# Anti-osteoporotic effect of *Urtica dioica* on ovariectomised rat

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**ABSTRACT**

This study was undertaken to check the anti-osteoporotic activity of *Urtica dioica* on ovariectomised (OVX) rats. Bilateral ovariectomy was performed in rats under aseptic condition and ether anaesthesia. Healthy albino rats were randomly divided in to 4 groups of 6 animals each. First group Assigned by sham operated and served as control, all remaining are Ovariectomy. Group (II) is ovearctomised control, group (III) *Urtica dioica* extract (20mg/kg), group (IV) treated with Raloxifene (5.4mg/kg as standard drug) respectively for 8 weeks. At the last of study blood wear collected to estimate serum calcium (Ca), phosphorus (Ph), bone specific alkaline phosphate (b-Alp) and osteocalcin (OC) level. Rats were euthanized femur, liver and uteri wear taken for bone analysis and weighed respectively. Result indicate that ovariectomy(OVX) gp show increased serum calcium (Ca), phosphorus (Ph), bone specific alkaline phosphate (b-Alp) and osteocalcin (OC) level also decreased Bone Mineral Density (BMD), $Ca^{+}$ in bone ash. Femur and uterine wear weighted. When OVX gp treated with *Urtica dioica* (200mg/kg) it normalise all elevated serum calcium (Ca), phosphorus (Ph), bone specific alkaline phosphate (b-Alp) and osteocalcin (OC) level and increased femur, uterine weight, it also increased BMD and Bone Volume. This study assess on the basis of biochemical, biomechanical parameter which show that administration of *Urtica dioica* is beneficial for women who are suffering for postmenopausal osteoporosis due to its good antiosteoporotic activity.

**KEY WORDS:** Ovariectomised rats, osteocalcin, alkaline phosphate, BMD, Bone fragility

## 1. INTRODUCTION

Osteoporosis is silent epidemic problems recently it become a major health hazard which afflicted over 2000 million people worldwide (Annie Shirwaikar, 2003). Osteoporosis is a systemic skeletal disease characterised by low bone mass caused by micro architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. The bone matrix is composed of organic and inorganic components. The organic components include collagen and glycoprotein and the inorganic include minerals mainly calcium and phosphorus, both organic and inorganic components provide strictness and strength to the bones (Annemieke, 1997).

The main region of Osteoporosis is due to lack of certain hormone like estrogen in women and androgen in men. In treatment with synthetic agent like selective estrogen receptor modulators like Raloxifene and droloxifene, hormone replacement therapy, all of them are associated with side effect such as hypercalciurea, increased risk of endometrial, hypocalcaemia, breast cancer, breast tenderness, and hot flushes (Bennet, 1984). Hormone replacement is currently considered the optimal therapy, both to protect against bone loss and to reduce the risk of coronary heart disease in postmenopausal women.

Bisphosphonates and the Calcitonin are antiresorptive agents that attenuate the decline in BMD in postmenopausal women, without side effects on reproductive tissue (Annie Shirwaikar, 2003) in the treatment of glucocorticoide, thyroxin & heparin bone mass decreased. Boron helps in osteoporosis without any toxicity or deleterious effect at 3mg/kg/day. Osteoporosis, defined as a decrease in bone mass, is a relatively common disease, a painless, 'silent disease'. If bone pain is present, there may be other underlying conditions such as osteoarthritis or small fractures that are responsible for the pain. Ovariectomy (OVX) provides the most popular model for studying events associated with postmenopausal osteoporosis with estrogens deficiency, and it has been well established that ovariectomy elicits bone loss and increased bone turnover in rats.( Bouxsein, 2003)

## 2. MATERIALS & METHODS

**Chemicals -** Raloxifene, pentobarbital sodium, Biochemical and electrolyte kits.

***Urtica dioica* (stinging nettle) -** *Urtica dioica* leaves (family urticacae) purchased from Tapan Singh, Good earth herbs, tupudana industrial area, Ranchi. Leaves were authenticated by a taxonomist at NISCAIR (National Institute of Science and information resources) Delhi.

Stinging nettle is rich in vitamin A, C, D, manganese, calcium, protein, etc. The protein quality of nettle is superior to that of meat and the same as eggs,

**Animal**: Twenty four, adult female rats of Wistar strain weighing 200-250 g body weight and 12-14

weeks old were used in this study. The rats were obtained from Animal Facilitation centre, R.V.Northland Institute Dadri, Greater Noida. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee. The animals were housed individually under hygienic conditions in metabolic cages. The rats were kept at a room temperature of 25 ± 2 °C with relative humidity of 50–60% and on 12hrs light/12 hrs dark cycles in the Animal House. Animals were caged with a maximum of two animals each in a polypropylene cage and were fed with standard food pellets (Hindustan Lever Ltd., India) and water ad libitum.

**Preparation of *Urtica* Extract:** The leaves of *Urtica dioica* were dried under the shade and ground to powder by an electrical grinder. It was soxhletted using a mixture of ethanol (80%) and doubly distilled water (20%) three times. The mixture was lyophilized. 200 mg/kg of the extract given to all group's of rat by gavages.

**Ovariectomy procedure:** Ovariectomy 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 cut almost together with the dorsal muscles and the peritoneal cavity was thus accessed. The ovary was found, surrounded by a variable amount of fat. 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. Three single catgut stitches were placed on the skin.

**Experimental procedure:** After complete surgical recovery animals (n= 24) were randomly distributed in to 4 groups of 6 rat each. Group (I) was sham operated (SHAM) and the other three groups were ovariectomised (OVX) and left after surgical operation for 3 weeks to ensure almost complete clearance of their bodies from estrogen hormone residues. At beginning and end of the experiment the rats were weighed and the changes in body weight gain were calculated. Group (II) was left OVX control non treated, while groups (III) were orally given Urtica extract in 200 mg.kg / day for 8 weeks,) and group (IV) were orally given Raloxifene as standard drug. At end of the experiment, blood and urine samples were collected for biochemical analyses. Both uterine horns of each rat were removed and weighed. Both femur bones of each rat were taken for bone analyses.

**Acute toxicity (OECD guidelines 425):** Twenty healthy Wistar albino rats were divided into two groups of 10 animals in each. Animals of both the groups were fasted overnight before the test. The first groups given Urtica dioica at 200mg/kg body wt. while the other gp given same volume of saline. The animal wear observed immediately and then after 30 min, 1, 2, 4, 6 h and thereafter daily for 14 days. At the end of the fourteenth day the animals were sacrificed with excess ether anesthesia and dissected for examination of vital organs and blood sample collected from portal hepatic vein for biochemistry & haematological examination.

**Evaluation parameter:**

**Biochemical parameters:** Clean blood collected into centrifuge tube and centrifuged at 3000 rpm for 10 min to separate serum and it stored in defreeze to maintain its enzymatic activity till biochemical analysis. Urine sample was collected and acidify with 12 Mol.HCL. Stored in defreeze until analysis.

**Serum calcium:** This test performed by diagnostic regent kit for in vitro calcium determining. Serum calcium wear precipitated with napthyl hydroxamic acid (calcium reagent). Precipitant dissolved in EDTA reagent and calcium wear complexed with colure regent to give colure complex and it measured with calorimeter.

**Serum alkaline phosphatase (ALP):** In vitro it is determined by kind and king's method was carried out by diagnostic kit. ALP from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of oxidizing agent potassium-ferricyanide and forms an orange red colored complex, which can be measured calorimetrically. The color intensity is proportional to enzyme activity (Canalis, 1998).

**Tart rate resistant acid phosphatase (TRAP):** In-vitro it determination by King's method was carried by using diagnostic reagent kits. Acid phosphatase from serum converts phenyl phosphate to phosphate and phenol at pH 4.8. Phenol so formed reacts in alkaline medium with aminoantipyrine in presence of oxidizing agent potassium-ferricyanide and forms an orange red colored complex, which can be measured colorimetric ally. The color intensity is proportional to enzyme activity. (Cummings, 1991).

**Osteocalcin determining:** Serum osteocalcin determined by enzyme-linked immunosorbend assay.(ELISA)

**Biomechanical parameters:** Selected bone (right femur, tibia and fourth lumbar vertebra) were examined for biomechanical strength by using the tensile strength testing machine. The biomechanical properties were determined included the following: max-stroke, max-strain, elastic, maxload, max-stress and energy. Different test wear examined.

**Organ weight**: After ovariectomy of all rats, body weight were taken of all group's of rat, and the end of the study all the rats were deprived of food for one night, On the next day, the animals were anesthetized with pentobarbital sodium (40 mg kg−1, i.p.), and blood were taken from retro orbital for the biochemical analysis, and then uterus removed and weighted.

**Femur ash weight and bone mineral calcium content:** The left femur bone was dried overnight at 100◦ C. The femur was then incinerated for 12 hrs at 1000◦ C in Muffle apparatus to obtain the ash weight and dissolve in 0.1 mol/L hydrochloric acid solution. Bone minerals calcium and phosphorus measured by U-V spectrophotometer.

**Femur physical parameters:** The soft tissues around the right femur bone were removed and the femur was weighed. Femur length was measured with vernier calliper and femur volume and density were calculated using Archimedes' principle. In brief, the femur was cut out at the mid diaphyses and bone marrow was washed out. Each bone was placed in an unstoppered vial filled with deionised water, and the vial was placed for 90 minutes in a vacuum desiccators. The desiccators were agitated periodically to ensure that the trapped air completely diffused out. The bone was removed from the vial, dried by blotted paper, weighed, and placed again in the vial containing deionised water. The bone was reweighed in a suspended vessel and should be not completely immersed in water before equilibrated at room temperature. Femur bone densities (bone weight/bone volume) were then calculated. (Fromm, 1991)

**Histopathology:** The femur bone wear fixed in 10% formalin for 12 h at 4 ◦C, decalcified in 5% ethyenediamine tetracetic acid (EDTA) for 7 days and fix in paraffin wax and cut into plane section of 5µm thickness by microtome. The sections were stained with haematoxyline and eosin (HE), and examined for histopathological changes under a light microscope.

**Statistical Methods:** The data obtained was compiled in excel and was analyzed using the Instate 5 The significance differences between the means was evaluated by the analysis of variance followed by Dunnett's Multiple comparison test ($p<0.01$).

## 3. RESULTS AND DISCUSSION

The results are summarized in tables given on following pages. The oral administration of *Urtica dioica* show no any clinical signs of morbidity in any of the four groups studied.

**Body weight:** All rats gained weight during the study but group's ovariectomised (OVX) had higher weight gains during the first 2 weeks of experiment and higher final body weight as compared to other three groups. However the difference did not reach statistical significance.

**Uterine weight:** The weight of uterus decreased in ovariectomised rats. Group when compared to the SHAM group and showed an increase when Raloxifene was administered, the difference being statistically significant

**Effect of Urtica *dioica extract* on biochemical of serum and urine parameter:** As compared to group 1, group 2 animals showed a significant rise in ALP level following ovariectomy while no significant change was seen in the serum calcium levels. Following ovariectomy while no significant change was seen in the serum calcium levels, ALP levels of groups 3 and 4 showed a further increase following administration of Raloxifene (at 5.4mg/kg). There is no significant difference in urine calcium and osteocalcin level as compared to sham operated. ($P < 0.05$, $P < 0.01$). Urine phosphorus level increased in 3 and 4 group.

**Effect of Urtica *dioica extract* on femur physical, femur ash weight, and calcium parameter:** The length, weight, ash weight and calcium level of femur wear significantly decreased ($p<0.001$) in OVX as compared to sham operated (tabel.3). Treatment with *Urtica dioica* and Raloxifene in OVX rats show significantly increased in length, weight, ash weight and calcium level which compared to the sham control group. However no significantly change in bone diameter.

**Effect of Urtica *dioica extract* on biomechanical parameters of femur:** Femur biomechanical parameters show no significant difference observed in max-load, max-stress, elastic, and energy, either in Sham-operated rats, OVX rats or in ovariectomised *Urtica dioica* administered rats. Three point bending test, load testing of femur decreased significantly in OVX gp as compared to sham gp. Treatment with *Urtica dioica and* Raloxifene to gp 3 & 4, the

biomechanical parameter significantly increased as compared to sham operated.

**Table.1. Body weight findings**

| Groups | Initial body wt **(gm) | Final body wt (gm) | Uterine weight **(mg) |
|---|---|---|---|
| **sham** | 200 ±6.45 | 272.5±3.81 | 270.2±5.00 |
| **OVX** | 204.1±7.68 | 285±1.35 | 155.0±0.001** |
| **OVX+Test Drug** | 195.8±7.68 | 267.5±3.81 | 163.0±0.04** |
| **OVX+raloxifene** | 200±9.12 | 264±3.83 | 175.0±0.009** |

All values expressed as mean ± S.D, **- differences significant at p<0.05

**Table.2. Biochemical profile Serum phosphorus, ALP levels, serum calcium**

| Group | Serum | | | | Urine | |
|---|---|---|---|---|---|---|
| | phosphorus** (mmol/L) | Alkaline phosphate** | Calcium** (mmol/L) | Serum osteocalcin (µg/L) | Calcium (mg/dl) | Phosphorus (mg /dl) |
| **Sham** | 2.50±0.03 | 41.01±0.01 | 10.4±0.03 | 0.75 ± 0.23 | 7.325 ± 0.023 | 6.600 ± 0.047 |
| **OVX** | 1.60±0.03** | 53.03±0.01** | 10.7±0.03** | 1.01 ± 0.10** | 7.973 ± 0.068[a] | 4.200 ± 0.206a |
| **OVX+T.D** | 2.04±0.06** | 55.09±0.01** | 10.3±0.05** | 0.89 ± 0.67** | 6.320 ± 0.125** | 5.875 ± 0.122** |
| **OVX+raloxifene** | 2.13±0.04** | 57.08±0.01** | 10.87±0.07** | 0.78 ± 0.95 | 7.892 ± 0.072** | 6.840 ± 0.206** |

[a] p < 0.01 as comparison to sham operated normal group, * p < 0.01 as comparison to OVX model group,
** p < 0.001 as comparison to OVX model group.

**Table.3. Bone parameters**

| Group | Bone | | | | |
|---|---|---|---|---|---|
| | Diameter** (mm) | Dry weight**(g) | Length** (mm) | Ash weight (g) | Calcium (mg/dl) |
| **Sham** | 3.84±0.06 | 0.55 ± 0.04 | 33.1±0.09 | 0.362±0.020 | 5.298±0.207 |
| **OVX** | 3.97±0.04** | 0.53±0.04** | 33.5±0.01** | 0.283 ± 0.014[c] | 2.653±0.166[c] |
| **OVX+Test Drug** | 3.91±0.04** | 0.54±0.03** | 33.6±0.03** | 0.315 ± 0.008* | 4.427±0.164** |
| **OVX+raloxifene** | 3.90±0.03** | 0.56±0.03** | 33.8±0.04** | 0.388±0.089** | 5.50 ± 0.093** |

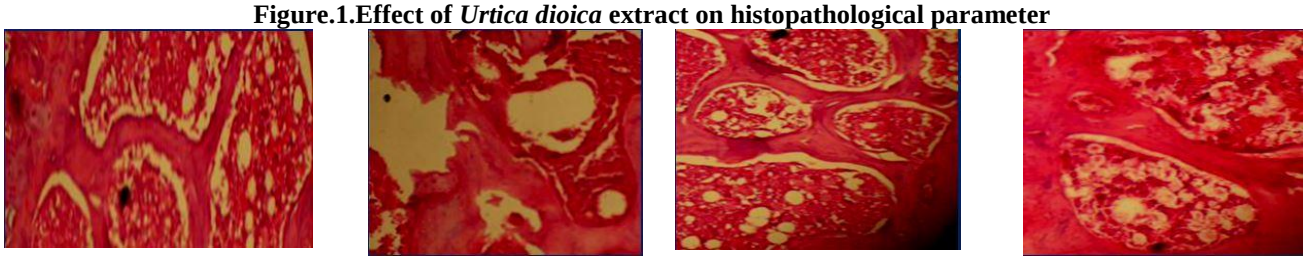
[b] p < 0.01 as comparison to sham operated normal group, [c] p < 0.001 as comparison to sham operated normal group,
* p < 0.05 as comparison to OVX model group, ** p < 0.001 as comparison to OVX model group,
**differences significant at p<0.05

**Table.4. Biomechanical parameters of femur**

| Group | Max-load (N) | Max-stress (N/mm2) | Max-stroke (mm) | Max-strain (%) | Elastic (N/mm2) | Energy (J) | Load testing of femoral head | Three point bending tibia |
|---|---|---|---|---|---|---|---|---|
| **Sham** | 81.9 ± 0.6 | 175.7 ± 1.25 | 0.900 ± 0.224 | 0.0346 ± 0.0046 | 714686 ± 78715 | 0.066 ± 0.010 | 75.33 ± 5.52 | 41.00 ± 1.03 |
| **OVX** | 81.5 ± 4.2 | 175.0 ± 8.48 | 0.848 ± 0.045 | 0.0290 ± 0.0017 | 767291 ± 74192 | 0.061 ± 0.022 | 43.23 ± 1.40* | 17.66 ± 1.60 |
| **OVX+Test Drug** | 82.095 ± 4.4 | 179.0 ± 00.60 | 0.860 ± 0.830 | 0.0345 ± 0.0148 | 783182 ± 86500 | 0.064 ± 0.028 | 96.33 ± 7.23**,**** | 77.00 ± 2.92** |
| **OVX+raloxifene** | 83.1 ± 5.0 | 182.4 ± 7.98 | 0.887 ± 0.204 | 0.0351 ± 0.0076 | 738206 ± 86354 | 0.067 ± 0.088 | 66.33 ± 4.67** | 75.66 ± 2.51** |

*$P < 0.05$ vs. Sham **$P < 0.05$ vs. Ovx, ***$P < 0.05$ vs. Raloxifene

**Figure.1.Effect of *Urtica dioica* extract on histopathological parameter**



**A** **B** **C** **D**

Sham control showing compact, normal, and uniform trabecular. (B) OVX model group of epiphyseal showing sparse, loss of interconnectivity, thinning of trabecular and widening of intertrabecular spaces, and lytic changes. (C) *Urtica dioica group* epiphyseal femoral region showing sparse, thinning of trabecular, loss of interconnectivity and narrowed intertrabecular spaces and showing restoration of normal architecture (40×). (D) 5.4 mg/kg Raloxifene showed trabecular restoration of normal architecture along with increasing bone cells (40×).

## 4. DISCUSSION

The present study was aimed to investigate the anti-osteoporotic activity of *Urtica dioica* extract in ovariectomised rats. It is well known that estrogen

deficiency is one of the important risk factors in the pathogenesis of osteoporosis. It is also evident that the bilateral ovariectomy results in dramatic decreases in uterine weight, bone mineral content, density and biomechanical strength due to estrogen loss,

*Urtica dioica* plant consist several important chemical constituent which are beneficial in osteoporotic women, i.e. calcium, potassium, and protein. In the present study, daily oral administration of *Urtica dioica* extract (200mg/kg) for 2 months significantly inhibited the increase of body weight gain induced by ovariectomy and maintained the body weight changes near the normal weight of SHAM operated rats. *Urtica dioica* extract restored the ovariectomy induced decrease in the uterine weight. It also normalized the biochemical changes in serum levels of calcium, phosphorus, bone – specific alkaline phosphatise.

*Urtica dioica* extract also restored the ovariectomy-induced changes in femur weight, diameter and density and normalized calcium content in femur bone ash. Calcium and phosphorus are widely accepted phenotype markers for bone formation. In the present study, the treatment with *Urtica dioica* extract restored the decreased serum calcium and phosphorus concentrations induced by ovariectomy to normal levels.

## 5. CONCLUSION

The current results showed the beneficial effects of *Urtica dioica* extract when given orally to rats with ovariectomy-induced osteoporosis. This study recommends that intake of *Urtica dioica* in foods could be considered as natural alternative to estrogen hormone replacement therapy for the prevention and treatment of osteoporosis due to estrogen deficiency in postmenopausal women.

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