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ORIGINAL ARTICLE **OPEN ACCESS**

Sesamol Alleviates and Strengthens Bone Health in Osteoporotic Rats Induced by Postmenopausal

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ABSTRACT

*Investigation of Protective Effect of Sesamol and Glyburide against Osteoporosis in Ovariectomized Rats. Sesame are Sesamum indicum rich in lignan content and beneficial for the health. The other sesame lignan compounds like sesamin and Sesamol, Antioxidants are a substance that can inhibit the oxidation reaction of free radicals. Sesamol is the natural products with antioxidant activity. It is widely used as an antioxidant in food, medicine and other fields. The antioxidant activity of Sesamol was tested and results reflected on the antioxidant potential. In our study total polyphenols and flavonoids contents, scavenging activity of DPPH and ABTS radicals were processed. They showed strong DPPH radical scavenging antioxidant activity with IC_{50} value of 19.98 ± 0.03 µM for ascorbic acid. The result of the experiment in DPPH exhibited potent antioxidant potential which is significant with respect to the reference standard ascorbic acid which showed an IC_{50} of and 14.07 ± 1.49 µM. We report that Sesamol exhibited strong ABTS radical scavenging potential with an IC_{50} of 3.49 ± 0.03 µM. Notably the ABTS radical quenching ability of Sesamol was far better than that reported in case of standard ascorbic acid (27.14 ± 0.03 µM) and result shows in ALP highly significant differences from the normal group (NC). ##P < 0.05 significant differences from the disease group ###p<0.001 highly significant from the toxic group. Furthermore, a decrease in calcium levels was seen in treated rats when compared to the disease group. However, SD***p<0.001 highly significant differences from the normal control group (NC). ##P < 0.05 significant differences from the disease group. ###p<0.001 is highly significant from the toxic group. SD. ***p<0.001 highly significant differences from the normal control group (NC). ##P < 0.05 significant differences from the disease group. ###p<0.001 is highly significant differences from the toxic group. Serum Creatinine Showed result of mean SD.***p<0.001 is highly significant differences from the normal control group (NC).##p<p0.05 is significant differences from the disease group.###p<0.001 is highly significant differences from the toxic group.*

Keywords: *Sesamol, DPPH, ABTS, Ovariectomy, Uterus histology, Bone histology,ALP, Creatinine, Phosphorus, Calcium*

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INTRODUCTION

Although various risk factors have been linked for the initiation, progression and complications of a set of diseases, oxidative imbalance has been known to fuel each and every step of the establishment of distinct ailments *i.e.*, atherosclerosis [1-3].diabetes [4, 5] and associated complications like nephropathy [6-8] neurodegenerative disorders [9-11] and ageing [11, 12].Considering the adverse events associated with the synthetic drugs, various plant-based metabolites i.e., lycopene, carvacrol, iridin, glycyrrhizic acid, and tocotrienol have been tested till date for their pharmacological effects against a spectrum of disease [1, 2, 9, 23]. Absorption of Sesamol takes place through (GIT) gastro intestinal tract and is metabolized by two-phase enzymes in the liver. Conjugated products such as Sesamol sulfate and glucuronide, and then transported to other tissues such as the brain and lungs, it is distributed in the lungs and kidneys and excreted in urine and faeces [14]. Bone biomarkers included bone formation, bone resorption and regulator are released during the bone remodeling processes [13]. Osteoporosis is a metabolic disease that is characterized by increase in alkaline phosphatase (ALP) levels. ALP, plays an important role in metabolism within the liver and bone [15]. As serum T-ALP levels have been considered a potential biomarker of bone formation [15].Serum ALP changes can be estimated in a variety of diseases, such as liver disease, cholestatic jaundice, arteriosclerosis, cognitive disorders, and even cerebrovascular diseases [16, 17].Total alkaline phosphatase (T-ALP) and bone-specific alkaline phosphatase (B-ALP) are byproducts are produced during bone remodeling. They can be measured in urine or serum so predict the bone turnover rate [18].

Creatinine

Renal function degrades with ageing [19].Because blood creatinine can be calculated as a measure of muscle mass, a decrease in the rate of skeletal muscle mass is associated with a decrease in bone mineral density. Serum creatinine is the beginning metabolite of creatine phosphate, and most of it can be detected in skeletal muscle. Plasma creatinine concentration is a constant, direct reflection of skeletal muscle mass because the level of creatinine per unit of skeletal muscle mass and the rate of creatine destruction remain constant [20].Serum creatinine correlates significantly with bone mineral density, particularly in patients with normal kidney function. Serum creatinine, on the other hand, has a positive relationship with bone mineral density. Serum creatinine can provide information regarding the bone and muscle health of those who have normal renal function. [21]. Phosphorus is a macroelement that plays a role in a variety of biological activities. Due to its mobility, it is an essential human intracellular anion that contributes to the body's acid-base balance by forming buffer systems in blood and urine [22, 23].Phosphorus is involved in the transmission of nerve impulses. Hydroxyapatites and phosphoproteins are bone-building materials, whereas pyrophosphates regulate osteogenesis and osteolysis (Penido et al; 2012).When there is a lack of calcium stored in the bones. This can result in osteoporosis (brittle bones) and gum and tooth problems, thus a balanced diet of calcium and phosphorus can reduce the incidence of osteoporosis [24].

Calcium

Calcium is a nutrient that is essential for the healthy functioning of the human body. This macroelement, which regulates many extracellular and intracellular processes, is required for bone the beginning, growth, and repair, as well as cellular cytoskeleton integrity [25].Calcium-rich diets are indicated to assist with prevent bone disorders. Due to the hormonal fall in estrogens, postmenopausal women are at a higher risk of developing this illness [26].

MATERIALS AND METHODS

Chemical reagents

Sesamol, standards (BHT, L-Ascorbicacid) and chemicals used were obtained from Hi-Media lab. Ltd, Mumbai, India. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radicals were purchased from Sigma Chemical Co, St, Louis, MO, USA.

In vitro antioxidant method

DPPH radical scavenging assay

The DPPH (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging capacity of the Sesamol was determined by standard protocol [9]. DPPH solution (132 mM) was prepared in methanol in a dark reagent bottle. 100µl of either Sesamol or ascorbic acid (concentration ranging from 0.0 to 250 µM) was added to 2 ml of DPPH solution and the reaction mixture was incubated for 15 minutes at 27ºC in a water bath and absorbance was measured at 517 nm. The reduced form of DPPH was generated, accompanied by the disappearance of the violet color. Ascorbic acid was used as a reference standard. Percent (%) scavenging of DPPH free radical was measured using the following equation:

$$\% \text{ DPPH Scavenging} = \frac{\Delta \text{ Abs. of control } - \Delta \text{ Abs. of test drug}}{\Delta \text{ Abs. of control}} \times 100$$

Further, IC_{50} value represented the concentration of Sesamol that caused 50% inhibition of DPPH radicals and was calculated by interpolation of linear regression analysis using Origin Professional for Windows.

ABTS radical scavenging assay

Radical-scavenging activity of Sesamol was determined according to the previously established protocol. ABTS radicals were pre generated by adding equal volume of 4.9 mM potassium persulfate solution and 14 mM ABTS solution and kept for 16 h in the dark. This solution was suitably diluted with distilled water to yield an absorbance of 0.90 at 734 nm and then used for antioxidant assay. Different concentrations of Sesamol (0.0–250 µM) were added to the above activated pre-generated ABTS solution. Ascorbic acid (0.0-250 µM) was used as a reference compound. The reaction mixture was vortexed for 10 s and the reduction in absorbance was recorded at 734 nm, using distilled water as a blank, on Eppendorf UV-visible spectrophotometer (Germany). Percent (%) scavenging of DPPH free radical was measured using the following equation:

$$\% \text{ ABTS Scavenging} = \frac{\Delta \text{ Abs. of control } - \Delta \text{ Abs. of test drug}}{\Delta \text{ Abs. of control}} \times 100$$

In vivo Animal method

EXPERIMENTAL DESIGN

Osteoporosis can be assessed using many models, including postmenopausal osteoporosis, disuse osteoporosis, and glucocorticoid-induced osteoporosis. In our investigation, we utilized the

postmenopausal osteoporosis technique. Ovariectomized is used for developing animal models of postmenopausal osteoporosis. In estrogen deficiency, bone loss occurs because the result of increased bone resorption and reduced osteoblast function. The estrogen receptors produce osteoclast death, yet the cause for decreased bone formation is unclear. We investigated the effect of Sesamol, an effective molecule obtained from seeds of *Sesamum indicum* [58].

Animals used in the study

Adult female Sprague Dawley rats were purchase from the Animal House Facility, Integral University, Lucknow. The animals kept in polypropylene cages (5 Animal in each cage) under standard laboratory conditions (12 hrs light and 12 hrs dark at day and night) and free access to appropriate diet and tap water *ad libitum.* The animal house temperature maintained at 25 ± 2°C & relative humidity was also maintained at (50 ±15%). They were fed standard laboratory diet and water *ad libitum*. Before starting the experiment, ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC) and IAEC Approval No: IU/IAEC/22/07 of Integral University, Lucknow.

Surgical procedure: ovariectomized

Animals were divided in 7 groups of 6 rats each. Animals of group 1 were administered with vehicle (1% CMC, 1ml/kg., p.o) and animals of group 2, 3, 4, 5, 6, 7 were bilaterally ovariectomized under Ketamine (40mg/kg, ip) and treated (p.o) with vehicle (1% CMC), Sesamol (low dose & high dose) and Alendronate (3 mg/kg) (Mustafa et al; 2018) respectively once for next 30 days (day 1: day of ovariectomy) Figure 1 (Arshad et al;2004). After 30 days of prescribed treatment, 5ml blood collected through cardiac puncture in centrifuge tube. After centrifugation serum was decanted in glass tube and stored at -20˚C till further investigation. each rat uterus removed, blotted and weighed and fixed in 5% formaldehyde in (pH 7.4) phosphate buffer for histology [59].5mm pieces from middle segment of uterus was dehydrated and stained with hematoxylin and eosin. Femur and tibia of each rat was dissected free of adhering tissue and fixed in 70% ethanol in saline and stored at -20˚C. Under Ketamine anesthesia, the bilateral ovariectomy was performed in rats by making two dorsolateral incisions using sharp dissecting scissors. The skin and dorsal muscles were then cut and the peritoneal cavity was thus reached. The uterine horn was picked out and the fatty tissue around the ovary was removed. The connection between the fallopian tube and the uterine horn was clamped by artery forceps and cut under the clamped area to remove the ovary. Skin was closed bilaterally with one simple catgut suture. Soframycin (antiseptic) was applied locally on the skin at both sites of the surgery [29].

Figure 1 Ovariectomized rat

Post-operative care

After suturing topical antibiotic such as soframycins will be applied to rat on the sutures. Analgesic such as tramadol (20mg/kg) will be injected intraperitoneally. Rats will be kept in disinfected cages. Antibiotic: Ceftriaxone Dose: 0.5g/kg for 3 days OD [27].

Analysis of biochemical markers

The blood samples of the experimented animals were collected separately in sterile tubes. Then the samples were allowed to clot, lysed and centrifuged at 1200*g* for 10 min at 4 °C. The serum was analysed for various biochemical markers such as calcium, phosphorus, ALP and creatinine

Assay of Alkaline phosphatase (ALP)

Assay of ALP was carried out by pNPP-AMP method by following the procedure described by (Kind et al;1954).using commercially available kits from Span Diagnostics, India. Briefly, 1ml of working ALP reagent containing substrate p-Nitrophenyl phosphate (pNPP) was added to the test samples (Serum). ALP catalyses the hydrolysis of colorless pNPP to yellow colored p-Nitrophenol and phosphate. Change in

absorbance due to yellow color formation is measured kinetically at 405nm and is proportional to ALP activity in the sample. The ALP activity was calculated by applying the following formula and expressed in IU/L

$$\text{ALP activity} = \text{Change in absorbance per min} \times \text{Kinetic factor}(2712)$$

Estimation of serum calcium

The estimation of serum calcium was carried out following the method of (Stern et al; 1958) briefly, 1ml of calcium reagent containing O-cresolphthalein complexone was added to 20µl of test samples. In alkaline solution, calcium binds with O-cresolphthalein complexone to form a bluish-purple complex, which is measured at 578nm.In the tube containing the standard was also processed similarly as the test samples. The intensity of color formed is proportional to calcium concentration in the sample. It is expressed in mg/dl. The amount of calcium present in the serum sample was calculated by applying the formula,

$$\text{Serum Calcium (mg/dL)} = \frac{\text{Absorbance of the test}}{\text{Absorbance of the standard}} \times 10$$

Estimation of serum phosphorus

The estimation of serum phosphorus was carried out following the method of (Brookes et al;1983) briefly, 1ml of molybdate reagent was added to 10µl of test samples. The inorganic phosphorus reacts with ammonium molybdate to from phosphomolybdate complex. This phosphomolybdate complex is measured at 340 nm and is directly proportional to the amount of inorganic phosphate in the sample, which is expressed in mg/dl. The amount of inorganic phosphate present in the serum sample was calculated by applying the formula

$$\text{Serum inorganic phosphate (mg/dL)} = \frac{\text{Absorbance of the test}}{\text{Absorbance of the standard}} \times 5$$

Creatinine

Serum creatinine levels were estimated as per Jaffe method using previously prepared solution (in equal volume NaOH and picric acid). Each serum samples and standards (50 µL) were gently mixed with 1000 µL of Jaffe working solution and incubated at 25 °C for 30 second. Creatinine was measured by using diluted urine in distilled water at a ratio of 1:50. Quality control samples were maintained to ascertain the accuracy of the results. Absorption of the reaction mix and measured by using a Stat Fax 3300 auto biochemistry analyzer [28]

Comparing organ weights

Organ weight is a significant pointer usually used to weigh of organ and their morphological characteristics. The uterus and femur, were extracted, weighed before and after drying at 65 °C to predict an invariable weight. Their equivalent organ coefficients were calculated as reported earlier using the weights of internal organs and the comparison of bodyweight ([29, 30].

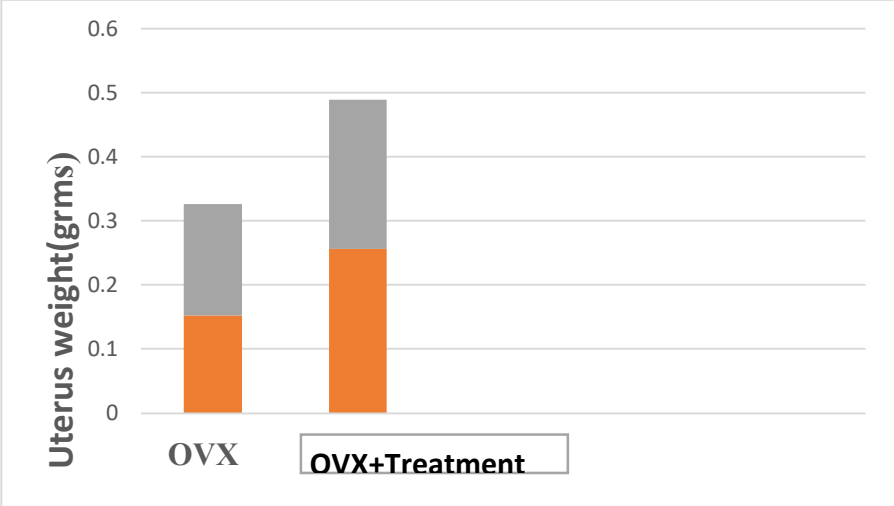


Figure 2. Effect of Sesamol on uterus weight in OVX and OVX+ Sesamol

Effect of Sesamol on uterine morphology

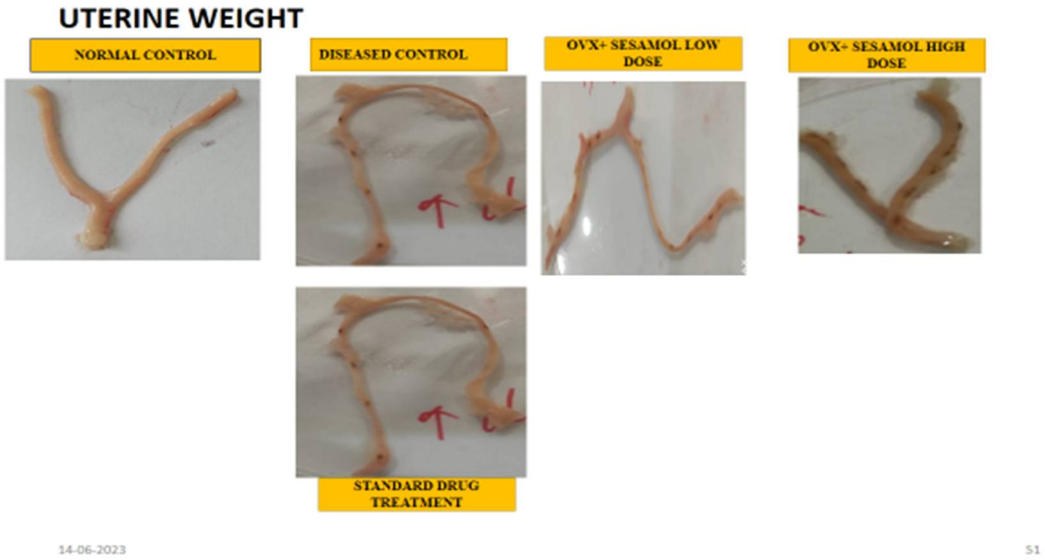


Figure 3.

Bone Morphometric parameters

The length and the femoral head width of all the bone samples were recorded with the instrument of digital Vernier Calliper (Mitutoyo corp. Japan).

Table 1: Effect of OVX and Sesamol on femur morphometric parameters

Groups	Groups Length (mm)	Ash content (g)
Normal control group	31.82±0.17	0.303±0.007
Diseased group	32.52±1.37	0.290±0.004
Treatment	33.46±1.76	0.342±0.008b

Each value is the mean ± SD (n=5). Values with a superscript are significantly different from the Normal control group p<0.001, p<0.01, <0.01)

Femoral length

The OVX group showed no significant change in the femoral length when compared to the normal control group. Sesamol dose to the OVX group showed significant increase (p<0.05) in the femur length when compared to the OVX group. However, Sesamol to the normal control animals showed no significant change in the femoral length in comparison to the control group.(Table 1)

Femoral ash content

The bone mineral content of the femur of OVX animals showed no significant change. Sesamol dose to the OVX animals significantly increased the treatment of the femur (p<0.01) (Table 1)

Table 2: Effect of OVX and Sesamol on tibia morphometric parameters

Groups	Groups Length (mm)	Ash content (g)
Normal control group	32.29±0.53	0.463±0.010
Diseased group	34.73±2.03	0.376±0.010
Treatment	36.36±1.55	0.462±0.011

Each value is the mean ± SD (n=5).Values with a superscript are significantly different from the normal control group p<0.001, d, p<0.05 p<0.001)

Tibia length

The OVX group showed no significant change in the length of tibia bone when compared to the normal control group. However, Sesamol to the OVX group showed significant increase (p<0.05) in the length of tibia. Sesamol to the normal control animals showed no significant alteration in the tibia length.(Table 2)

Tibia ash content

A significant decrease in the Sesamol of tibiae from OVX animals was observed. Sesamol to the OVX animals significantly increased the mineral contents of tibia (p<0.001) and restored them to normal.(Table 2)

Effect of Sesamol on body weight

Significant weight loss occurred in the Sesamol treatment groups (control and OVX treated) ($p < 0.05$) in comparison to normal control untreated animals. Due to loss of estrogen many of the metabolic pathways play role in ovariectomized group leading to significant gain in the body weight in comparison to the control untreated group ($p < 0.05$) (Fig. 2,3) which was significantly decrease by Sesamol.

Effect of Sesamol on uterus weight

Increase in the uterus weight was evident in the OVX + Sesamol group in comparison to the OVX although the difference failed to achieve any significance ($p > 0.05$) (Fig. 2,3).

Results and discussion

DPPH radical scavenging activity of Sesamol

DPPH radical scavenging activity is widely used to evaluate antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. In the same context, we also analyzed the DPPH free radical scavenging activity of Sesamol. The results indicated the presence of the experiment exhibited potent antioxidant potential with IC_{50} values of 19.98 ± 0.03 µM, which is almost comparable to the reference standard ascorbic acid which showed an IC_{50} of and 14.07 ± 1.49 µM (Fig. 4). This substantial scavenging of DPPH was then attempted for co-relation with osteoporotic principles.

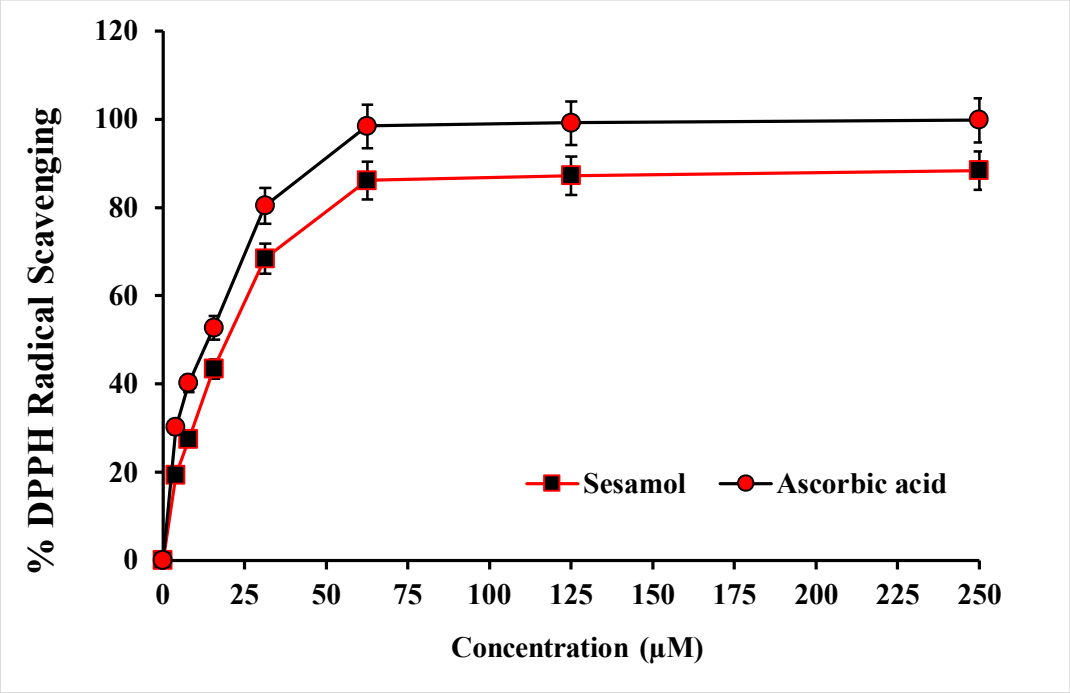


Fig. 4. DPPH scavenging assay of Sesamol.

ABTS radical scavenging activity of Sesamol

In addition to the DPPH free radical scavenging activity, the ABTS quenching ability of Sesamol was also assessed as ABTS is also another most widely used method for the determination of antioxidant potential of various plant extracts and their bioactive secondary metabolites. We report that Sesamol exhibited strong ABTS radical scavenging potential with an IC_{50} of 3.49 ± 0.03 µM. The ABTS radical quenching ability of Sesamol was far better than that of reported in case of standard ascorbic acid (27.14 ± 0.03 µM) (Fig. 5).

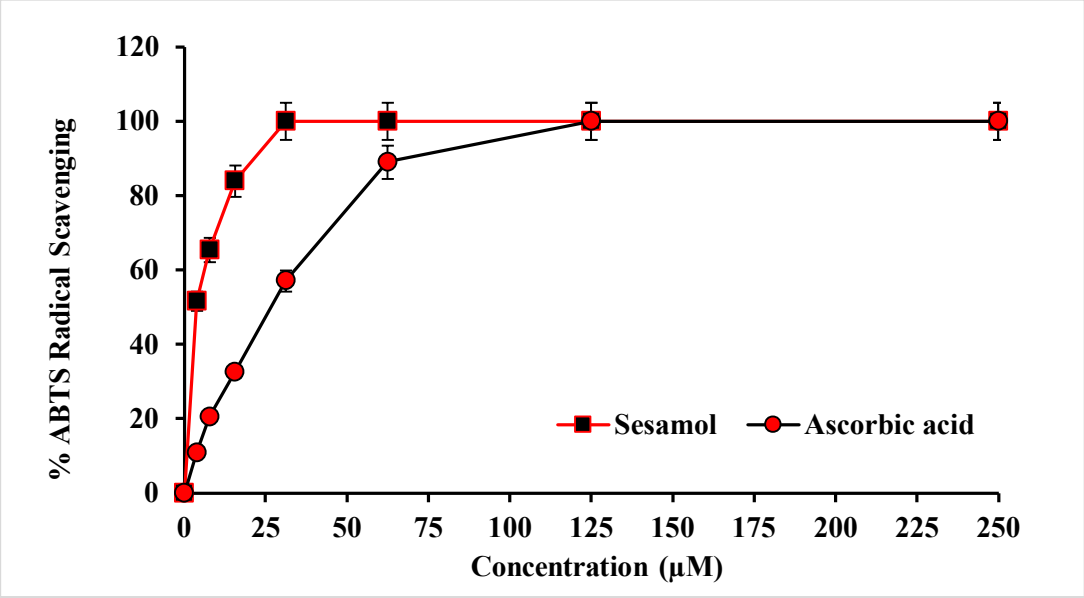
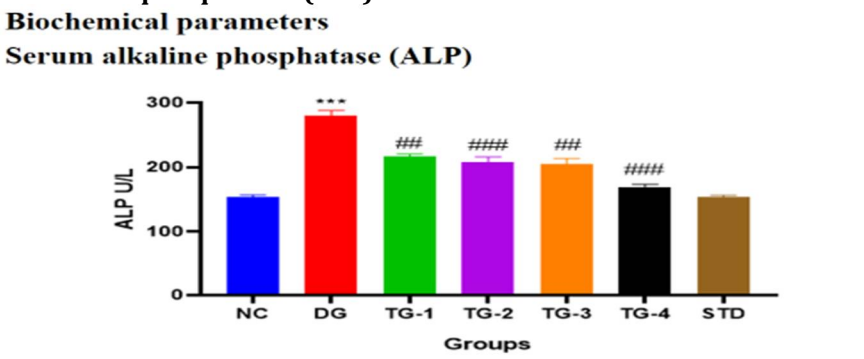


Fig. 5. ABTS radical scavenging activity of Sesamol

Results of biochemical analysis

Further, biochemical analysis was done through the levels of bone markers like ALP, Serum calcium, Serum phosphorus, Creatinine.

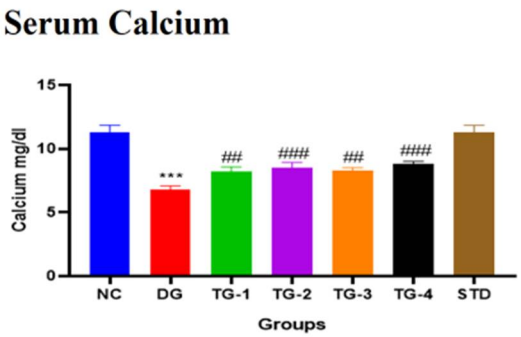
Estimation of Alkaline phosphatase (ALP)



All values are expressed as Mean ± SD. ***p < 0.001 = highly significant differences from the normal control group (NC). ##p<0.05- significant differences from the disease group. ###p< 0.001= highly significant differences from the Toxic group (TG)

Figure.6

Ovariectomized rats treated with Sesamol indicate a significant reduction in the serum alkaline phosphate levels in all treated groups. Highly significant results were obtained in comparison with normal control (##*P* < 0.05) and the Toxic group (###p<0.001) (Fig. 6).

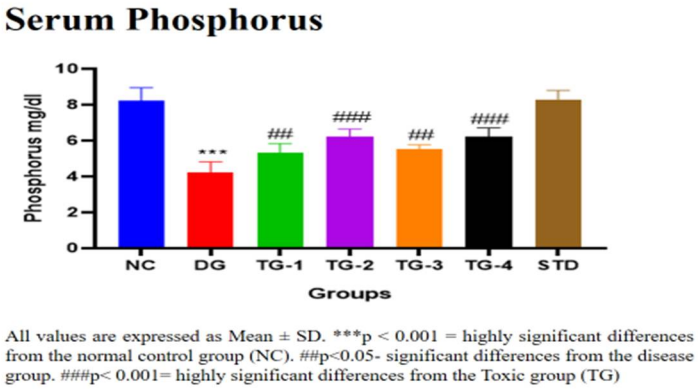


All values are expressed as Mean ± SD. ***p < 0.001 = highly significant differences from the normal control group (NC). ##p<0.05- significant differences from the disease group. ###p< 0.001= highly significant differences from the Toxic group (TG)

Figure. 7Serum Calcium

Ovariectomized rats indicate a significant increase in the serum calcium levels in all treated groups. Highly significant results were obtained in both the cases that is in comparison to control (##$P < 0.05$) and the Toxic group (###p<0.001) (Fig. 7).

Serum Phosphorus

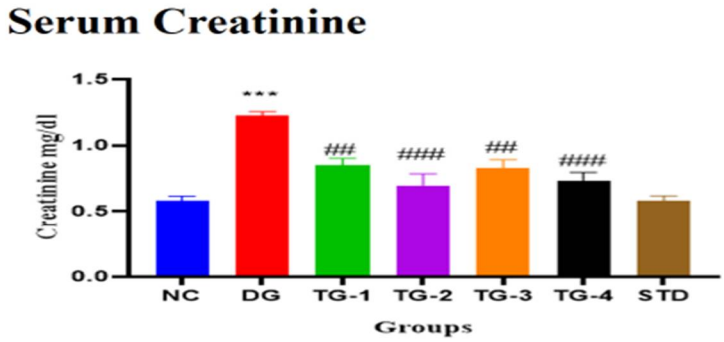


All values are expressed as Mean ± SD. ***p < 0.001 = highly significant differences from the normal control group (NC). ##p<0.05- significant differences from the disease group. ###p< 0.001= highly significant differences from the Toxic group (TG)

Figure 8. Serum Phosphorus

A significant increase in the serum phosphorus levels was observed in all treated groups. OVX rats treated with Sesamol indicate a significant increase in the serum phosphorus levels. Highly significant results were obtained in both the cases with respect to normal control (##$P < 0.05$) and the Toxic group (###p<0.001). (Fig. 8).

Serum Creatinine



All values are expressed as Mean ± SD. ***p < 0.001 = highly significant differences from the normal control group (NC). ##p<0.05- significant differences from the disease group. ###p< 0.001= highly significant differences from the Toxic group (TG)

Figure 9.

A significant increase in the serum creatinine levels was observed in all treated groups. OVX rats treated with Sesamol indicate a significant increase in the serum creatinine levels. Highly significant results were obtained with respect to normal control (##$P < 0.05$) and Toxic group (###p<0.001). (Fig. 9).

Table 3: Effect of OVX and Sesamol on Uterus histology

Groups	Epithelial cell height (µm)	Thickness of endometrium(µm)	Thickness of myometrium(µm)	Diameter ofuterus (µm)
Normal control	8.44±0.49	50.48±05.14	30.67±1.90	312.37±15.14
Disease	18.46±1.06**	199.02±18.64**	91.55±8.30**	743.81±41.77**
Treatment	13.5±0.1*	83.02±02.97*	58.27±4.96*	454.74±27.77*

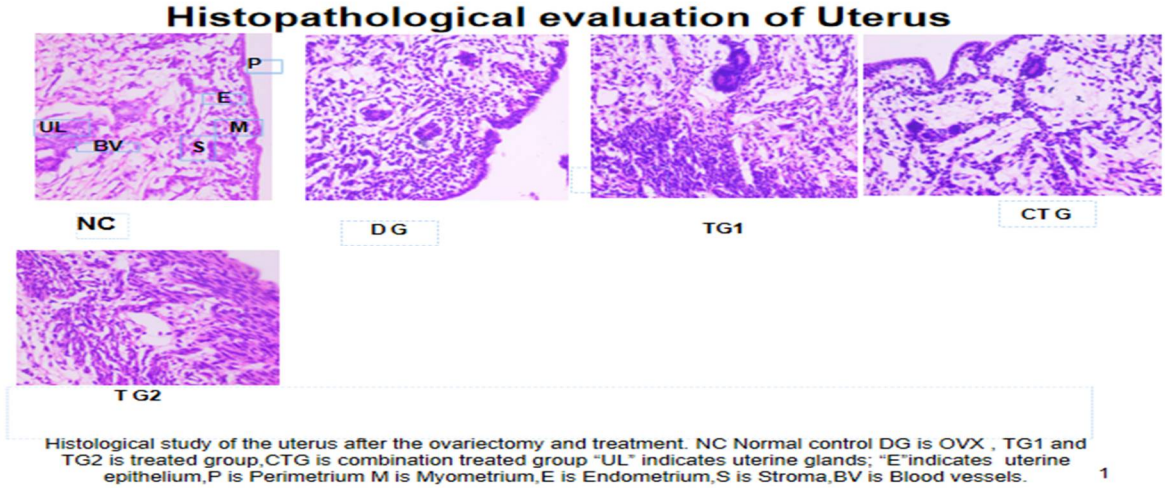


Figure 10.

Histological study of the uterus after the ovariectomy and treatment.The uterus of CMC treated control OVX mice (A; x20) showing a smooth luminal epithelium (E) of the endometrium. The endometrial gland (G) are poorly developed and less in number. The endometrium (E), myometrium (M) and the luminal epithelial cells (E) poorly developed . NC Normal control DG is OVX group , TG1 and TG2 is treated group,CTG is combination treated group "UL" indicates uterine lumen; "E"indicates uterine epithelium, P is Perimetrium M is Myometrium, E is Endometrium,S is Stroma, BV is Blood vessels.The Alendronate is used as a standard treated OVX mice however showed endometrial epithelial cells proliferated with increase in the number of endometrial glands. The endometrium (E) and the myometrium (M) also increased in thickness in the treated group. The oral administration of Sesamol to OVX mice was also found to increase the number of endometrial glands (E). Moreover, it also increased the endometrial luminal epithelium (E), the myometrium (M) and the endometrium (E) of the uterus in treated mice, uterine lumen.

Effect of the Sesamol on histological changes of the uterus:

The histo-architecture of the uteri of all the experimental group of animals was studied using eosin-haematoxylene stained slides and result which are shown in Table-3 and figure 10. Investigation of uterine histological sections reveals Also, the endometrial glands became more frequent followingtreatment with the Sesamol to the OVX mice. Histological changes in the thickness of endometrium, myometrium and epithelial cell height and diameter of the uterus after treatment with Sesamol and Glyburide in ovariectomized mice.in normal control endometrium (thick layer of smooth muscle) ,myometrium (Thick layer) and perimetrium (dense and connective tissue detected) and uterine lumen (irregular), Stroma,tiny blood vessel observed. In disease group Stromal tissues loose and increase the number,Lumen dilated (thin uterine layer).In treatment group Uterine layer thick, stromal cells increase and treated group showing the result thickness of endometrium.it may be due to either estrogenic effect in (Fig. 10).

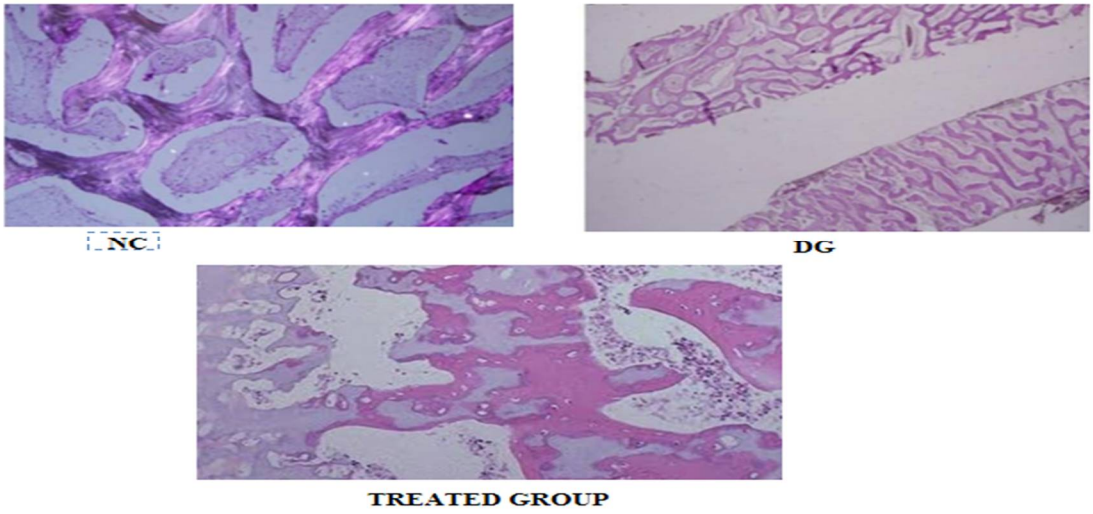


Figure 11.

Epiphyseal region showing normal compact trabeculae with inter trabecular spaces in NC (normal control group). Epiphyseal part showing sparse, trabeculae thinning, and widening of inter trabecular spaces in DG (disease group). Epiphyseal region showing moderately thick elongated trabeculae and narrowed inter trabecular spaces in treated group it also showed trabecular restoration of normal architecture along with normal bone cells.

Histopathological evaluation of femur bone

Histopathological evaluation of the femur section showed normal architecture and normal bone compactness in normal control (Fig. 11), while disease group showed disruptive, lytic changes, thinning of the trabecule resulting in widening of intertrabecular spaces (Fig. 11). Alendronate and treated groups showing significant restorative progress with increased ossification, mineralization, and increased osteoclastic activity and reduced bone resorption, which indicates the recovery of normal bone (Fig. 11).

DISCUSSIONS

Osteoporosis is characterised by decreased bone density, bone tissue degradation, and disturbance of bone microarchitecture, all of which contribute to an increased risk of fracture. It affects quite a few of people, however the incidence may grow with age. Apart from fractures, it also causes serious alternate ailments [31, 32]. Sesamol is a phenol group, which is found in a variety of plants and glyburide is thiazolidinedion group. Various studies have been conducted on the therapeutic efficacy of these substances with a wide variety of pharmacological activities [33]. Given the favourable benefits of Sesamol, animal models were used to assess the molecule's efficacy in osteoporosis. With all of these prior research findings, we selected Sesamol as our suggested drug and provided the first original study in which it was studied for OVX-induced osteoporosis. The current work investigated the pharmacological effect of Sesamol on postmenopausal osteoporosis using OVX female rats, a frequently used animal model for the treatment of estrogens lack. While experimenting with estrogen-deficient rats, it was shown that body weight is a common phenomenon. An outcome indicating that postmenopausal women are prone to weight increase and osteoporosis [34, 35]. In the current study, rats' body weights increased much more after weeks of ovariectomy than in the control group. After weeks of therapy with low and high doses of Sesamol, OVX-induced body mass gain in rats was reduced, and the results were highly helpful. Menopause is the most common cause of oestrogen deficiency in women, which causes calcium breakdown and secretion, act bone decomposition [35]. In the current study, the loss of calcium in OVX rats remain measured by the treatment of Sesamol (figure 7) and this result was supported the previous study that indicated high calcium excretion during insufficient estrogen in women [36].Histopathological examination of the femurs of either extract treated groups suggested ossification, mineralization, calcified cartilaginous deposit and marginal osteoclastic activity.Remodelling of bone is a bright advancement in metabolism and a dynamic friend in modification and rearrangement of bone through the lifetime. The main role is enact by stable the no. of osteoblasts and osteoclasts. When this instability is altered, whole conformation is alteration in the inclination of extravagant natural process, bone loss thus resolve in occurrence of osteoporosis [38-43] It is well established that the biomarkers of bone formation like ALP, Creatinine, Ca and Phosphorus are used to assess bone modifications [44, 45]. During accretion, the changes of ALP have been reported to increase discouragingly during estrogen insufficiency in rats [46-50].which is supported by the results of current

research. Remarkably, the treatment of Sesamol in the present study remarkably reduced the levels of ALP in OVX. Taken together, it can help bone absorption connected with blood indicators in OVX and prevent the effect of osteoporosis.In addition, Sesamol and glyburide was helped to improve the femoral safe modification and determined weight in OVX rats, which conforming to the previous studies [56].Free radicals are known to be the main cause of oxidative stress which is grossly implicated in the pathogenesis of various diseases such as cancer, diabetes, cardiovascular diseases, and osteoporosis. Natural antioxidants have gained much aid from go through because they are considered safer than synthetic antioxidants. Natural antioxidants derived from seeds, fruits, vegetables, spices, and cereals are very effective and can protect the human body from oxidative effect caused by ROS [51-59]. treated group showing the result thickness of endometrium.it may be due to either estrogenic effect. Similarly, Sesamol did not affect the position of the trabecular bone in OVX female rats. Generally, these fall outs while treatment with Sesamol did not have any destructive result on the skeleton structure of OVX animals. Uterus weight was measured because after ovariectomized the uterus is pervert. So i wanted to get whether Sesamol low dose being a phytoestrogen had the potential to risen bring up the uterus weight. In our study, Sesamol at dose of 5 mg/kg (low dose), had a significant effect in OVX rats. The outcome of this study also opinions available that Sesamol an glyburide might be responsible for the anti-osteoporotic effect.

CONCLUSIONS

Estrogen is the most potent inhibitor of osteoclastic bone resorption (loss), so estrogen deficiency is a major risk factor in the pathogenesis of osteoporosis [12]. Bilateral ovariectomy in rats caused dramatic decreases in uterine weight, bone mineral content, density and biomechanical strength due to estrogen deficiency [23, 15, 20, 55]. Postmenopausal osteoporosis is commonly treated by estrogen replacement therapy and/or by some drugs such as Alendronate (one of Bisphosphonates series) which inhibits osteoclast-mediated bone resorption[41]. The present study provides experimental evidence that Sesamol possesses potent antioxidant properties in addition to its various pharmacological activities. This new finding may contribute to the understanding of beneficial effects in a variety of diseases where oxidative stress has long been known to contribute to the respective pathogenesis. Our result reported that Sesamol exhibited strong ABTS radical scavenging potential and Sesamol was far better than that of reported in case of standard ascorbic acid. ALP is found in most organs of all mammalian species. ALP is primarily found in epithelial tissue, liver, kidney, and placenta. Bone is the only connective tissue shown to produce ALP. Hence, results of this part of the study associate that ovariectomy induced estrogen deficiency and postmenopausal circumstances were stately by changes in the bone macroarchitecture, uterine morphology, biochemical and pathological changes in the bone tissue.

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