation in latter groups. However the change in bone weight is not significant between different doses the test drug indicating no dose related effects. There is a significant change in raloxifene treated as well as in combination treated groups in comparison to all the doses of test drug showing the better effectiveness of raloxifene.

Histopathology of Bones

Femoral sections from ovariectomised rats revealed highly significant decrease in number of osteoblasts. Most of osteocytes appeared degenerated with pyknotic nuclei. There were some empty lacunae devoid of cells. Besides, multiple osteoporotic cavities of different sizes were also seen. Collagen fibers were apparently decreased. Periosteum and endosteum appeared irregular with multiple notches. Femoral sections of both control and NS treatedrats were more or less similar showing compact bone with smooth bone surfaces (periosteum & endosteum). Active osteoblasts having cubical nuclei appeared onbone surfaces. Osteocytes in their lacunae were present between Haversian canals. Collagen fibers were regularly arranged. Sections from rats with raloxifen showed nearly normal osteoblasts. Most of osteocytes were normal but few cells were degenerated. There were few small osteoporotic cavities and some cement lines indicating bone repair. Collagen fibers were more or less normal. Outer and inner bone surfaces appeared slightly irregular [Figure 5-8].

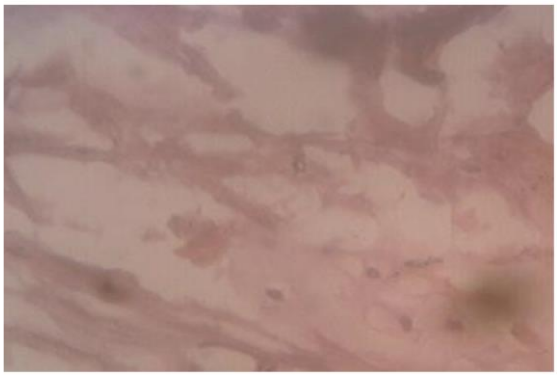

Fig 5: Histopathology of normal rat femur bone show normal appearing bony trabeculae with normal osteoblasts and osteoclasts within their lacunae and smooth contours with normal bone marrow elements.

Fig 6: Histopathology of ovx rat femur bone showing irregularly and widely spaced thinned out and eroded bony trabeculae with increased osteoclastic pitting.

Fig 7: Histopathology of rat femur bone raloxifene treated shows thick elongated trabeculae and marrow cavity showing restoration of normal bony architecture

Fig 8: Histopathology of ovx-ns treated rat femur bone show widened and broad smooth edged bony trabeculae with normal osteoblasts and osteoclasts within their lacunae along with some marrow elements

Radiological Changes

The radiological findings of sham rats showed normal bone architecture density, alignment and cortico-medullary ratioin comparison to ovariectomised rats which showed decreased bone mass density and bone softening as evidenced by bending of the mid shaft and thinning of the cortex especially at the medial aspect of the lower 2/3 rd of the femur. Femur of the test drug treated group showed remodeling with still slightly decreased cortical thickness comparable to the standard drug raloxifene [Fig 9A-D].

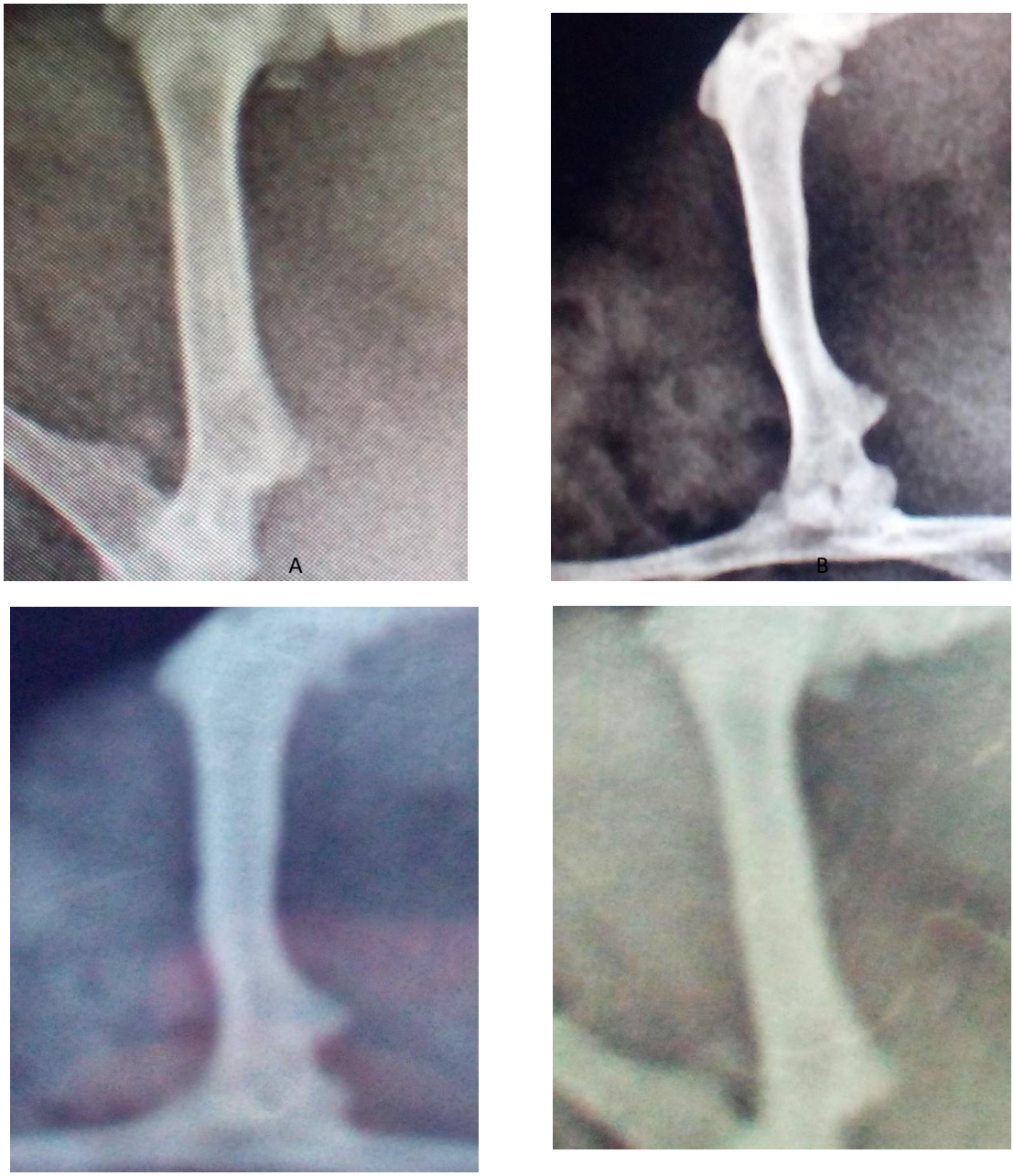

Fig 9: (A) Femur of control rat shows normal bone architecture, bone density, alignment and normal mineralization; (B) Femur of ovariectomized rat shows abnormal bony architecture, decreased

bone density, bone softening evidenced by bending of mid shaft; (C) Femur of OVX-R rat shows remodeling of bone and (D) Femur of OVX-NS rat shows remodeling with slightly decreased cortical thickness.

Discussion

The work embodied in this research work comprises of study of the effect of the traditional herbal product of Nigella sativa on bone mass density in albino rats. This plant is widely cultivated in India and its seed is frequently used as a spice in Indian cuisines. Besides, the seeds are also used frequently by rural people all over the state for diverse ailments as well as nutritional supplement. Literature survey revealed the scientific basis of many of its medicinal uses. Recently its anti-osteoporotic effect has been reported in some studies. However, sufficient scientific validation of its use in treatment and prevention of osteoporosis is still lacking which demanded a study as regards this important clinical potentiality of the indigenous drug. Ovariectomy leads to decreased trabecular bone volume, BMD and strength in rats. Therefore a model for osteoporosis which could clearly reveal the loss of bone mechanical strength is desirable. Since same effect is seen in the post menopausal women, the ovariectomised rat model was selected for this study. As evident from observation table 2, ovariectomy in group II resulted in significant hypocalcaemia. Nigella sativa in all doses i.e., 400 (Gr III), 800 (Gr IV) and 1200 mg/kg (Gr V) significantly increased the serum calcium level in comparison to ovariectomy alone (Gr II). However, there was no significant difference between the groups treated with NS 800 mg/kg and 1200 mg/kg, showing that there is no dose related effects. These effects were comparable with that of standard drug raloxifene 5.4 mg/kg. Treatment with raloxifene and NS 800 combination (Gr VII) showed statistically significant improvement when compared with NS 800 or raloxifene alone indicating that the combination is better than individual drugs alone.These results are in agreement with Mattix-Kramer who reported that ovariectomised rats had impaired Ca^{+2} balance that could have contributed to ovariectomy-induced osteoporosis. Moreover, menopause is associated with impaired intestinal Ca^{+2} absorption that could be due to reduced plasma 1,25 dihydroxyvitamin D levels, as well as to the resistance of the gastrointestinal system to the action of 1,25 dihydroxyvitamin D [23, 24]. Estrogen has been shown to modulate the end organ effect of 1,25 dihydroxyvitamin D on intestinal calcium absorption [23]. Furthermore, menopause is associated with increased renal excretion of calcium [25, 26]. The results of our study show that NS supplementation in ovariectomised rats was effective in preventing hypocalcaemia caused by ovariectomy in these rats. This finding corroborates with the findings of Gennari C (1990) et al [27]. Sheweita SA et al (2007) reported that NS seed oils contain high amount of unsaturated fatty acid linoleic acid being the major one followed by oleic acid. Oleic acid was found to increase Ca^{+2} levels in some studies [28]. As regards serum phosphorous level; there is no significant difference between sham and all other groups. Similarly ovariectomy did not produce any change in phosphorous level. Serum phosphorous levels showed no significant changes in any of the doses of Nigella sativa. No significant change inthe serum phosphorous level was observed in standard drug treatment group as well as in combination group in comparison to any dose of the test drug. This is in corroboration with the findings of Seif A A (2014) [11]. In the current study, serum alkaline phosphatase (ALP) was significantly increased in Gr I ovariectomised rats compared to Gr II sham rats, indicating increased osteoblastic activity with increased bone formation. NS supplementation in OVX-NS rats lowered serum ALP levels, though still significantly higher compared to sham rats, indicating more stable bone formation [29]. Similar results were obtained with standard drug raloxifene and combination of test and standard drugs [30].It is evident from measurement of dry bone weight that there is significant bone loss in the ovariectomised rats in comparison to sham rats. Treatment with Nigella sativa extract significantly improved the bone weight, though there is no dose related improvement. There is a significant change in raloxifene treated as well as in combination treated groups in comparison to all the doses of test drug showing the better effectiveness of raloxifene. The histopathological examination of femur of the group I Sham Control showed normal bone micro-architecture as compared with group II ovariectomised rats, where significant pathological alterations like discontinuous network of bone trabeculae with widening of bone marrow space were observed indicating osteoporosis. However, restoration of bone micro-architecture was observed in the form of increased matrix, less intratrabeular space, increased mineralisation and trabecular components in all the

groups (Gr III to VII) rats treated with NS and raloxifene as compared with ovariectomised rats. This result shows that NS seed extracts strongly increased bone mass density by enhancing bone formation and inhibiting bone resorption.

The results of the present study are in agreement with the previous data showing that plasma calcium levels and plasma alkaline phosphatase levels, the important marker of bone resorption, are significantly increased in ovariectomised rats compared to normal rats and Nigella sativa seed extract effectively reverses this and increases the bone density, which is further supported by histopathological as well as radiological studies. The radiological findings of OVX rats also revealed the bone forming action of the test drug Nigella sativa which is comparable to that of raloxifene. This further supports the biochemical as well as histopathological findings. Saif A A [11] observed a significant increase in plasma amino terminal collagen type 1 telopeptide (NTx) levels compared to sham rats indicating increased bone resorption. Treatment with Nigella sativa lowered plasma NTx levels to levels of control sham rats, indicating decreased bone resorption. Moreover, tibias of ovariectomised rats showed eroded cavities with widened bone marrow spaces indicating increased bone resorption which is reversed by treatment with Nigella sativa. These results are in accordance with Grassi who linked estrogen deficiency to accelerated bone remodelling, where bone resorption outpaced bone formation [31]. Besides that, previous literatures on Nigella sativa and thymoquinone have highlighted two properties i.e., antioxidative and anti-inflammatory properties might be responsible for their antiosteoporotic effect [11]. Recently, a number of studies have supported the role of inflammation in pathogenesis of osteoporosis [32]. Kireev reported that pro-inflammatory cytokines TNF- α, IL-1β and IL-6 measured in liver homogenates were significantly increased and anti-inflammatory IL-10 decreased during ageing and after ovariectomy in rats. Moreover, Levels of lipid peroxides in the liver homogenates as well as inducible NO synthase (iNOS) protein expression and NO levels were increased in old rats as compared to young animals; this effect was more evident in ovariectomised animals. They also opined that administration of the different hormonal replacement therapies was able to inhibit the induction of pro-inflammatory cytokines and iNOS, decreased the levels of oxidative stress markers and had therapeutic potential in the prevention of osteoporosis [32, 33]. Moreover, Pighon showed that exercise training in ovariectomised rats acted like estrogen in properly regulating the expression of inflammatory biomarkers in liver of these rats. Thymoquinone was believed to exert anti-inflammatory effects by inhibiting the synthesis of prostaglandins and leukotrienes which are the main mediators of inflammation [34]. Büyüköztürk S et al stated that a possible anti-inflammatory mechanism of thymoquinone might suppress nitric oxide production by macrophages. Another study has shown that the alveolar bone loss due to periodontitis was reduced by gastric feeding of TQ to rats [35]. This was accompanied by reduction in osteoclast number and raised osteoblastic activity in TQ-treated rats. In studies using rheumatoid arthritis model, TQ was reported to reduce the serum levels of IL-1 and TNF-α [35, 36]. The production of cytokines including TNF and IL-17 can increase osteoclastogenesis and bone loss in inflammatory liver conditions [37]. Bone protective effects of estrogen might involve suppression of inflammatory cytokines such as IL-1 and TNF-α**,** which in turn activate i-NOS. The results of the present study are in agreement with the previous data showing that plasma calcium levels and plasma alkaline phosphatase levels, the important marker of bone resorption, are significantly increased in ovariectomised rats compared to normal rats and Nigella sativa seed extract effectively reverses this and increases the bone density, which is further supported by histopathological as well as radiological studies. Based upon the results of the present study in animal model, it is quite justified that this indigenous drug may be tried as an adjunct therapy in the treatment as well as prevention of osteoporosis. However, there are certain limitations of the study viz, the study was conducted only in a single animal model of osteoporosis. This study has to be carried out in other animal models as well as analysis of more biochemical parameters like amino terminal collagen type 1 telopeptide (NTx), malondialdehyde (MDA), nitrates, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) that may reveal its antioxidative and anti-inflammatory properties , before this beneficial activity can be documented beyond any doubt.

Conclusion

The present study was undertaken with an aim to investigate the effect of *Nigella sativa* on bone mass density. The study was conducted in an animal model, ovariectomised rats, which mimic the postmenopausal osteoporosis. The results of the present study show that

serum Ca^{+2} levels were significantly decreased in ovariectomised rats compared to both sham and OVX-NS rats. NS supplementation in the entire OVX-NS treated group restored Ca^{+2} levels to be insignificantly different from control sham rats, but significantly higher compared to OVX rats. In this study, *Nigella sativa* was found to increase the bone mineral density significantly which was comparable to that of raloxifene, taken as standard drug. Serum alkaline phosphatase levels were significantly increased in ovariectomised rats compared to SHAM group. Although ALP levels were decreased in OVX-NS compared to OVX rats, these levels remained significantly higher compared to SHAM group. Further, the histopathological studies as well as radiographic finding of bone showing high osteoblastic activity and minimum osteoclastic activity indicating bone formation, also supports its anti-osteoporotic activity. Hence, further study in this line on other animal models, isolation of active principles responsible for antiosteoporotic activity, understanding their mechanism of action and finally, clinical trials are necessary to pass on its beneficial effect from bench to bed side of patients.

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