RESEARCH PAPER

Evaluation of Osteoprotective Effects of Phytoestrogen-Rich *Glycine Max* Extract in Ovariectomized Rat Model

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ABSTRACT

Background: Osteoporosis is a progressive metabolic bone disorder characterized by decreased bone mass and deterioration of bone microarchitecture, leading to increased bone fragility and fracture risk. Estrogen deficiency following menopause is a major cause of osteoporosis. Phytoestrogens derived from plants have gained attention as safer alternatives to conventional hormone replacement therapy.

Objective: The present study aimed to evaluate the osteoprotective effects of phytoestrogen-rich *Glycine max* (soybean) extract in an ovariectomized (OVX) rat model of osteoporosis.

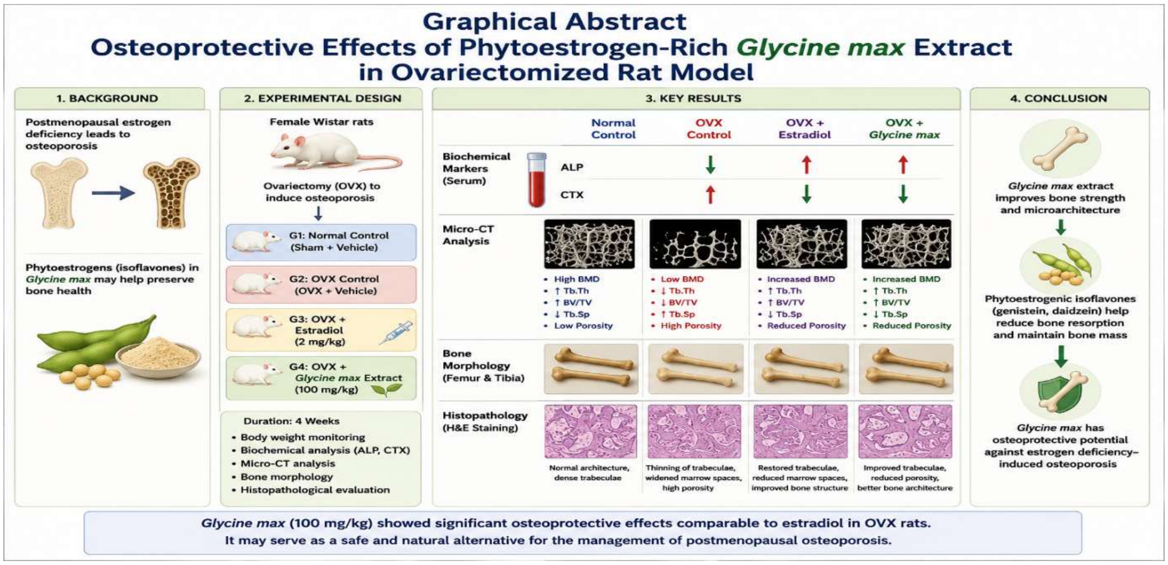
Methods: *Glycine max* seeds were collected, authenticated, and extracted using ethanol. Preliminary phytochemical screening and HPLC analysis were performed to identify phytoestrogenic constituents. Osteoporosis was induced in female Wistar rats by bilateral ovariectomy. Animals were divided into experimental groups and treated with *Glycine max* extract for the study period. Various parameters including body weight, serum calcium, phosphate, alkaline phosphatase (ALP), estradiol, C-terminal telopeptide of type I collagen (CTX), bone morphology, and histopathological changes were evaluated.

Results: Treatment with *Glycine max* extract significantly improved biochemical markers associated with bone metabolism. The extract increased serum calcium, phosphate, and estradiol levels while reducing elevated ALP and CTX levels induced by ovariectomy. Histopathological examination revealed preservation of trabecular architecture and reduced bone deterioration in treated groups compared to OVX controls.

Conclusion: The findings suggest that phytoestrogen-rich *Glycine max* extract possesses significant osteoprotective activity and may serve as a promising natural therapeutic agent for the prevention and management of postmenopausal osteoporosis.

Keywords: *Glycine max*, Phytoestrogens, Osteoporosis, Ovariectomy, Isoflavones, Genistein, Daidzein, Bone Health.

How to cite this article: Burli S, Waghmare B, Kumbhar P, Dhole V, Mugade M, Jadhav K. Evaluation of Osteoprotective Effects of Phytoestrogen-Rich Glycine Max Extract in Ovariectomized Rat Model. Int J Drug Deliv Technol. 2026;16(55s): 1236-1252. DOI: 10.25258/ijddt.16.55s.119



1. INTRODUCTION

Reduced bone mass, deteriorating bone microarchitecture, and an increased risk of fractures are the hallmarks of osteoporosis, a chronic metabolic bone condition. It is one of the most common skeletal disorders in the world and a serious public health issue, especially for postmenopausal women and the elderly. Because bone loss develops slowly and shows no signs until a fracture occurs, the disorder is frequently referred to as a "silent disease." Hip, vertebral, and wrist fragility fractures are prevalent clinical presentations that are linked to high rates of morbidity, mortality, and medical costs.[1]

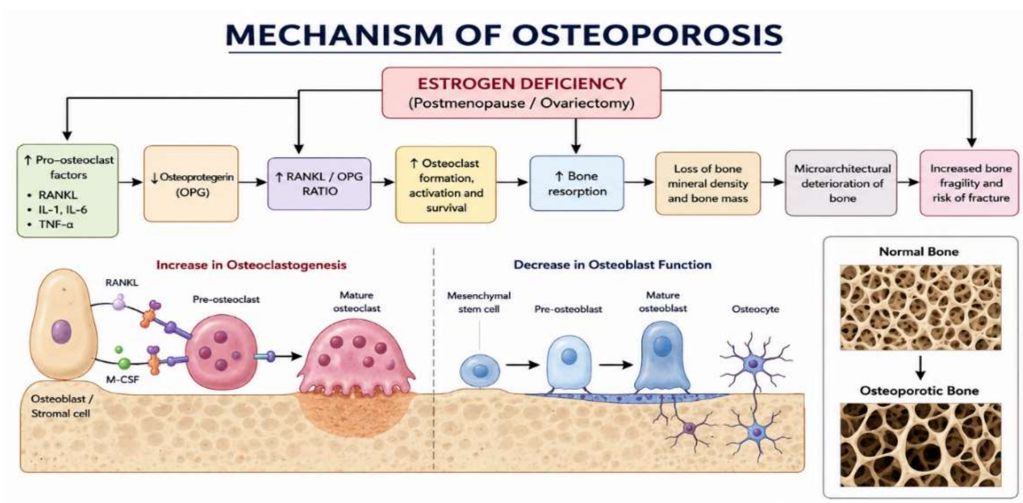


Figure No.1 mechanism of osteoporosis

Osteoblasts, osteoclasts, and osteocytes work together to continuously remodel bone tissue, which is a dynamic organ. Under healthy settings, osteoblast-mediated bone production is counterbalanced by osteoclast-mediated bone resorption. Estrogen is one of several hormonal and molecular elements that strictly maintain this equilibrium. While encouraging osteoblast survival and bone production, estrogen inhibits osteoclast differentiation and activity.

This balance is upset after menopause by a sharp drop in estrogen levels, which leads to increased bone resorption, decreased bone production, and a gradual loss in bone mineral density.[2]

Postmenopausal osteoporosis is caused by a variety of cellular and molecular processes. Tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) are examples of pro-inflammatory cytokines that are produced when estrogen levels are low. These cytokines stimulate osteoclastogenesis by activating the receptor activator of nuclear factor kappa-B ligand (RANKL) signaling pathway. Increased osteoclast activity speeds up the breakdown of the bone matrix, resulting in skeletal fragility, cortical bone loss, and trabecular thinning. Furthermore, oxidative stress and persistent inflammation exacerbate bone degeneration and hinder bone regeneration. Several pharmacological approaches, including hormone replacement therapy, bisphosphonates, calcitonin, and selective estrogen receptor modulators, are currently employed for the management of osteoporosis. Although these therapies effectively reduce fracture risk, their long-term use is often associated with adverse effects such as gastrointestinal disturbances, thromboembolic complications, cardiovascular disorders, and hormone-dependent malignancies. Consequently, increasing attention has been directed toward the development of safer and more effective therapeutic alternatives derived from natural sources.[3]

Phytoestrogens are naturally occurring substances generated from plants that can bind to estrogen receptors and share structural similarities with endogenous estrogen. These substances have biological actions similar to those of estrogen and have garnered a lot of attention due to their potential to prevent illnesses associated with estrogen insufficiency. Glycine max, or soybean, is one of the phytoestrogen-rich plants that has been studied the most because of its high isoflavone content, especially genistein and daidzein. By promoting osteoblastic activity, preventing osteoclast-mediated bone resorption, and increasing bone mineral density, these bioactive components have been shown to influence bone remodeling.[4]

Soybean isoflavones have anti-inflammatory and antioxidant qualities in addition to their phytoestrogenic qualities, which may help protect the skeleton. Genistein and daidzein have been shown in both experimental and clinical research to improve bone turnover markers, maintain trabecular bone architecture, and lessen osteoporotic alterations linked to estrogen shortage. According to these results, glycine max may be a viable natural substitute for managing and preventing postmenopausal osteoporosis.[5]

Because it closely resembles the estrogen deficiency-induced bone loss seen in humans, the ovariectomized (OVX) rat model is widely recognized as a trustworthy experimental model for studying postmenopausal osteoporosis. An efficient platform for assessing possible osteoprotective drugs is provided by the hormonal changes, accelerated bone turnover, and progressive decrease in bone mineral density that follow surgical removal of the ovaries.[6]

In order to assess the phytoestrogen-rich Glycine max extract's osteoprotective properties in an ovariectomized rat model, the current study was conducted. The study sought to determine how it affected bone shape, histological changes linked to osteoporosis, and biochemical indicators of bone metabolism. The results may offer scientific proof of Glycine max's medicinal promise as a safer, natural substitute for treating postmenopausal osteoporosis.[7]

2. MATERIALS AND METHODS

2.1 Materials

2.1.1. Instruments

Torson's vacuum desiccators, IME pattern Vernier calipers, Growell Instrument Muffles Furnace, Singla Scientific Work Pfizer hardness compressor, Remi rescarch centrifuge (C-24), Afcoset Digital balance (E-R-180A), Klenzieds Laminar air flow, and Stat fax® Autoanalyser 2000

2.1.2. Diagnostic kit

a) Calcium kit
b) Phosphorus kit
c) Alkaline phosphates kit
d) CTX kit
e) Estradiol kit

2.2 Plant Material

Glycine max (L.) Merr. seeds were collected from a local agricultural farm in Kolhapur district, Maharashtra, India, during November 2025. The collected seeds were thoroughly cleaned to remove dust and extraneous matter, washed with distilled water, and shade-dried at room temperature. The plant material was authenticated by Mr. M. N. Patil, Assistant Professor and Head, Department of Botany, Yashwantrao Chavan Warana Mahavidyalaya, Warananagar, Kolhapur. A voucher specimen (Voucher No. BW01_YC2527) was deposited in the departmental herbarium for future reference. The dried seeds were coarsely powdered using a mechanical grinder and stored in airtight containers until further use.

Figure No.2 Seed of *Glycine Max*

2.3 Preparation of Plant Extract

Using ethanol as the extraction solvent, the maceration method was used to extract 100 g of powdered Glycine max seeds. To guarantee effective phytoconstituent extraction, the powdered material was steeped in 1000 mL of ethanol and left at room temperature for 72 hours with sporadic stirring. Whatman filter paper and muslin cloth were used to filter the resultant slurry. A water bath was used to concentrate the filtrate at a lower temperature until a thick, semi-solid extract was produced. The percentage yield was computed after the concentrated extract was further dried to eliminate any remaining solvent. The dried ethanolic extract was stored in an amber-colored airtight container under refrigerated conditions until further phytochemical and pharmacological investigations.[8]

The following formula was used to compute the percentage yield:

Percentage Yield (%) = (Weight of Dried Extract / Weight of Plant Material) × 100

2.4 Qualitative Phytochemical Analysis

Standard qualitative chemical tests were used to perform a preliminary phytochemical screening of the ethanolic extract of Glycine Max in order to detect the presence of several groups of secondary metabolites.[9]

2.5 Isolation and Identification of Flavonoid Compounds by High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) analysis was used to identify the flavonoid and isoflavone contents in the ethanolic extract of Glycine max seeds. Before analysis, the dried extract was precisely weighed, diluted in methanol, sonicated for ten to fifteen minutes, and filtered through a 0.45 µm membrane filter. A Shimadzu HPLC system with a C18 reverse-phase column and a photodiode array (PDA) detector was used for the HPLC analysis. Methanol and 0.1% acetic acid (53:47, v/v) made up the mobile phase, which was supplied at an ideal flow rate. The detection was done at 254 nm. The system was injected with a 20 µL sample volume. The presence of several phytoconstituents was indicated by the chromatographic profile's significant peaks at retention periods of 3.023, 3.400, 4.075, 5.109, and 7.166 minutes. The presence of significant flavonoid and isoflavone components, including phytoestrogenic elements responsible for the osteoprotective effect of Glycine max extract, is suggested by the greater peak area percentages of the main peaks seen at retention durations of 4.075- and 5.109-min.[10,11]

Figure No.3 Shimadzu HPLC system

2.6 Experimental Animals

A CPCSEA-certified animal facility contributed the healthy adult Wistar albino female rats, which weigh between 150 and 250 g. The animals were kept in polypropylene cages in a controlled environment with a 12-hour light/dark cycle, a temperature of 25 ± 2°C, and a relative humidity of 55 ± 5%. Throughout the study, the animals were given a regular pellet meal and free access to water. Before being evaluated, the animals had a week to acclimate to the lab setting. The study's experimental strategy was examined and authorized by the Institutional Animal Ethics Committee (IAEC) of Ashokrao Mane College of Pharmacy in Peth Vadgaon, Kolhapur, in compliance with CPCSEA norms for the care of lab animals.

2.7 Experimental Design

For the study, 8–10-week-old, healthy adult female Wistar rats weighing 150–200 g were employed. Before the trial started, the animals were given free access to a regular pellet food and unlimited water while being acclimated to standard laboratory settings for one week. Bilateral ovariectomy (OVX) employing the dorsolateral surgical technique under suitable anesthesia was used to induce osteoporosis. In order to promote the development of osteoporotic alterations caused by estrogen deprivation, the animals were given two to three weeks to recover after surgery.[12]

Figure no. 4 Ovariectomy Surgery of Rat

The rats were split into four groups at random, each with six animals (n = 6). Group I did not have surgery and acted as the Normal Control. Group II served as the Ovariectomized Control (OVX) and received no treatment. Estradiol (2 mg/kg) was administered to ovariectomized rats in Group III, which was the standard treatment group. Glycine max ethanolic extract (100 mg/kg, p.o.) was administered to ovariectomized rats in Group IV. For the duration of the planned experiment, the treatments were given every day. In order to evaluate the osteoprotective activity of Glycine max extract, animals were sacrificed at the end of the study and biochemical parameters such as serum calcium, phosphate, estradiol, alkaline phosphatase, and C-terminal telopeptide of type I collagen were assessed. Additionally, bone morphology and histopathological examination were performed.[13,14]

Group	Treatment	Number of Animals
G1	Normal Control	6
G2	OVX Control	6
G3	OVX + Estradiol (2 mg/kg)	6
G4	OVX + *Glycine max* Extract (100 mg/kg)	6

2.8 Body Weight

A calibrated digital weighing balance was used to record each animal's body weight prior to the start of therapy and on a weekly basis during the trial. Changes in body weight were used to assess the physiological effects of ovariectomy and treatment.

2.9 Micro-CT Analysis

Micro-computed tomography (Micro-CT) analysis was performed on collected femur and tibia bones at the conclusion of the investigation. Bone mineral density (BMD), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) were evaluated to assess bone microarchitecture.[15,16]

2.10 Bone-Specific Alkaline Phosphatase (ALP)

At the conclusion of the experiment, blood samples were taken, and centrifugation was used to separate the serum. Bone-specific ALP levels were estimated using a commercial diagnostic kit to evaluate bone formation activity.

2.11 C-Terminal Telopeptide of Type I Collagen (CTX)

A rat-specific ELISA kit was used to measure serum CTX levels in accordance with the manufacturer's instructions. As a biochemical indicator of osteoclast activity and bone resorption, CTX was assessed.[17,18]

2.12 Serum Calcium

A common biochemical diagnostic kit was used to estimate the serum calcium concentration. The obtained values were expressed as mg/dL and used to evaluate bone mineral metabolism.

2.13 Serum Phosphate

A commercially available biochemical kit was used to measure serum phosphate levels in accordance with the manufacturer's instructions. The results were expressed as mg/dL and used to assess mineral homeostasis.

2.14 Serum Estradiol

A rat estradiol ELISA kit was used to measure the amount of estradiol in the serum. To verify estrogen deficiency and assess the estrogenic impact of treatment, the assay was carried out in accordance with the manufacturer's instructions.[19]

2.15 Bone Morphology

Following sacrifice, femur and tibia bones were carefully isolated, cleaned, and examined for gross morphological changes. To evaluate the protective impact of therapy against osteoporosis-induced bone loss, measurements of bone weight and length were made.[20]

2.16 Histopathological Examination

Samples of tibia and femur bones were decalcified, processed, embedded in paraffin wax, and fixed in 10% neutral buffered formalin. Hematoxylin and eosin (H&E)-

stained sections of 4–5 µm thickness were examined under a light microscope to assess the trabecular architecture and bone integrity histologically.[21]

2.17 Statistical Analysis

The mean ± SEM was used to express all experimental data. One-way analysis of variance (ANOVA) and a suitable post hoc test were used for statistical analysis; values of $p < 0.05$ were deemed statistically significant.[22]

3 RESULTS

3.1 Extraction

Percentage yield of *Glycine Max* seed extract.

Percentage yield (%) = (weight of semi solid component(g)/ weight of plant material used (g)) *100

Percentage yield (%) = (32 g/ 400gm) *100

Percentage yield (%) = 8 %

Table No. 1 Percentage Yield

Parameter	Value
Weight of dried seed powder used (g)	100 g
Weight of semi solid component used (g)	32 g
Percentage yield (%)	8 %

3.2 Qualitative Phytochemical Analysis

Alkaloids, flavonoids, glycosides, steroids, tannins, phenolic compounds, and saponins were among the bioactive phytoconstituents found in the ethanolic extract of Glycine Max, according to preliminary phytochemical screening.

Table 3. Qualitative Phytochemical Screening of Ethanolic Extract of *Glycine Max*

Phytochemical constituent	Result
Alkaloids	+
Flavonoids	+
Glycosides	+
Steroids	+
Tannins	+
Phenolic compounds	+
Saponins	+
Proteins	–
Carbohydrates	±

(+): Present; (−): Absent

3.3 Characterization of Isolated Flavonoids

1. High performance liquid chromatograph

Column : c18 (250 mm * 4.6 mm, 5um)

Mobile phase : Methanol: water (40:30v/v)

Flow rate : 1.0 mL/min

Injection volume : 20 uL

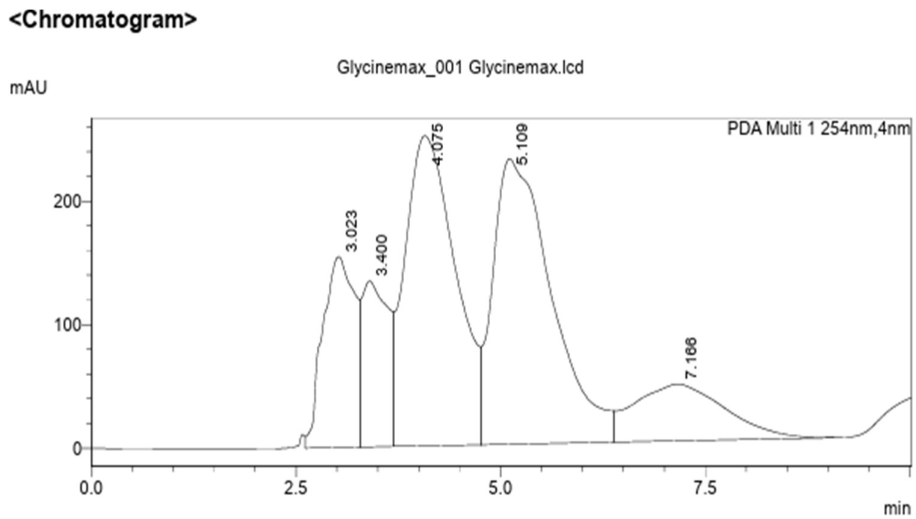
Detection : PDA at 254 nm

Column temperature : 30 c

Rum time : 15 min

Table No.2 Retention time for *Glycine Max* compound

Peak no.	Retention time (min)	Area (mAU)	Hight (mAU)	Area	Compound
1	5.109	1524876	274532	100.00	Genistein
Total		1524876	274532	100.00	-



HPLC Analysis

Five distinct peaks with differing retention times were found in the ethanolic extract of Glycine max by HPLC analysis, suggesting the presence of diverse phytoconstituents. The presence of bioactive isoflavone compounds was shown by the main peaks at 5.109 min (35.020% peak area) and 4.075 min (32.520% peak area). The chromatographic profile confirmed the presence of phytochemicals that may contribute to the osteoprotective activity of Glycine max extract.

3.4 Ovariectomized (OVX) Rat Model

A week prior to research, healthy adult female Wistar rats weighing 150–200 g and aged 8–10 weeks were acclimated to conventional laboratory settings. Osteoporosis was induced by bilateral ovariectomy under aseptic conditions and appropriate anesthesia through a dorsolateral incision, followed by careful removal of both ovaries. Following surgery, animals were given analgesics and antibiotics as part of their post-operative care, and they were given two to three weeks to recuperate. Osteoporotic alterations that closely mirror postmenopausal osteoporosis in rats occurred during this time due to increased bone resorption, decreased bone production, and degradation of trabecular bone architecture caused by estrogen insufficiency.

Figure no. 5 Recovery of Ovariectomised Rat

3.5 Effect of *Glycine Max* component on Body Weight in ovariectomized rat

Table No.3 Effect of *Glycine Max* component on Body weight in OVX rats

Group	Initial Body Weight (g) Mean ± SEM	Week 1 Mean ± SEM	Week 2 Mean ± SEM	Week 3 Mean ± SEM	Week 4 Mean ± SEM

Normal Vehicle	206.3 ± 0.8819	206.3 ± 0.8819	210.5 ± 0.7638	213.3 ± 0.8819	220.7 ± 0.7149
OVX Induced	190.2 ± 0.4773 ***	190.2 ± 0.4773 ***	185.0 ± 0.3651 ***	187.3 ± 0.4944 ***	190.8 ± 0.6009 ***
OVX + Estradiol (2mg/kg)	203.2 ± 0.7491 ***	203.2 ± 0.7491 ***	197.7 ± 0.5578 ***	198.7 ± 0.5578 ***	201.3 ± 0.4216 ***
OVX + *Glycine Max* component (100mg/ kg)	207.2 ± 0.9458 **	207.2 ± 0.9458 **	200.7 ± 0.7601 **	202.8 ± 0.6540 **	205.7 ± 0.7601 **

Over the course of the investigation, body weight assessments revealed a considerable decline in the OVX-induced group relative to the normal control group, suggesting the onset of osteoporosis. Treatment with estradiol (2 mg/kg) and Glycine max extract (100 mg/kg) significantly improved body weight compared to the OVX control group. From week two to week four, the Glycine max-treated group's body weight gradually increased, indicating its protective impact against metabolic changes brought on by ovariectomy.

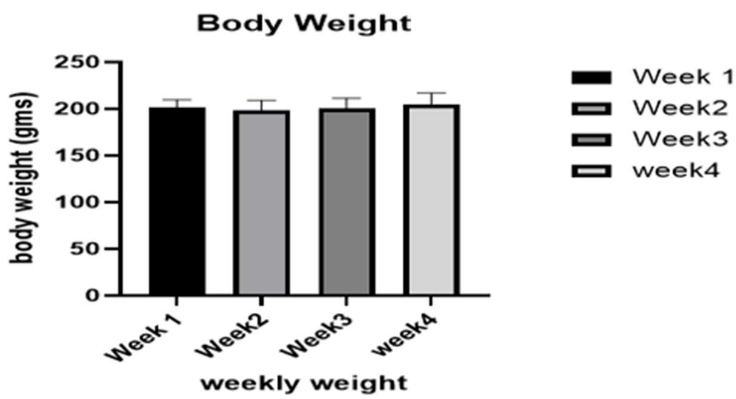
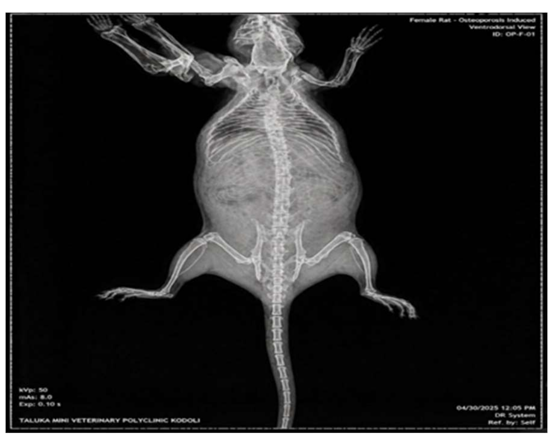


Figure No.6 Body Weight in ovariectomized rats

3.6 Effect of *Glycine Max* component on Micro CT in ovariectomized rats



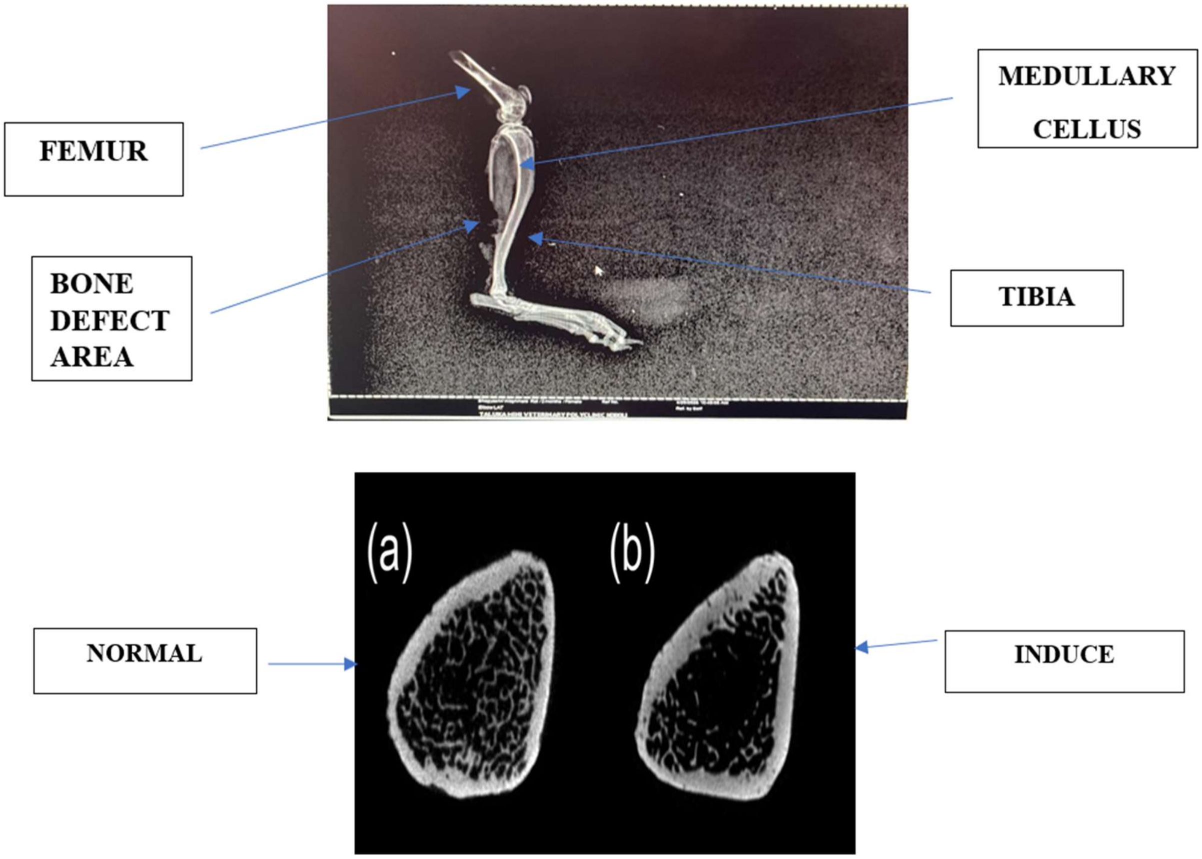


Figure No.7 Micro- CT Analysis of Ovariectomised Rats

Effect of Glycine max component on Bone Microarchitecture (Micro-CT Analysis)

The normal control group had normal trabecular architecture and bone mineral density according to micro-CT analysis, but the OVX control group showed significant osteoporotic alterations, such as decreased trabecular thickness, increased bone porosity, and decreased bone mineral density. In comparison to the OVX control group, treatment with estradiol and Glycine max extract markedly enhanced bone microarchitecture and maintained trabecular bone integrity. These results imply that Glycine max has osteoprotective properties and successfully reduces bone degradation brought on by ovariectomy.

3.7 Effect of *Glycine Max* component on Bone-specific ALP (Alkaline phosphatase) in ovariectomized rats

Table No.4 Effect of *Glycine Max* component on Bone-specific ALP in OVX rats

Group	Mean ± SEM (U/L)
Normal	104.0 ± 1.844
OVX Induced	325.5 ± 4.380***
OVX + Estradiol (2mg/kg)	184.0 ± 1.807***
OVX + *Glycine Max* component (100mg/ kg)	168.3 ± 2.171***

When compared to the normal control group, the ovariectomized control group's bone-specific ALP levels were considerably higher, suggesting accelerated bone turnover brought on by estrogen shortage. Increased osteoblastic response linked to quick bone remodeling is suggested by elevated ALP activity in OVX rats. When compared to OVX rats who were not treated, treatment with estradiol and *Glycine Max* 100 mg component decreased ALP levels. The improvement in bone metabolism and potential osteoprotective action of the therapies are shown by the decrease in ALP levels. The phytoestrogenic activity of soybean isoflavones may have contributed to the group treated with *Glycine Max*'s apparent recovery.

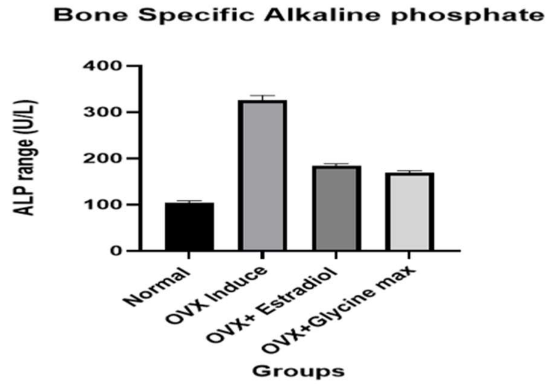


Figure No.8 Bone Specific ALP in ovariectomized rat

3.8 Effect of *Glycine Max* component on CTX (C-Terminal Telopeptide) Resorption in ovariectomized rats

Table No.5 Effect of *Glycine Max* component on CTX in ovariectomized rat.

Group	Mean ± SEM (ng/mL)
Normal	2.150 ± 0.04282
OVX Induced	6.783 ± 0.07032 ***
OVX + Estradiol (2mg/kg)	3.550 ± 0.04282 ***
OVX + *Glycine Max* component (100mg/ kg)	3.083 ± 0.04773 ***

The ovariectomized control group had significantly higher CTX levels, suggesting accelerated bone resorption brought on by an estrogen shortage. Increased CTX reflects elevated osteoclast activity and excessive breakdown of bone tissue in OVX rats. When compared to the OVX control group, estradiol treatment dramatically reduced CTX levels. In a similar vein, the Glycine Max 100 mg component similarly decreased CTX levels, indicating that bone resorption activity was inhibited. The osteoprotective activity of phytoestrogen-rich Glycine Max extract is supported by the reduction in CTX levels in treated groups.

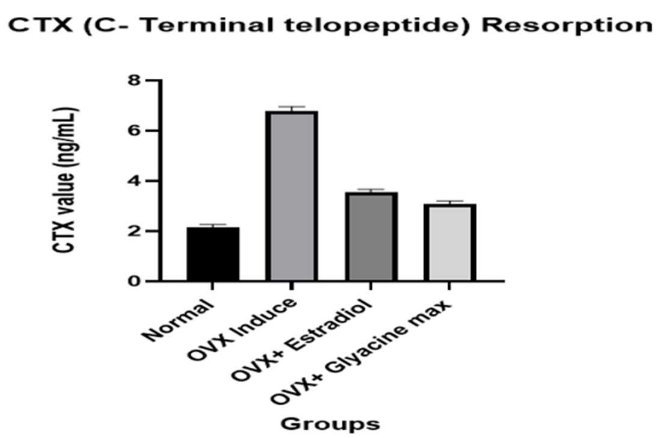


Figure No.9 C- Terminal Telopeptide Level in Ovariectomized Rats

3.9 Effect of *Glycine Max* component on Serum Calcium in ovariectomized rats.

Table No.6 Effect of *Glycine Max* component on Serum calcium in OVX rat.

Group	Mean ± SEM (mg/dL)
Normal Vehicle	10.28 ± 0.06009
OVX Induced	6.783 ± 0.07032 ***
OVX + Estradiol (2mg/kg)	9.150 ± 0.04282 ***
OVX + *Glycine Max* component (100mg/ kg)	8.500 ± 0.05774 ***

When compared to the normal control group, the serum calcium levels of ovariectomized rats were considerably lower, indicating disrupted calcium metabolism and bone mineral imbalance brought on by osteoporosis. After an ovariectomy, a lack of estrogen may change calcium homeostasis and promote bone resorption. Estradiol treatment brought serum calcium levels back to normal. When compared to OVX rats, the *Glycine Max*-treated group also displayed improved calcium levels. *Glycine Max* component may help preserve bone mineral balance and lessen osteoporotic changes, according to this improvement.

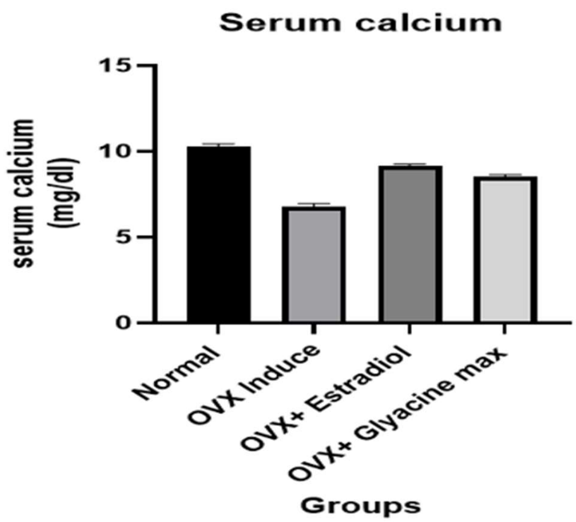


Figure No.10 Serum Calcium Level in Ovariectomized Rat

3.10 Effect of *Glycine Max* component on Serum Phosphatase in ovariectomized rats

Table No.7 Effect of *Glycine Max* component on Serum phosphatase in OVX rats.

Group	Mean ± SEM (mg/dL)
Normal Vehicle	6.283 ± 0.06009
OVX Induced	3.950 ± 0.04282 ***
OVX + Estradiol 2mg/kg	5.550 ± 0.04282 ***
OVX + *Glycine Max* component 100mg/ kg	5.000 ± 0.05774 ***

When compared to the normal control group, the ovariectomized control group's serum phosphate levels were lower, suggesting poor bone metabolism linked to estrogen shortage. Increased bone resorption and altered mineral metabolism in osteoporosis may be linked to decreased phosphate levels. In OVX rats, estrogen treatment markedly raised serum phosphate levels. When *Glycine Max* 100 mg component was administered to ovariectomized rats, their phosphate levels improved as well. The positive impact of phytoestrogen-rich soybean component in preserving normal bone mineralization is suggested by the observed result.

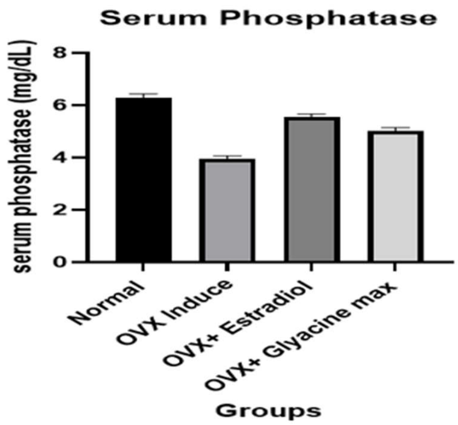


Figure No.11 Serum Phosphatase Level in Ovariectomized Rats

3.11 Effect of *Glycine Max* component on Serum Estradiol in ovariectomized rats

Table No.8 Effect of *Glycine Max* component on Serum Estradiol in OVX rats

Group	Mean ± SEM (pg/mL)
Normal Vehicle	69.00 ± 0.9661
OVX Induced	11.00 ± 0.5774 ***
OVX + Estradiol 2mg/kg	49.33 ± 0.8819 ***
OVX + *Glycine Max* component 100mg/ kg	35.50 ± 0.7638 ***

The ovariectomized control group's serum estradiol levels significantly dropped, indicating that the induction of estrogen insufficiency after ovariectomy was successful. Increased bone resorption and the advancement of osteoporosis are directly linked to lower levels of estradiol. Estradiol treatment brought hormone levels back to baseline, demonstrating successful estrogen replacement therapy. When compared to untreated OVX rats, the *Glycine Max* 100 mg treated group similarly showed improvements in serum estradiol levels. The inclusion of isoflavones, which have phytoestrogenic action and lessen bone loss linked to estrogen deprivation, may be the cause of the rise seen in the treated group.

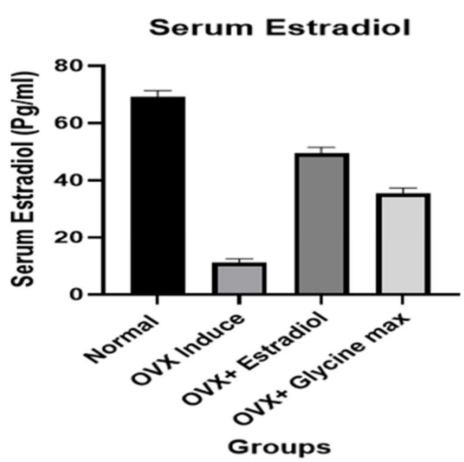


Figure No.12 Serum Estradiol Level in Ovariectomized Rats

3.12 Effect of Glycine max component on Bone Morphology

Morphological assessment of the femur and tibia revealed normal bone weight, length, thickness, and structural integrity in the normal control group, whereas the OVX control group exhibited reduced bone weight, decreased compactness, thinning of bone structure, and increased fragility, indicating osteoporosis-induced bone loss. Treatment with estradiol and *Glycine max* extract improved bone morphology by preserving bone mass, maintaining structural integrity, and reducing bone fragility when compared with the OVX control group. These findings demonstrate the osteoprotective potential of *Glycine max* in preventing ovariectomy-induced deterioration of bone quality

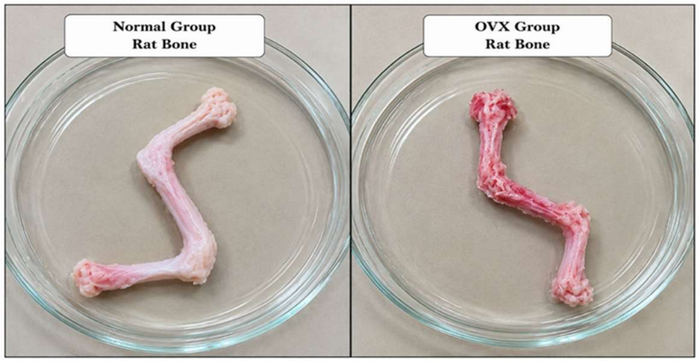


Figure No. 13 Normal group rat bone and OVX group rat bone

Figure No. 14 Estradiol treated OVX rat bone and *Glycine Max* treated OVX rat bone

3.13 Histopathological Evaluation of Femur and Tibia

Histopathological examination of femur and tibia sections revealed normal bone architecture with dense trabecular bone and intact cortical structure in the normal control group, whereas the OVX control group showed marked osteoporotic changes, including trabecular thinning, widened marrow spaces, increased bone porosity, and deterioration of bone microarchitecture. Treatment with estradiol and *Glycine max* extract significantly improved bone histology by restoring trabecular arrangement, reducing bone porosity, and preserving cortical bone integrity compared to the OVX control group. These findings indicate the osteoprotective potential of *Glycine max* against ovariectomy-induced bone loss.

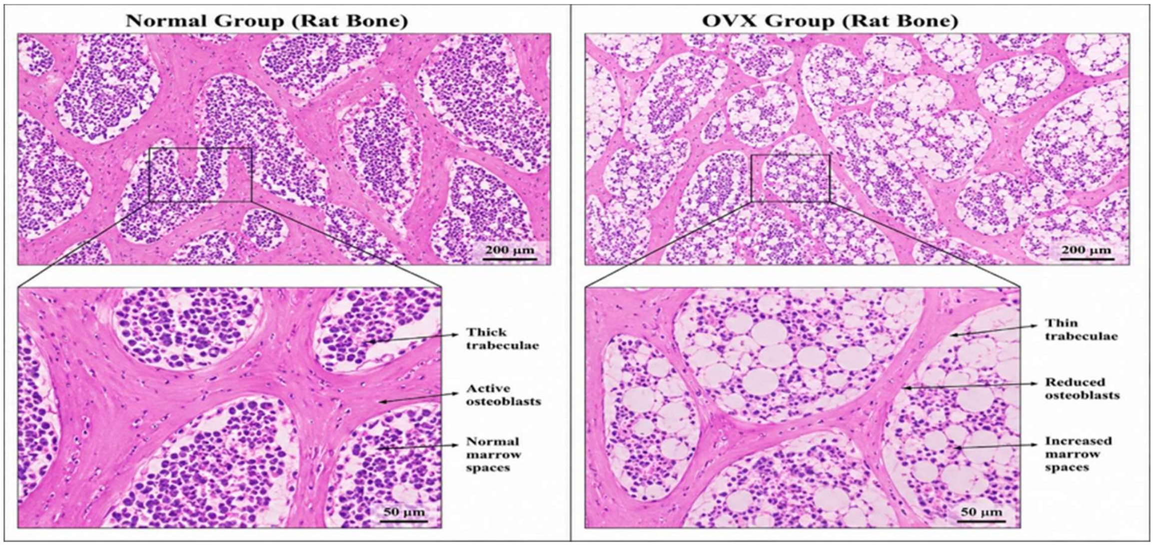


Figure No. 15 Normal group rat bone and OVX group rat bone

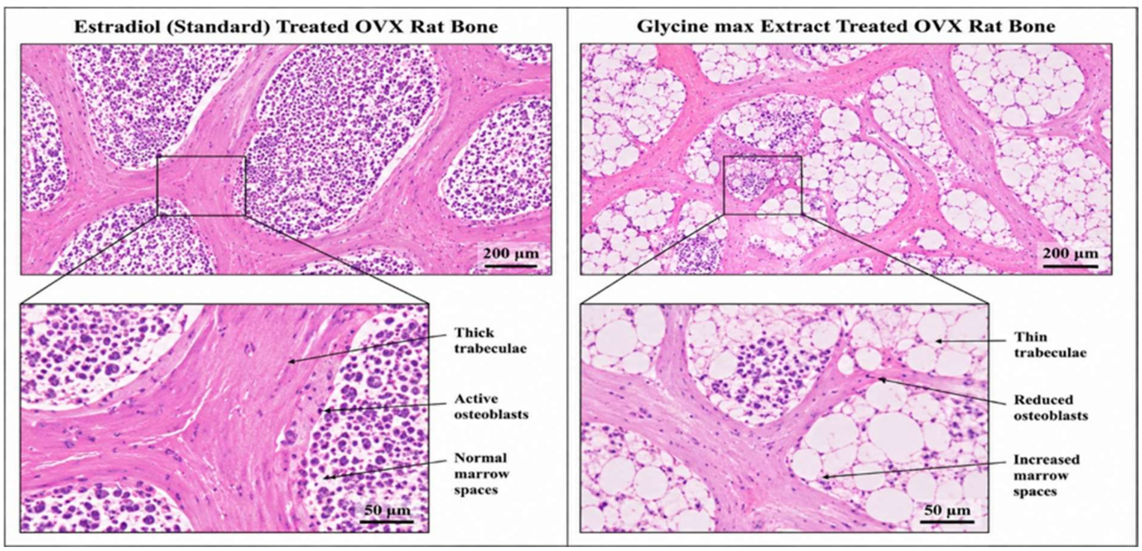


Figure No. 16 Estradiol treated OVX rat bone and *Glycine Max* treated OVX rat bone

4. DISCUSSION

Osteoporosis is a progressive skeletal disorder characterized by reduced bone mass, deterioration of bone microarchitecture, and increased susceptibility to fractures. Estrogen plays a vital role in maintaining bone homeostasis by regulating the balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption. In the present study, an ovariectomized (OVX) rat model was employed to mimic postmenopausal osteoporosis, as estrogen deficiency following ovariectomy closely resembles the pathological changes observed in postmenopausal women. The findings of the study confirmed successful induction of osteoporosis, as evidenced by alterations in body weight, biochemical markers, serum mineral levels, hormonal status, micro-CT parameters, and histopathological changes in bone tissue.[23]

Body weight assessment revealed progressive weight gain in the normal control group, whereas OVX-induced rats exhibited altered body weight patterns following estrogen deficiency. These changes may be attributed to metabolic disturbances associated with ovarian hormone depletion. Treatment with estradiol and *Glycine max* extract improved body weight profiles compared to OVX control animals, suggesting a beneficial effect on overall metabolic regulation. The observed improvement may be related to the phytoestrogenic activity of soybean isoflavones, which can partially compensate for estrogen deficiency.[24]

Biochemical evaluation demonstrated a marked elevation of bone-specific alkaline phosphatase (ALP) and C-terminal telopeptide of type I collagen (CTX) levels in OVX rats. Increased ALP levels indicate enhanced bone turnover, while elevated CTX levels reflect excessive osteoclast-mediated bone resorption and degradation of the bone matrix. These findings are consistent with the accelerated bone remodeling commonly observed in estrogen-deficient osteoporosis. Administration of estradiol and *Glycine max* extract significantly reduced ALP and CTX levels compared with the OVX group, indicating suppression of excessive bone turnover and restoration of bone remodeling balance. The reduction in bone resorption markers suggests that *Glycine max* possesses anti-osteoporotic activity comparable to standard estrogen therapy.[25]

Serum biochemical analysis further supported these observations. Ovariectomy resulted in a significant reduction in serum calcium, phosphate, and estradiol levels, indicating impaired mineral metabolism and successful induction of estrogen deficiency. Reduced mineral availability may adversely affect bone mineralization and contribute to skeletal fragility. Treatment with estradiol restored these parameters toward normal values, while *Glycine max* extract also significantly improved serum calcium, phosphate, and estradiol levels. These effects may be attributed to the presence of phytoestrogenic isoflavones, particularly genistein and daidzein, which exhibit estrogen-like activity and help maintain mineral homeostasis and bone metabolism.[26]

The micro-CT and histopathological findings provided strong structural evidence of the osteoprotective effect of *Glycine max*. OVX control rats showed substantial deterioration of bone architecture, characterized by reduced bone mineral density, decreased trabecular thickness, increased trabecular separation, enlarged marrow spaces, and increased bone porosity. Histopathological examination of femur and tibia sections revealed thinning of trabeculae, disruption of bone architecture, and loss of bone mass. In contrast, estradiol-treated and *Glycine max*-treated groups exhibited preservation of trabecular structure, improved bone density, reduced porosity, and restoration of normal bone architecture. These improvements indicate that *Glycine max* effectively protects against estrogen deficiency-induced bone loss and helps maintain skeletal integrity.[27]

Overall, the findings of the present study suggest that phytoestrogen-rich *Glycine max* extract possesses significant osteoprotective activity in ovariectomized rats. The beneficial effects observed in biochemical; hormonal, micro-CT, and histopathological parameters indicate that *Glycine max* may serve as a promising natural alternative for the prevention and management of postmenopausal osteoporosis. The osteoprotective action is likely mediated through the phytoestrogenic isoflavones genistein and daidzein, which help regulate bone remodeling, suppress excessive bone resorption, and preserve bone microarchitecture.[28]

5. CONCLUSION

The goal of the current investigation was to assess the phytoestrogen-rich *Glycine Max* 100mg extract's osteoprotective potential in rats with ovariectomy-induced osteoporosis. Osteoporosis-like changes, including estrogen deficiency, altered body weight, elevated bone turnover markers, decreased serum calcium and phosphate levels, decreased serum estradiol levels, deterioration of bone microarchitecture, and histopathological changes in the femur and tibia bones, were successfully induced by ovariectomy. These results verified that osteoporosis was successfully OVX induced in animals.[29]

When compared to ovariectomized rats, treatment with *Glycine Max* 100mg component significantly improved biochemical, hormonal, radiological, and histological markers. In micro-CT and histological investigations, the component decreased high ALP and CTX levels, enhanced blood calcium and phosphate levels, restored estradiol levels, and maintained trabecular bone architecture. The phytoestrogenic isoflavones included in *Glycine Max* 100mg extract, such as *genistein and daidzein*, which aid in lowering bone resorption and preserving bone metabolism, may be responsible for the reported osteoprotective action.

Overall, the results of this study indicate that *Glycine Max* 100mg component may be a promising natural therapeutic agent for the treatment of postmenopausal osteoporosis and has considerable anti-osteoporotic potential. To determine its long-term safety, effectiveness, and therapeutic use in humans, more thorough pharmacological and clinical research is necessary.[30]

Acknowledgement

The authors would like to thank the peer reviewers for their insightful comments which helped improve this manuscript.

Abbreviations

ALP – Alkaline Phosphatase, BMD – Bone Mineral Density, CTX – C-Terminal Telopeptide of Type I Collagen, ELISA – Enzyme-Linked Immunosorbent Assay, H&E – Hematoxylin and Eosin, HPLC – High-Performance Liquid Chromatography, IL-1 – Interleukin-1, IL-6 – Interleukin-6, Micro-CT – Micro-Computed Tomography, OVX – Ovariectomized, PDA – Photodiode Array, RANKL – Receptor Activator of Nuclear Factor Kappa-B Ligand SEM – Standard Error of Mean, Tb.N – Trabecular Number, Tb.Sp – Trabecular Separation, Tb.Th – Trabecular Thickness, TNF-α – Tumor Necrosis Factor-Alpha, BSP – Bone-Specific Protein , CPCSEA – Committee for the Purpose of Control and Supervision of Experiments on Animals, BMC – Bone Mineral Content, BSA – Bovine Serum Albumin.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Financial support and sponsorship

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Conceptualization: Miss. Bhagyashree Waghmare; Literature Search and Data Acquisition: Dr. Sanganna Burli; Writing – Original Draft: Miss. Bhagyashree Waghmare, Dr.

Sanganna Burli; Writing – Review and Editing: Miss. Bhagyashree Waghmare; Final Approval: All authors.

Summary

This study evaluated the osteoprotective effects of phytoestrogen-rich *Glycine max* extract in an ovariectomized rat model of postmenopausal osteoporosis. Treatment with *Glycine max* improved bone biochemical markers, preserved bone microarchitecture, and reduced osteoporosis-induced histopathological changes. These findings suggest that *Glycine max* may serve as a promising natural alternative for the prevention and management of postmenopausal osteoporosis.

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