

## THE BENEFICIAL EFFECT OF ETHANOLIC EXTRACT OF MORINGA OLEIFERA ON OSTEOPOROSIS

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### ABSTRACT

Several animal and clinical studies have shown that phytoestrogens, plant derived estrogenic compounds can be useful in treating post menopausal osteoporosis. Phytoestrogens and phytoestrogen containing plants are currently under active investigation for their role in estrogen related disorders. The present study deals with anti-osteoporotic evaluation of phytoesterol rich plant Moringa oleifera, commonly known as Drumstick.

3 Month old female wistar rats were bilaterally ovariectomised (OVX) and randomly assigned to 3 groups (6 rats/group). Additional 6 animals were sham operated. OVX and sham control groups were orally administered with vehicle while the other two OVX groups were administered 0.15 mg/kg estradiol and 1 g/kg of ethanolic extract of Moringa oleifera leaves in two divided doses for 6 weeks. At the end of the study blood, bones of the animals were collected. Serum was evaluated for Calcium, phosphorus, alkaline phosphatase. Results were analyzed using ANOVA.

Ethanolic extract of Moringa oleifera (600mg/kg, p.o.) significantly reduced urinary calcium excretion and significantly increased calcium content of bones in comparison to OVX control. Unlike estradiol it did not affect body weight gain in OVX animals. Ethanolic extract of Moringa oleifera prevented Ovariectomy induced bone loss in rats. The osteoprotective effect was comparable with estradiol.

**Key words:** Ovariectomised rats, Osteoporosis, Estradiol, Moringa Oleifera. Phytosterol

### [I] INTRODUCTION

Osteoporosis is a multifactorial disease in which there is a diminution in the quality of trabecular cortical bone mass, leading to a progressively increasing frequency of fractures with age<sup>[1]</sup>. The literature study of osteoporosis reveals that the therapeutic efficacy of synergistic effect of garlic oil, 1,25 dihydroxy vit-D3 and calcium in osteopenic ovariectomized rats has been performed by Wafaa I and coworkers<sup>[2]</sup>. Efficacy of OST-6, a polyherbal formulation in the management of osteoporosis in postmenopausal women was reported<sup>[3]</sup>. Osteoporosis is the most frequent metabolic condition experienced by

elderly individuals. It is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture. Osteoporosis that is associated with ovarian hormone deficiency following menopause is by far the most common cause of age related bone loss. Menopause results in elevated bone turnover, an imbalance between bone formation and bone resorption and net bone loss. Postmenopausal osteoporosis has become a major problem with significant morbidity and mortality<sup>[4]</sup>. Estrogen replacement therapy is approved for the prevention of bone loss in

postmenopausal women and is efficacious in reducing the incidence of skeletal fractures. However, estrogen use and compliance are limited due to its numerous undesirable side effects such as uterine and breast cancer. Hence, it would be most helpful to explore naturally occurring substances especially of plant origin that could prevent bone loss and free from any adverse effects. Many consumers would also prefer to avoid synthetic molecules in favor of natural products.

The Indian system of medicine mentions several plants that are used to heal fractures and cure many metabolic bone disorders. Ayurvedic preparations contain natural forms of calcium, which prevent development of fractures in osteoporosis. *Withania somnifera* is a rejuvenator that helps in relieving pain associated with osteodystrophic conditions<sup>[5]</sup> and is also useful in people with general debility, nervous problems and muscular pain. *Commiphora wightii* increases the mineralization of bones. *Sida cordifolia* contains phytosterol and potent phytoestrogens. *Vanda roxburghii* has anti-inflammatory activity, which is useful for relief of bone pains of osteoporosis. *Godanti bhasma* and *Kukkutandatvak bhasmas* are rich in natural calcium. The calcium present in this formulation is extracted in a traditional way that can be easily absorbed in the intestines<sup>[6]</sup>.

The main objectives of the proposed study are to evaluate the preparation of ethanolic extract of *moringa oliefera*

leaves for its usefulness in preventing bone loss in estrogen deficient ovariectomized rats. The OVX model was chosen for the study because OVX rat has been particularly useful to study the efficacy of various agents for the prevention and for reversal of bone loss. The study was designed to investigate the effects in estrogen deficient ovariectomized rats.

## [II] MATERIALS AND METHODS:

### **Animals and Diet:**

This study was carried out with 24 female white albino rats weighing 150-200g. They were reared and maintained individually in an environmentally controlled animal laboratory (12h light / dark schedule at 25±2oC). The rats were acclimatized to the local vivarium conditions for 2 weeks and allowed free access to water and pelleted commercial diet. They were fed a standard chow throughout the study. The animals were either subjected to bilateral ovariectomy (OVX) or to sham operation (SH) under ether anesthesia. The beginning of therapy started 3 months after OVX and sham operations. The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee.

The animals were divided into 4 groups 6 rats in each group.

**Group I:** Sham operated rats (SH) as control group.

**Group II:** Bilaterally ovariectomized rats (OVX).

**Group III:** Bilaterally ovariectomized rats (OVX) received Estrodiol (0.15 mg/kg).

**Group IV:** Bilaterally ovariectomized rats (OVX) received Ethanollic Extract (600 mg/kg).

**Methodology.** Ovaiectomy was made by two dorso-lateral incisions, approximately 1 cm long above the ovaries .With the use of a sharp dissecting scissors, the skin was

**The sutures were made as shown in figure.**

cut almost together with the dorsal muscles and the peritoneal cavity was thus accessed. After peritoneal cavity was accessed, the ovary was found, surrounded by a variable amount of fat. The surgery was done under anesthesia, using a ketamine 50 mg/kg, interaperotonially. Ligation of the blood vessels was necessary. The connection between the Fallopian tube and the uterine horn was cut and the ovary moved out. Because of muscle bleeding, its incision required suturing <sup>[7]</sup>.

*Figure 1.* Longitudinal, dorsal midline skin incision.



*Figure 2.* Three single catgut stitches on the skin wound.



*Figure 3.* Two dorso-lateral incisions of the skin and muscles.



*Figure 4.* One single catgut suture on the skin wound.



**Sample Collection:**

**Blood Collection:**

After 6 months post-operative i.e 3 months after starting the treatment animals were anaesthetized by diethyl ether and the blood was withdrawn from the optical vein. The samples were collected in clean polypropylene tubes, left to clot at 37°C for 10 minutes, then centrifuged and sera were separated. All samples were frozen at -20°C until used<sup>[8]</sup>.

**Histology of femur bone:**

The right femurs were fixed in 10% NBF for 12 h at 4 °C, decalcified in 5% ethylenediamine tetraacetic acid (EDTA) for 7 days, embedded in paraffin and cut into longitudinal sections of 5 µm thickness. The sections were stained with haematoxylin and eosin (H&E) and

tartrate-resistant acid phosphatase (TRAP), a cytochemical marker for osteoclasts and finally counterstained with haematoxylin. The number of positively stained osteoclasts in the sections of the median portion of the whole femora was enumerated for the three groups<sup>[9]</sup>.

**[III] RESULTS:**

**Effect of *Moringa oleifera* extract on ovariectomized induced rats body weight changes:**

Table 1, shows significant changes in body weight in ovariectomized rats comparable to sham operated control. It was also noticed that significant changes in body weight in animals treated with *Moringa oleifera* Ethanolic (600 mg/kg p.o) extract compared Estrodiol treated group to ovariectomized rats.

**Table 1**

**Effect of *Moringa Oleifera* extract on Body weight (gms) in ovariectomized rats**

Treatment	Initial body weight	Week 1	Week 2	Week 3	Week 4
Sham Control	220.83±13.57	220.83±13.57	226.7 ± 13.60	223.3 ± 12.36	233.0 ± 14.65
Ovariectomized Rats	204.2 ± 16.35	204.2 ± 16.35	195.3 ± 15.76	199.7 ± 16.14	202.7 ± 16.18
Ovariectomized Rats + Estrodiol (0.15 mg/kg)	216.7 ± 16.67	216.7 ± 16.67	211.3 ± 15.38	216.0 ± 15.94	217.8 ± 16.43
Ovariectomized Rats + Ethanolic Extract (600 mg/kg)	216.7 ± 12.36	216.7 ± 12.36	200.8 ± 13.37	207.7 ± 13.66	212.8 ± 12.12

Effect of *Moringa oleifera* extract on body weight in ovariectomized rats was

calculated from first day of study and every week regularly. The body weight of each animal was recorded one hour prior to administration of drug extract on recording day. The results are depicted as mean  $\pm$  S.E.M. and analysed by student 't' test for coming to conclusion.

**Effect of *Moringa Oleifera* extract on biochemical marker in ovariectomized rats.**

Significant differences were found in serum level of alkaline phosphatase in ovariectomized rats to normal and treated ovariectomized rats. In below mentioned table No 2, significance  $7.50 \pm 0.95$  ( $p < 0.01$ ) decreased in serum calcium ion level was found. Treatment of ovariectomized rats with Estrodiol and Ethanolic extract of *Moringa Oleifera* show significant change in serum level, however, Ethanolic extract significantly

( $p < 0.01$ ) increased serum calcium level when compared to ovariectomized rats. Significantly ( $p < 0.01$ ) serum phosphorus level was decreased in ovariectomized rats  $4.5 \pm 0.45$ , but significant change in serum phosphorous level was observed with estrodiol and ethanolic extract treatment. Ethanolic extract of *Moringa Oleifera* significantly ( $p < 0.01$ ) reduced renal creatinine clearance when compared to ovariectomized rats, however estrodiol has no significant influence in this process. Loss of calcium ion in urine was significantly ( $p < 0.01$ ) increased when rats were treated with estrodiol and ethanolic extract at dose (600 mg/kg) when compared to ovariectomized rats. Urine phosphorous level was significantly increased to 7.5 in ovariectomized rats when compared to sham-operated rats, but no significant change in Ethanolic extract treated ovariectomized rats.

**Table 2**

**Effect of *Moringa Oleifera* extract on Serum and Urinary Biochemical markers in ovariectomized rats.**

Treatment	Serum			Urine		
	Alkaline phosphatase (Iu/L)	Calcium (mg %)	Phosphorous (mg %)	Creatinine (mg %)	Calcium (mg %)	Phosphorous (mg %)
Sham Control	138.66 $\pm$ 4.82	8.97 $\pm$ 0.41	5.15 $\pm$ 0.08	12.7	1.6	4.2
Ovariectomized Rats	171.24a $\pm$ 10.70*	7.50 $\pm$ 0.95*	4.50 $\pm$ 0.45 *	6.38	9.0*	7.5*
Ovariectomized Rats + Estrodiol (0.15 mg/kg)	145.41b $\pm$ 7.80*	8.73 $\pm$ 0.32	5.38 $\pm$ 0.18	9.5	2.1*	5.8
Ovariectomized Rats + Ethanolic Extract (600 mg/kg)	142.5b $\pm$ 8.21*	8.39 $\pm$ 0.29	5.22 $\pm$ 0.14	1.3	4.2	2.3

Effect of *Moringa Oleifera* extract on ovariectomized rats was studied by serum and urine biochemical analysis. Biochemical parameters were estimated by using Span diagnostic kits purchased from local market. Result obtained from serum biochemical analysis are expressed as Mean  $\pm$  SE and evaluated by student 't' test. Urine biochemical results were collected from each group and statistically evaluated by Chi-square test for coming to conclusion. p value less than 0.05 was considered as significant. \*p<0.01.

**Effect of *Moringa Oleifera* extract on Histopathological changes in ovariectomized Rats.**

Histopathological observation of femur were shown epiphyseal region in ovariectomized rats were sparse, thinning of trabeculae with tendency of disappearance, loss of connectivity and widening of intertrabecular spaces as compare to normal and estrogen treated rats. Ethanolic extract treated (600mg/kg) rats had shown, lesser, moderately thick elongated trabeculae and narrowed intertrabecular spaces.

Observation of reddish stained TRAP shown positive osteoclasts and increased in number of osteoclasts in ovariectomized rats as compare to normal and estrodiol treated groups. The treatment of Ethanolic extract (600 mg/kg) rats were shown moderately decreased in number of osteoclasts, where as compared to Estrodiol treated groups.

(Figure 1.) SHAM OPERATED CONTROL GROUP.



Figure 1. (a) Epiphyseal region showing normal compact trabeculae with intertrabecular spaces in a SHAM rat.

(figure 2.) OVARIECTOMIZED GROUP

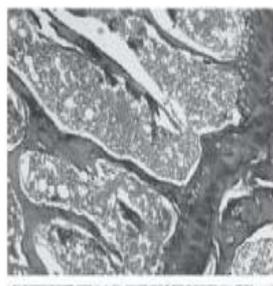


Figure 2. (b) Epiphyseal region showing sparse, thinning of trabeculae with tendency for disappearance, loss of connectivity and widening of intertrabecular spaces in an OVX rat.

(Figure 3.) ETHANOLIC EXTRACT OF MORINGA OLEIFERA GROUP.

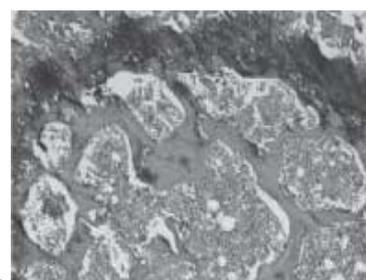


Figure 3. (c) Epiphyseal region showing moderately thick elongated trabeculae and narrowed intertrabecular spaces in an Ethanolic extract treated rat.

#### [IV] DISCUSSION:

The objective of this study was to evaluate the efficacy of Ethanolic Extract of Moringa Oliefera in the prevention of bone loss under calcium and oestrogen deficient conditions. The results from this experiment combined with estrodiol indicate that Ethanolic extract prevents the progress of bone loss even in conditions of severe calcium and oestrogen deficiency. The present study creating an experimental model of the menopause by Ovariectomized model (OVX) and indicated that the model provided the needs of the study.

Because of its incidence and the health-related problems it gives rise to, postmenopausal osteoporosis has become a social problem requiring appropriate management strategies.

Therefore our work aimed to study the efficacy of Ethanolic extract of Moringa Oliefera in suppressing ovariectomy induced bone resorption.

The phytoestrogenic active principle in the Ethanolic extract of Moringa Oliefera possibly stimulated estrogen synthesis at extragonadal sites. Such extragonadal estrogen synthesis finds support from earlier observations that circulating estrogen concentration elevates gradually with time after ovariectomy in rats.

Furthermore even in postmenopausal women, estradiol is produced in a number of extragonadal sites and acts locally at these sites. These sites include the mesenchymal cells of adipose tissue,

osteoblasts, chondrocytes of bone, numerous sites in the brain and breast vasculature.

Our results also proved that increased bone turnover rate as indicated by the higher serum alkaline phosphatase level in the OVX group compared to the SH control group.

These results are in agreement with those of Goseki *et al.*, 1996 and Mukherjee *et al.*, 2004, who demonstrated that bone remodelling in rats is accelerated after ovariectomy.

In our experiment such high rate of boneturnover was well corrected in all groups but best results were obtained in the Ethanolic Extract of Moringa Oliefera group. Therefore the high rate of bone turnover was well corrected by Ethanolic Extract of Moringa Oliefera suggesting that Ethanolic extract of Moringa Oliefera may have protective action against ovarian hormone insufficiency – related bone resorption.

In another study by Choi they proved that phytoestrogens perform their antiosteoporotic effect by stimulating osteoblastic activity through an estrogen receptor mediated action, or by increasing the production of insulin 1 like growth factor-1 (IG-F) which is known to enhance osteoblastic activity. And positively affect bone mass in postmenopausal women.

*Moringa Oliefera* used traditionally in ayurveda, for various kind of the disease and disorders and antiinflammmtory activity and anti asthamatic drugs. The earlier phytochemical analysis is demonstrated the

presence of the flavonides, saponins, sterols, Glycosides, Phytosterols, Flavanoids, Phenolic compounds, Proteins.

The antiosteoporotic activity by flavones and steroids has been ascertained in their ability in bone remodeling. After consumption of the phytosterone and isoflavone precursors, metabolic conversions occur in the gastrointestinal tract resulting in the formation of heterocyclic phenols that are similar in structure to estrogens. These phytoestrogens have a diphenolic ring in chemical structure that is very similar to endogenous estrogens, estradiol and diethylstilbestrol, accounting for their weak estrogen-like effect. These phytoestrogen found to bind two subtypes of estrogenic substance but more strongly to the  $\beta$ -estrogens. Therefore, they are considered as natural selective estrogen receptor modulators, as they appear to be estrogen agonists for cardiovascular system, bone and brain. As steroidal estrogens are of benefit in preventing osteoporosis, phytoestrogens may have a protective effect in the postmenopausal women.

In the current study significant decrease in the mean serum calcium level was noticed in the OVX compared to the SH control group. This decrease was improved in Ethanolic Extact of Moringa Oliefera groups although the change is statistically significant.

These results found support from an earlier proposed hypothesis that menopause and estrogen deficiency are associated with

increased renal excretion of calcium and intestinal resistance to 1,25 dihydroxy vitamin D , and thereby reduced calcium absorption, this will result in a rapid decrease in calcium content within the bones leading to demineralization and eventually development of postmenopausal osteoporosis. Also proved the effect of Ethanolic Extact of Moringa Oliefera in the improvement of serum calcium level in the OVX animals and they suggested that the phytoestrogen present in Ethanolic Extact of Moringa Oliefera possibly could restore hydroxylation of vitamin D and thereby an increase in the intestinal transference of calcium. Histological examination also revealed the antiosteoporotic property of OST-6 as demonstrated by the restoration of trabecular bone with less TRAP positive cells in OST-6 treated group compared with the OVX group.

#### **[V] CONCLUSION:**

Treatment our experimental animal groups with Ethanolic Extact of Moringa Oliefera could effectively restore the reduced calcium level, correct the high rate of bone turnover, reduced the elevated serum alkaline phosphates level.

In conclusion, Ethanolic Extact of Moringa Oliefera treatment in ovariectomized rats further affirmed the beneficial effects and thus indicates its potential in preventing osteoporosis in a natural way through herbal resources.

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