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Therapeutic Potential of Hydroalcoholic Bark Extract of *Terminalia arjuna* in Dexamethasone-Induced Osteoporosis in Rats

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KEYWORDS

Osteoporosis, Dexamethasone, GIOP, Terminalia arjuna, Biochemical analysis.

ABSTRACT:

Background: Glucocorticoid-induced osteoporosis (GIOP) is a common secondary osteoporosis caused by prolonged corticosteroid therapy, leading to reduced bone mass and increased fracture risk. Long-term use of conventional therapies may cause adverse effects, highlighting the need for safer alternatives. Terminalia arjuna possesses bioactive compounds with potential osteoprotective effects.

Objectives: The present study aimed to evaluate the protective effect of hydroalcoholic T. arjuna bark extract against dexamethasone-induced osteoporosis in rats by assessing biochemical, functional, biomechanical, and histopathological parameters.

Methods: Twenty rats were randomly divided into five groups (n = 4). Group I served as normal control, while Group II received dexamethasone (7 mg/kg, i.m., 2×/week for 2 weeks) as a negative control. Group III received dexamethasone followed by alendronate (0.2 mg/kg, p.o. 3 weeks). Groups IV and V received T. arjuna extract at doses of 100 mg/kg and 500 mg/kg orally for 3 weeks. Body weight, serum calcium, phosphorus, and alkaline phosphatase (ALP) levels were estimated. Motor coordination and nociceptive responses were evaluated using rotarod and hot-plate tests, respectively. Bone weight, length, and femur histopathology were also assessed. Statistical analysis was performed using one-way ANOVA.

Results: Dexamethasone significantly altered biochemical markers, reduced body weight, impaired motor performance, and increased pain sensitivity, confirming osteoporosis induction. Treatment with T. arjuna extract produced dose-dependent improvements in biochemical parameters, functional performance, bone indices, and histological architecture, with the higher dose showing effects comparable to alendronate.

Conclusion: Hydroalcoholic T. arjuna bark extract demonstrates significant osteoprotective activity against glucocorticoid-induced osteoporosis and represents a promising natural therapeutic option.

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1. Introduction

Osteoporosis is a prevalent skeletal condition characterized by reduced bone mass and microarchitectural deterioration of bone tissue, leading to increased bone fragility and a higher risk of fractures. Osteoporosis is the most common condition impacting bones and joints globally, especially among the elderly population.[1] This illness poses a serious threat to world health, particularly for the elderly and postmenopausal women. The chance of breaking a bone over a lifetime is about the same as the risk of developing heart disease.[2] The balance between bone growth and bone degradation is controlled by the cooperation of osteoblasts and osteoclasts.[3] Osteoporosis comes in two varieties: primary and secondary. Primary osteoporosis, sometimes referred to as postmenopausal osteoporosis, is more common in women after menopause and occurs after the age of 75. The development of secondary osteoporosis can occur at any age and affects both men and women. Prolonged use of medications like glucocorticoids or long-term underlying conditions might cause secondary osteoporosis. Glucocorticoids, like dexamethasone, are strong immunosuppressive and anti-inflammatory drugs that are frequently recommended for a number of ailments. GIOP, also known as glucocorticoid-induced osteoporosis, is a disorder caused by bone loss brought on by long-term glucocorticoid medication. Moreover, intestinal calcium absorption is reduced by glucocorticoids, increasing the risk of osteoporosis.[4] Dexamethasone exposure leads to bone loss primarily by suppressing osteoblast differentiation and activity, while simultaneously promoting osteoclastogenesis and apoptosis of osteoblasts and osteocytes.[5] Back pain, humpback, height loss, and even fractures are common in adult GIO patients due to rapid, dose-dependent bone loss.[6] According to early research, osteoporosis developed in 30% of patients who took glucocorticoids for longer than six months. Certain medications, such as heparin, prednisone, methotrexate and dexamethasone can seriously harm bones over time and eventually result in bone loss. Certain endocrine and gastrointestinal disorders also raise the risk of getting osteoporosis. [7] Bone loss affects trabecular bone more frequently than cortical bone.[8] Glucocorticoids influence bone formation through two main processes: they impact the Wnt/β-catenin signaling pathway and activate PPAR-γ, which is involved in regulating metabolism. Additionally, these hormones promote bone resorption by increasing the production of RANKL and M-CSF, which are important for the activity and development of osteoclasts. At the same time, glucocorticoids decrease the production of osteoprotegerin (OPG) by bone cells like osteocytes and osteoblasts. This reduction in OPG leads to more active and numerous osteoclasts, which break down bone tissue. Additionally, there are few studies using animal models that indicate glucocorticoids affect the mineralization and morphology of osteocytes. Oxidative stress is brought on by glucocorticoids because they induce ROS to build up. ROS initiates RANK/TRAF6/RANKL signaling, which increases the expression of NFATc1, IKK/NF-κB pathway, and osteoclast-specific genes including ACP. Increased RANKL/RANK signaling and suppressed Nrf2/HO1 signaling cause bone resorption, which is a significant role in the initiation and progression of osteoporosis.[1]

Currently available anti-osteoporotic drugs such as bisphosphonates, SERMs, hormone replacement therapy, and parathyroid hormone analogues are effective but often associated with adverse effects like gastrointestinal disturbances, thromboembolic risk, and osteonecrosis on long-term use.[8] These limitations have created a growing interest in exploring safer, plant-based alternatives. *Terminalia arjuna*, rich in flavonoids and tannins, has shown antioxidant and bone-protective potential in in-vitro studies, warranting its evaluation for osteoprotective efficacy in-vivo. One of the most widely used and advantageous medicinal plants in the traditional medical system for the treatment of numerous serious illnesses is *Terminalia arjuna* (T. arjuna). It is a member of the Combretaceae family, which has about 200 species worldwide. Different triterpenoids, phenolics, flavonoids, tannin, glycosides, minerals, and trace elements include magnesium, calcium, zinc, aluminium, silica and copper can be found in T. arjuna bark. Terminic acid, arjunic acid, arjunolic acid, terminoltin, arjunin, and arjunenin are examples of triterpenoids. According to Ayurveda, *Terminalia arjuna* has long been used as a cardioprotective and cardiotonic. T. arjuna has a variety of therapeutic qualities, including antidiabetic, antioxidant, cardioprotective, antimicrobial, antiarthritic, anti-dysenteric, antidiarrheal, CNS stimulator, analgesic action, abortifacient, and diuretic in liver cirrhosis.[9] Although a few clinical and preclinical studies have reported the pharmacological activities of *Terminalia*

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arjuna, there is no literature available on osteoprotective evaluation of its bark extract in dexamethasone-induced osteoporosis rat models. Hence, in this study, the bark of *Terminalia arjuna* was selected to evaluate its potential in preventing bone loss and structural deterioration in a glucocorticoid-induced osteoporotic rat model. This approach focuses on exploring the bark's osteoprotective properties against the adverse skeletal effects commonly associated with prolonged glucocorticoid therapy.

2. Materials and Methods

2.1. Plant collection and authentication:

Terminalia arjuna bark was collected from Gummidipoondi village, Tamil Nadu, India. The plant material was authenticated by Dr. KN. Sunil Kumar and Dr. P. Elankani, Research officers, Department of Pharmacognosy at Siddha Central Research Institute, Arumbakkam, Chennai. A specimen sample of *Terminalia arjuna* bark has been allotted voucher sample reference number T28052501A.

2.2. Preparation of hydroalcoholic bark extract of T. arjuna:

The bark of T. arjuna was carefully washed with tap water. The bark was cut into small pieces and shade dried for approximately 30 days and minced before pulverizing; coarsely powdered bark was passed through a sieve of no.10 to obtain uniform particle size. The hydroalcoholic extract of Terminalia arjuna bark was prepared using the Soxhlet extraction method. Approximately 50 g of coarsely powdered bark was packed into the Soxhlet apparatus and extracted continuously with a 1:1 mixture of ethanol and distilled water for 24 hours. After extraction, the solvent was removed under reduced pressure using a rotary evaporator, and the extract was dried to a constant weight in a desiccator. The extraction yield in percentage was calculated by the formula [(W1/W2) X 100], where W1 is the net weight of extracted bark powder in gm and W2 is the total weight of bark powder in gm taken for extraction.[9]

2.3. Phytochemical analysis:

***Salkowski Test for Phytosterols**:* To 0.5 ml of the chloroform extract, 1 ml of concentrated sulfuric acid was carefully added along the sides of the test tube. The development of a reddish-brown coloration in the chloroform layer confirmed the presence of phytosterols.

Liebermann–Burchard Test for Triterpenoids: A small portion of the extract was treated with a few drops of acetic anhydride, gently heated, and allowed to cool. Concentrated sulfuric acid was then added along the test tube wall. The appearance of a brown ring at the interface and a deep red coloration indicated the presence of triterpenoids.

Foam Test for Saponins: A small quantity of extract was mixed with water and vigorously shaken. The formation of stable foam that persisted for about 10 minutes was indicative of saponins.

***Dragendorff's Test for Alkaloids**:* Each extract was dissolved in chloroform, and the solvent was evaporated. The residue was acidified with a few drops of Dragendorff's reagent (potassium bismuth iodide solution). The appearance of an orange-red precipitate confirmed the presence of alkaloids.

Molisch's Test for Carbohydrates: The extract was treated with Molisch's reagent, followed by the careful addition of concentrated sulfuric acid along the sides of the test tube to form layers. The formation of a reddish-violet ring at the interface indicated the presence of carbohydrates.

Lead Acetate Test for Flavonoids: To the alcoholic extract, a few drops of 10% lead acetate solution were added. The formation of a yellow precipitate confirmed the presence of flavonoids.

Legal's Test for Lactones: The extract was mixed with sodium nitroprusside and pyridine, followed by the addition of sodium hydroxide. A deep red coloration signified the presence of lactones.

***Ferric Chloride Test for Phenolic Compounds and Tannins**:* Two millilitres of the extract were treated with a few drops of ferric chloride solution. The appearance of a bluish-black coloration indicated the presence of phenolic compounds and tannins.

Ninhydrin Test for Proteins and Amino Acids: A few drops of ninhydrin solution were added to the extract and heated gently. The development of a blue coloration confirmed the presence of amino acids, while proteins may occasionally yield a weak positive result.

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Keller–Killiani Test for Cardiac Glycosides: To the extract, 1 mL of glacial acetic acid containing a trace amount of ferric chloride was added, followed by careful addition of concentrated sulfuric acid along the sides of the tube. The formation of a reddish-brown ring at the interface confirmed the presence of deoxysugars, indicating cardiac glycosides.[10]

2.4. Experimental animals:

Sprague-Dawley rats (6-8 weeks old, female) were obtained from the Mass Biotech Chennai, Tamil Nadu. Experimental procedures and protocol were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) CCSEA/IAEC approval no: 185/KKCP/2024. Experiments were performed following the committee for the control and supervision of experiments on animals (CCSEA) guidelines for laboratory animal facility, the gazette of India, 2018. Animals were housed under standard laboratory conditions, air conditioned with adequate fresh air supply (air changes 12-15 per hour), room temperature of 22 ± 3°C and relative humidity of 30-70 %, with 12 hours' light and 12 hours' dark cycle was maintained in the experimental room. A group of three animals was housed in standard polycarbonate cages with stainless steel mesh tops having facilities for holding pelleted feed and drinking water. Clean sterilized corn cob was provided as bedding material. Animals were fed with laboratory animal feed throughout the experimental period. Animals were provided with ad libitum filtered drinking water in polypropylene bottles.[11]

2.5. Induction of Osteoporosis:

Osteoporosis was experimentally induced using dexamethasone, a glucocorticoid, administered intramuscularly at a dose of 7 mg/kg body weight, two times per week for two consecutive weeks, to all groups except Group I, which served as the normal control. The development of osteoporosis was assessed through behavioral evaluations of motor coordination using the rota-rod test and nociceptive response using Eddy's hot plate method. Biochemical assessment of bone loss was carried out by estimating serum calcium, phosphorus, and alkaline phosphatase levels.[4]

2.6. Experimental design:

Twenty healthy female Sprague Dawley rats were weighed and grouped into five groups (n=4). Group I was designated as the control group and did not receive any treatment. Group II was treated with dexamethasone 7mg/kg in the intramuscular route twice a week for 2 weeks. Group III kept as standard control group was treated with dexamethasone 7mg/kg twice a week for 2 weeks intramuscularly and standard drug alendronate 0.2 mg/kg p.o daily for another 3 weeks. Group IV and Group V were considered as treatment groups (low dose and high dose) that received dexamethasone 7mg/kg twice a week for 2 weeks intramuscularly and T. arjuna bark extract at a dose of 100 mg/kg and 500 mg/kg orally from 3rd week to 5th week. The induction period was for first 2 weeks and the treatment period was for next 3 weeks.

2.7. Evaluation parameters:

2.7.1. Body weight:

Body weight was measured on the first day of the study (baseline), and subsequently every week until the end of the 5-week study period using a digital weighing balance. Changes in body weight were analyzed and compared between the initial measurement (week 0), mid-study (week 2, following dexamethasone induction), and the end of the study (week 5).

2.7.2. Behavioral Assessments

Motor coordination and pain sensitivity were evaluated at the end of the 2nd week to assess dexamethasone-induced impairments and again at the 5th week to determine treatment-related improvements.

Rota Rod Test: Motor coordination and balance were assessed using the rota rod apparatus. Prior to testing, rats were acclimatized for 2–3 consecutive days by placing them on the rotating rod at a low speed to minimize stress and ensure familiarity. On the test day, each rat was placed individually on the rod rotating at a constant speed of 16–20 rpm, and the time spent without falling (fall latency) was recorded, with a cut-off time of 180 seconds. Each animal underwent three trials with a 10–15-minute rest interval, and the mean latency time was used for analysis. A reduction in latency indicated motor impairment, whereas an increase following treatment reflected improvement. The apparatus was cleaned with 70% ethanol after each trial to avoid olfactory interference.[12]

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Hot Plate Test: Thermal pain sensitivity was measured using the hot plate method. Each rat was placed individually on the plate maintained at 50 ± 0.5 °C, and the latency to the first nociceptive response (hind paw licking, shaking, or jumping) was recorded. A cut-off time of 10–15 seconds was applied to prevent tissue damage. Three trials were conducted per animal with 10–15 minutes of rest between trials, and the mean latency was used for analysis. A decreased response latency indicated increased pain sensitivity, while an increase after treatment suggested recovery. The apparatus surface was cleaned with 70% ethanol between trials to remove residual odor cues.[13]

2.7.3. Biochemical analysis:

At the end of the 2nd week, blood samples were collected from all experimental groups via retro-orbital puncture under light ketamine–xylazine anaesthesia. Final blood collection was performed at the end of the 5th week by cardiac puncture under deep isoflurane anaesthesia. Samples were collected in clot activator tubes, transferred to microcentrifuge (Eppendorf) tubes, and allowed to clot at room temperature for 10–15 minutes. The clotted samples were centrifuged at 3000 rpm for 15 minutes to separate the serum, which was subsequently used for the estimation of serum calcium, phosphorus, and alkaline phosphatase (ALP) levels using standard biochemical methods.[4,6]

2.7.4. Bone Collection and Morphometric Analysis

Following euthanasia by isoflurane overdose, each animal was positioned on a dissection board, and the femur was carefully isolated by exposing the hip joint through gentle incision and retraction. After dislocation, the femoral bone was cleaned of adhering soft tissues and preserved in 10% neutral buffered formalin for further analysis. Morphometric parameters, including bone weight and bone length, were recorded. Bone weight was measured using a calibrated digital balance, and bone length was determined with a standard measuring scale.[6,7,8]

2.8. Histopathological Examination

Femoral bones were fixed in 10% neutral buffered formalin for at least 48 hours, followed by decalcification in 5% formic acid. The tissues were then processed through graded alcohols and xylene, embedded in paraffin wax, and sectioned longitudinally at a thickness of approximately 5 µm using a rotary microtome. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for histopathological alterations. [6,7,8]

2.9. Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Dunnett's and Sidak's multiple comparison tests, employing GraphPad Prism software (version 10.6.1). One-way ANOVA was applied to evaluate differences among groups. A p-value of <0.05 was considered statistically significant, with additional thresholds reported as $P<0.01$, $P<0.001$, and $P<0.0001$ where applicable.

3. Results:

3.1. Extraction yield: The extractive value of hydroalcoholic extract of T. arjuna was found to be 16.4% w/w

3.2. Phytochemical analysis:

A preliminary phytochemical evaluation was conducted to identify the chemical constituents present in the hydroalcoholic bark extract of T. arjuna. The screening indicated the presence of phytosterols, triterpenoids, saponins, alkaloids, carbohydrates, flavonoids, lactones, proteins, glycosides, phenolic compound and tannin.

3.3. Body Weight Changes

A progressive increase in body weight was observed in the control rats from Week 0 to Week 5, indicating normal physiological growth during the experimental period. In contrast, the negative control group exhibited a gradual reduction in body weight following dexamethasone induction. The positive control, low-dose, and high-dose treatment groups showed an initial decrease in body weight during Weeks 1–2, followed by gradual recovery and improvement during the treatment phase. However, these intergroup variations were not statistically significant. ***(Table 1: Changes in body weight across different groups over the study duration) (Figure 1: Body weight variations among experimental groups during the study duration)***

3.3. Behavioral assessments:

Rotarod Test: At Week 2, all dexamethasone-treated groups showed a significant reduction in fall latency

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compared with the control group (P < 0.0001), confirming impaired motor coordination due to osteoporosis induction. By Week 5, both *Terminalia arjuna*-treated groups exhibited improved performance, with the high-dose group (500 mg/kg) showing results comparable to the standard treatment. Significant recovery was observed in the standard drug group (P < 0.0001 vs. negative control), whereas the negative control group continued to display reduced motor function. ***(Table 2: Motor activity assessment using Rotarod) (Figure 2: Evaluation of motor performance at Week 2 and 5 using the rotarod test)***

Hot plate test: At Week 2, all dexamethasone-treated groups exhibited a reduction in response latency compared with the normal control, indicating increased pain sensitivity following osteoporosis induction. A significant difference was observed only in the high-dose *Terminalia arjuna*-treated group (P < 0.05 vs. control). By Week 5, the disease control group showed a marked decrease in response latency relative to the normal control (P < 0.0001), confirming persistent hyperalgesia. In contrast, the standard drug and *T. arjuna*-treated groups demonstrated significant improvement compared with the negative control (P < 0.001 for positive control; P < 0.05 and P < 0.01 for low- and high-dose groups, respectively). The high-dose (500 mg/kg) group produced a greater latency increase than the low-dose group, suggesting a dose-dependent analgesic effect, with the standard treatment showing the highest efficacy. ***(Table 3: Pain sensitivity assessment using hotplate test) (Figure 3: Evaluation of pain sensitivity at Week 2 and 5 using the hotplate test.)***

3.4. Biochemical Analysis

At the end of Weeks 2 and 5, blood samples were collected from all experimental groups to assess serum biochemical parameters, including calcium, phosphorus, and alkaline phosphatase (ALP) levels. The results are summarized in the corresponding tables and figures**.**

(Table 4: Effect of T. arjuna bark extract on serum levels of calcium, phosphorous and ALP on 2nd week)

(Table 5: Effect of T. arjuna bark extract on serum levels of calcium, phosphorous and ALP on 5th week)

(Figure 4: Graphical representation of serum calcium, phosphorous and ALP analysis on week 5)

(Figure 5: Graphical representation of serum calcium, phosphorous and ALP analysis on week 2)

Serum Calcium: At Week 2 (post-induction), a significant decline in serum calcium was observed in the negative control group compared with the normal control (P < 0.001), confirming successful osteoporosis induction. The positive control and both *Terminalia arjuna*-treated groups (100 and 500 mg/kg) also showed significant reductions (P < 0.001 vs. control) during this phase. By Week 5 (post-treatment), serum calcium levels in the negative control group remained significantly reduced (P < 0.001 vs. control). In contrast, the positive control group exhibited a marked increase (P < 0.001 vs. negative control). Both *T. arjuna*-treated groups demonstrated significant improvement (P < 0.05 and P < 0.001 for low- and high-dose groups, respectively), with the high dose showing a more pronounced effect.

Serum Phosphorus: At Week 2 (post-induction), the negative control group showed a pronounced decrease in serum phosphorus (P < 0.0001 vs. control), reflecting disturbed mineral balance following glucocorticoid induction. The positive control and both *T. arjuna*-treated groups also exhibited significant reductions (P < 0.001 vs. control). By Week 5 (post-treatment), the negative control group maintained significantly low phosphorus levels (P < 0.0001 vs. control). The positive control group showed marked recovery (P < 0.001 vs. negative control), while the low- and high-dose *T. arjuna*-treated groups displayed significant improvements (P < 0.01 and P < 0.001, respectively), indicating effective restoration of phosphorus homeostasis.

Serum Alkaline Phosphatase (ALP): At Week 2 (post-induction), serum ALP activity was significantly elevated in the negative control group (P < 0.05 vs. control), indicating increased bone turnover associated with glucocorticoid-induced osteoporosis. A similar elevation was observed in the high-dose group (P < 0.01 vs. control), while other groups showed moderate increases. By Week 5 (post-treatment), the negative control group continued to exhibit markedly elevated ALP levels (P < 0.001 vs. control). In contrast, the positive control group showed a significant reduction (P < 0.001 vs. negative control). Both *T. arjuna*-treated groups displayed notable decreases in ALP activity (P < 0.01 for both vs. negative control), suggesting

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normalization of bone metabolic activity following treatment.

3.5. Biomechanical Analysis

At the end of the study period, all animals were sacrificed, and femur bones were isolated for the evaluation of biomechanical parameters, including bone weight and bone length. The corresponding results are summarized in the respective table and figure. ***(Table 6: Effect of T. arjuna bark extract on biomechanical analysis) (Figure 6: Graphical representation of biomechanical analysis)***

Bone Weight: A significant reduction in bone weight was observed in the negative control group, indicating bone deterioration following dexamethasone induction. The standard drug group demonstrated a highly significant increase ($P < 0.0001$ vs. negative control). Similarly, *T. arjuna* at 100 mg/kg produced a significant improvement ($P < 0.01$), while the 500 mg/kg group showed a highly significant recovery in bone weight ($P < 0.0001$ vs. negative control), reflecting substantial restoration of bone mass.

Bone Length: The negative control group exhibited a significant decrease in bone length compared with the normal control. Treatment with the standard drug resulted in a significant increase ($P < 0.01$ vs. negative control). The low-dose *T. arjuna* group showed a mild, non-significant improvement, whereas the high-dose group produced a significant increase in bone length ($P < 0.05$ vs. negative control). These findings indicate that *T. arjuna* extract mitigates bone loss and supports bone growth, particularly at higher doses.

3.6. Histopathological Examination

Histological analysis of femur sections showed normal cortical structure, intact cartilage, and healthy marrow in the control group **(Figure 7a).** The negative control group exhibited irregular lamellar bone, cartilage erosion, and fatty marrow infiltration, confirming osteonecrotic changes **(Figure 7b).** The positive control group showed mild lamellar degeneration and limited fatty infiltration, indicating partial recovery (**Figure 7c).** The low-dose Terminalia arjuna group demonstrated mild degeneration and minimal fatty infiltration within the marrow **(Figure 7d),** while the high-dose group exhibited nearly normal lamellar organization with minimal necrosis and limited cartilage erosion **(Figure 7e).** Both *Terminalia arjuna*-treated groups displayed improved bone architecture, with the high-dose (500 mg/kg) group showing near-normal histology. These findings suggest a dose-dependent osteoprotective and restorative effect of *T. arjuna* against glucocorticoid-induced bone damage. ***(Figure 7a, b, c, d, e: Histopathological examination of femur bone sections stained with H&E showing structural variations among groups at 10× magnifications)***

4. Discussion

Body weight is a reliable indicator of general health and metabolic stability during pharmacological studies. In the present investigation, normal control rats exhibited a steady increase in body weight throughout the experimental period, indicating normal physiological growth. Conversely, dexamethasone administration caused a progressive reduction in body weight in the negative control group, reflecting glucocorticoid-induced muscle wasting and metabolic imbalance. Treatment with *Terminalia arjuna* bark extract at both 100 and 500 mg/kg, as well as sodium alendronate (0.2 mg/kg), promoted gradual recovery in body weight during the treatment phase. Although these changes were not statistically significant, the overall trend suggested a protective influence of *T. arjuna* against steroid-induced catabolic effects. Serum calcium and phosphorus are key indicators of bone mineral metabolism. A marked reduction in both parameters was observed in the negative control group after dexamethasone induction, confirming the establishment of osteoporosis. Post-treatment, sodium alendronate significantly restored these mineral levels ($P < 0.001$ vs. negative control). Similarly, *T. arjuna* bark extract produced dose-dependent improvement, with the high-dose group showing a more pronounced effect ($P < 0.001$). These outcomes suggest that *T. arjuna* enhances mineral retention, likely through stimulation of osteoblastic activity and inhibition of bone resorption. Serum alkaline phosphatase (ALP), a marker of bone turnover, was elevated in the negative control group ($P < 0.05$–0.001 vs. control), reflecting increased bone resorption and metabolic disturbance. Treatment with *T. arjuna* bark extract and sodium alendronate significantly reduced ALP activity ($P < 0.01$–0.001 vs. negative control), indicating normalization of bone metabolism. This suggests that *T. arjuna* supports balanced bone remodeling by maintaining equilibrium between

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osteoblastic and osteoclastic functions. Dexamethasone-induced osteoporosis also resulted in significant reductions in bone weight and bone length compared with normal controls, confirming bone loss. Sodium alendronate (0.2 mg/kg) markedly improved both parameters ($P < 0.0001$ and $P < 0.01$ vs. negative control). Likewise, *T. arjuna* bark extract enhanced bone weight significantly at both doses and increased bone length notably at the higher dose ($P < 0.05$ vs. negative control). These findings indicate restoration of bone mass and structural integrity, possibly due to the presence of flavonoids, tannins, and triterpenoids known for their antioxidant and osteoprotective activities. Functional performance further supported the biochemical outcomes. In the Rotarod test, dexamethasone-treated rats exhibited reduced fall latency, indicating impaired motor coordination associated with bone weakness. Treatment with *T. arjuna* extract significantly improved fall latency, particularly at 500 mg/kg, with results comparable to the standard drug, suggesting improved neuromuscular coordination. In Eddy's hot plate test, the negative control group demonstrated decreased response latency (hyperalgesia), reflecting increased pain sensitivity. Treatment with *T. arjuna* significantly increased latency times, indicating its potential analgesic effect, likely due to improved skeletal integrity and attenuation of inflammation. Histopathological findings corroborated these biochemical and functional improvements. The negative control group showed severe bone and cartilage degeneration, necrosis, and fatty marrow infiltration characteristic of glucocorticoid-induced osteonecrosis. Conversely, treatment with *T. arjuna*, especially at the higher dose, markedly reduced necrotic and fatty changes, preserving normal lamellar organization and cartilage structure. The high-dose group displayed histoarchitecture comparable to that of the standard drug group, confirming a strong osteoprotective and restorative effect. Collectively, biochemical, biomechanical, histological, and functional assessments demonstrate that the hydroalcoholic bark extract of *Terminalia arjuna* exerts a potent osteoprotective effect comparable to sodium alendronate. The osteoprotective action of *T. arjuna* may be attributed to its rich phytochemical profile, including flavonoids, tannins, and triterpenoids, which possess antioxidant, anti-inflammatory, and osteogenic properties. Therefore, *Terminalia arjuna* bark extract holds promise as a natural therapeutic agent for the prevention and management of glucocorticoid-induced osteoporosis, meriting further molecular and clinical investigation.

5. Conclusion

The present study demonstrates that the hydroalcoholic bark extract of *Terminalia arjuna* exerts significant protective effects against steroid-induced osteoporosis, as evidenced by improvements in serum biochemical markers, biomechanical parameters, motor activity, pain sensitivity, and bone histoarchitecture. While these findings confirm the osteoprotective potential of *T. arjuna*, the precise molecular mechanisms underlying its effects on bone mass, quality, and long-term maintenance remain unclear. Further investigations are warranted to elucidate the specific actions of the bioactive phytoconstituents and to explore the therapeutic potential of *T. arjuna* in the management of osteoporosis.

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7. Conflict of interest: No conflict of interest

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Table:1 Changes in body weight

Group (n=4)	Baseline	Body weight during induction		Body weight during treatment		
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Group I Control	139.25±10.27	150.5±15.58	156.25±12.25	163±9.41 *	167.25±8.13 **	172.75±6.94 **
Group II Negative control	162±13.58	146.75±20.02	141.75±22.88	137.25±23.38	134±21.18	131±20.54
Group III Positive control	172.5±19.26	159±20.60	153.25±17.80	169.75±18.33	168.75±22.85	180.5±18.04
Group IV Low dose	149.75±8.73	144.25±12.09	141.5±15.37	153.5±13.96	153.25±17.15	157±13.36
Group V High dose	161.5±12.44	149.5±14.47	147.25±7.84	157.25±15.90	165.75±17.05	169.25±19.05

Legends: Changes in body weight of experimental animals across different groups over the study duration

Table 2: Motor activity assessment using Rotarod

Animal Group	Fall latency (Week 2)	Fall latency (Week 5)
Group I Control	**158.25±10.90**	**160.25±11.38** ####
Group II Negative control	**93.75±5.05** ####	**71.5±3.41**
Group III Positive control	**97.75±3.5** ####	**153±5.29** ****

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Group IV Low dose	**95.75±3.5 ####**	**136±3.65 ******
Group V High dose	**96.75±3.5 ####**	**152±2.94 ******

Analyzed by One-way analysis of variance (ANOVA) followed by Dunnett's & Sidak's multiple comparison test. #### P < 0.0001 compared with Control; ****P < 0.0001 compared with Negative control

Table 3: Pain sensitivity assessment using Hot Plate test

Animal Group	Response latency (Week 2)	Response latency (Week 5)
Group I Control	**9±1.82**	**9.5±1.29 ####**
Group II Negative control	**7.5±1.29**	**5±0.81**
Group III Positive control	**7±2.58**	**8.5±0.57 *** **
Group IV Low dose	**6.5±2.38**	**7±0.81 ***
Group V High dose	**4.25±2.21 #**	**7.5±0.57 ** **

Analyzed by One-way analysis of variance (ANOVA) followed by Dunnett's & Sidak's multiple comparison test. #### P < 0.0001, # P < 0.05 compared with Control; ***P < 0.001, ** P < 0.01, * P<0.05 compared with Negative control

Table 4: Effect of T. arjuna bark extract on serum levels of calcium, phosphorous and ALP on 2nd week

Animal Group	Serum calcium mg/dl	Serum phosphorous mg/dl	Serum Alkaline phosphatase IU/L
Group I Control	**11.4±2.21**	**10.66±1.34**	**134.83±33.36**
Group II Negative control	**5.53±0.87 ###**	**4.4±1.15 ####**	**347.33±90.52 #**
Group III Positive control	**5.4±0.5 ###**	**5.06±0.75 ###**	**254.66±8.14**
Group IV Low dose	**5.46±0.83 ###**	**4.76±0.47 ###**	**278.33±66.15**
Group V High dose	**4.63±1.05 ###**	**5.7±1.30 ###**	**367.66±109.69 ##**

Analyzed by One-way analysis of variance (ANOVA) followed by Dunnett's & Sidak's multiple comparison test. #### P < 0.0001, ### P<0.001, ## P<0.01, # P<0.05 compared with control.

Table 5: Effect of T. arjuna bark extract on serum levels of calcium, phosphorous and ALP on 5th week.

Animal Group	Serum calcium mg/dl	Serum phosphorous mg/dl	Serum Alkaline phosphatase IU/L
Group I Control	**11.93±1.54 ###**	**11.83±1.43 ####**	**139.33±31.89 ###**
Group II Negative control	**5.13±0.50**	**4.33±1.05**	**399.5±113.92**
Group III Positive control	**13.46±1.95 *** **	**11.6±0.75 *** **	**143±32.18 *** **
Group IV Low dose	**9.86±1.35 ***	**9.68±1.53 ** **	**174.66±17.24 ** **

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Group V High dose	**13.03±1.68** ***	**11.11±1.17** ***	**157.66±23.71** **

Analyzed by One-way analysis of variance (ANOVA) followed by Dunnett's & Sidak's multiple comparison test. #### P < 0.0001, ### P<0.001 compared with Control; ***P < 0.001, ** P<0.01, * P<0.05 compared with Negative control.

Table 6: Effect of T. arjuna bark extract on biomechanical analysis

Animal Group	Bone weight (gm)	Bone length (cm)
Group I Control	**0.96±0.01** ####	**3.23±0.20** ###
Group II Negative control	**0.63±0.03**	**2.36±0.30**
Group III Positive control	**0.83±0.03** ****	**3.03±0.15** **
Group IV Low dose	**0.74±0.04** **	**2.80±0.10**
Group V High dose	**0.82±0.02** ****	**2.90±0.10** *

Analyzed by One-way analysis of variance (ANOVA) followed by Dunnett's & Sidak's multiple comparison test. #### P < 0.0001, ### P<0.001 compared with Control; ****P < 0.0001, ** P<0.01, * P<0.05 compared with Negative control

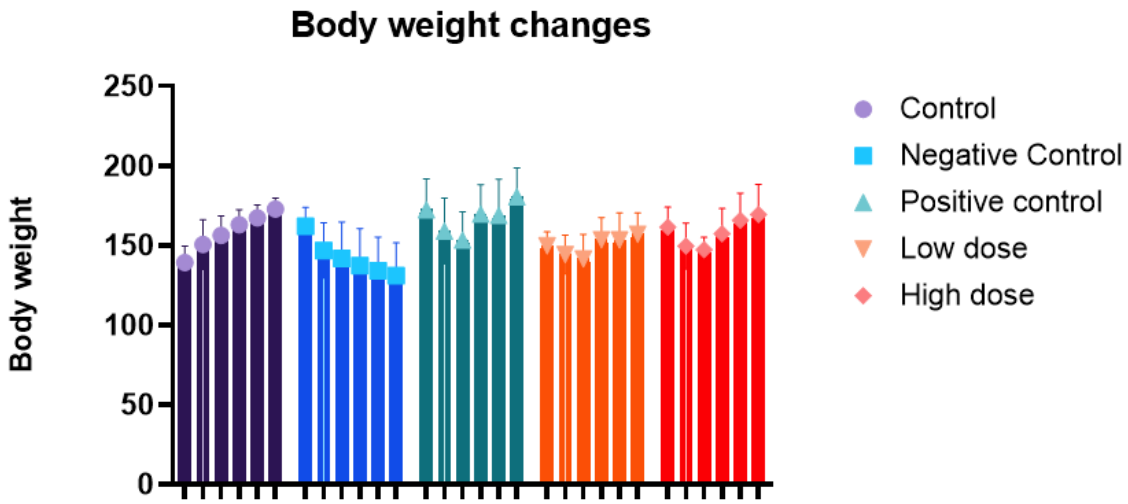

Figure 1: Body weight variations among experimental groups during the study duration

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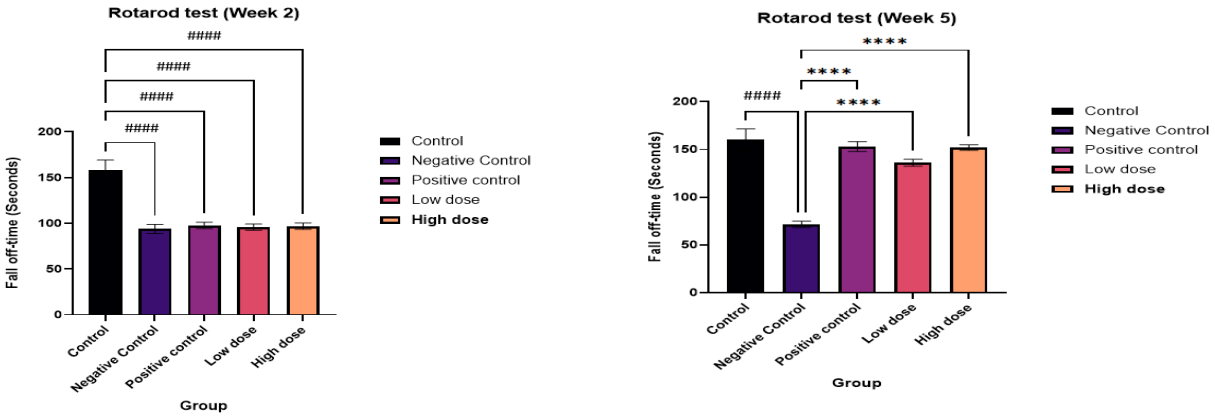

Figure 2: Evaluation of motor performance at Week 2 and 5 using the rotarod test

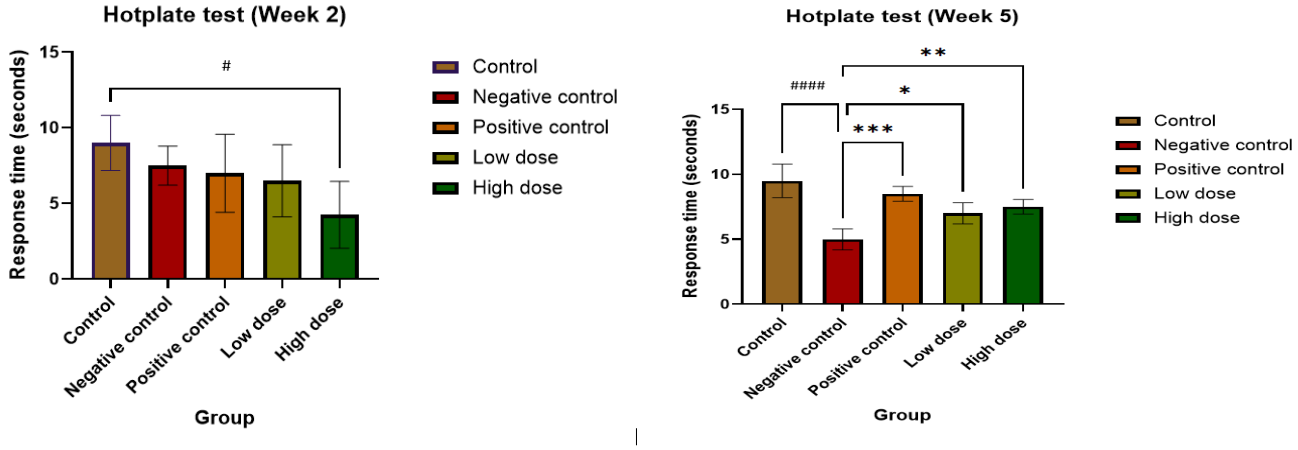

Figure 3: Evaluation of pain sensitivity at Week 2 and 5 using the hotplate test.

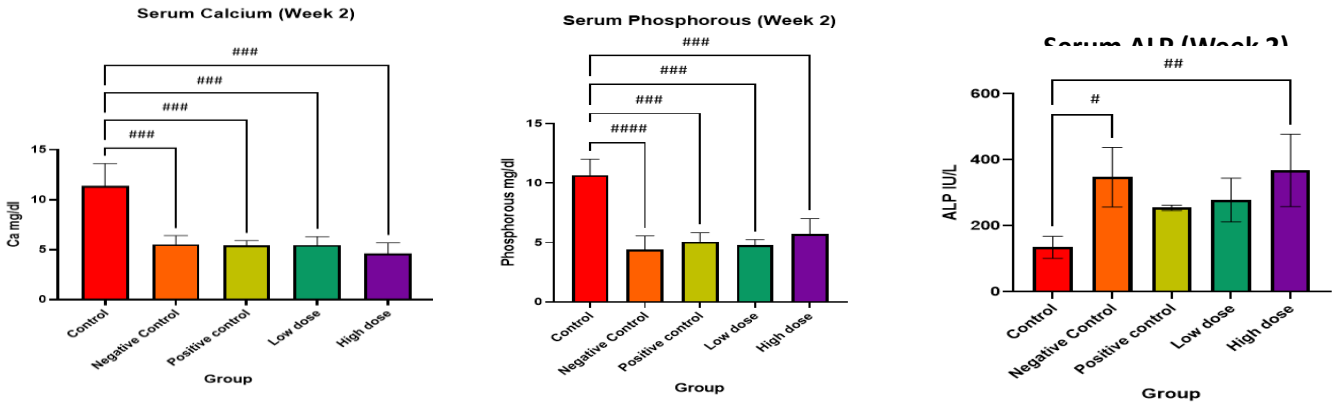

Figure 4: Graphical representation of serum calcium, phosphorous and ALP analysis on week 2.

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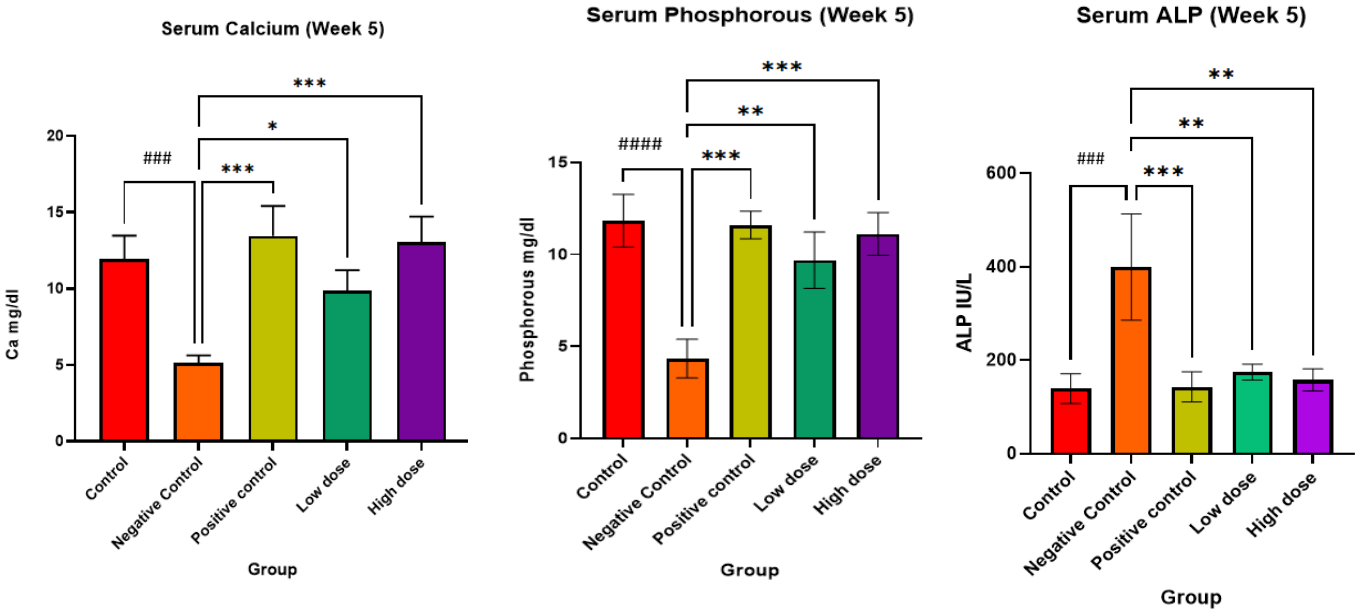

Figure 5: Graphical representation of serum calcium, phosphorous and ALP analysis on week 5.

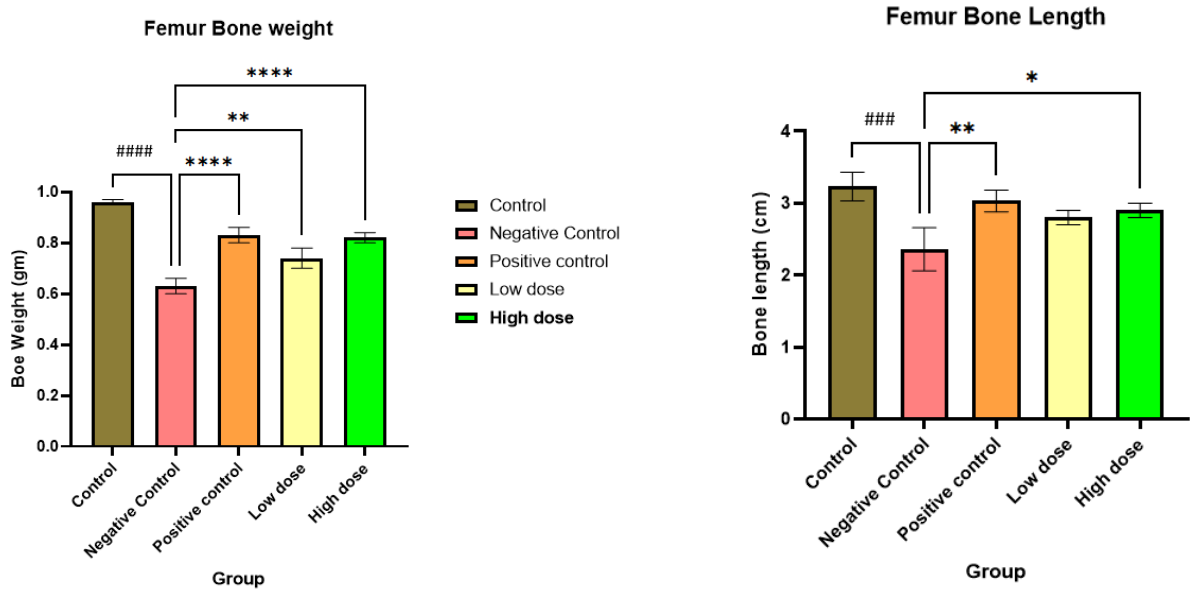

Figure 6: Graphical representation of biomechanical analysis (Bone weight and length)

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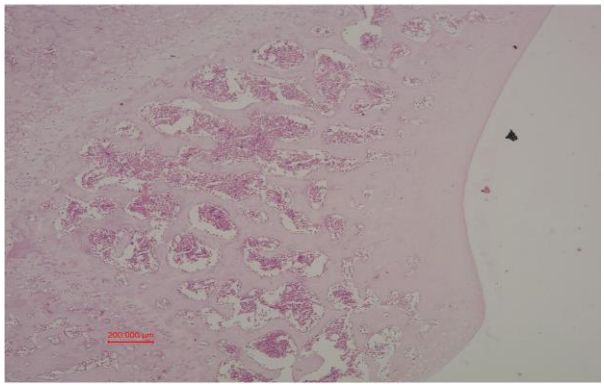

Figure 7a) Group 1 Control

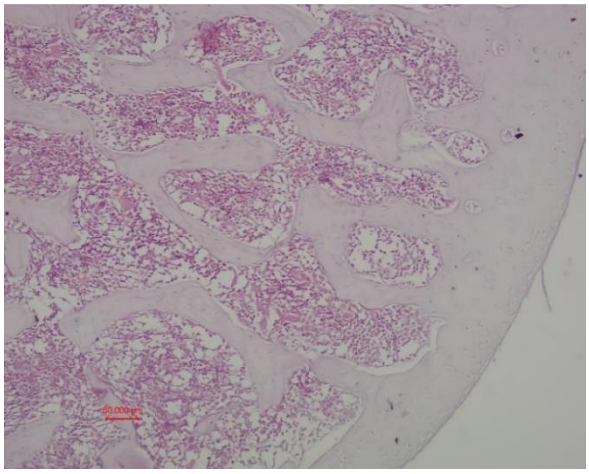

Figure 7b) Negative control

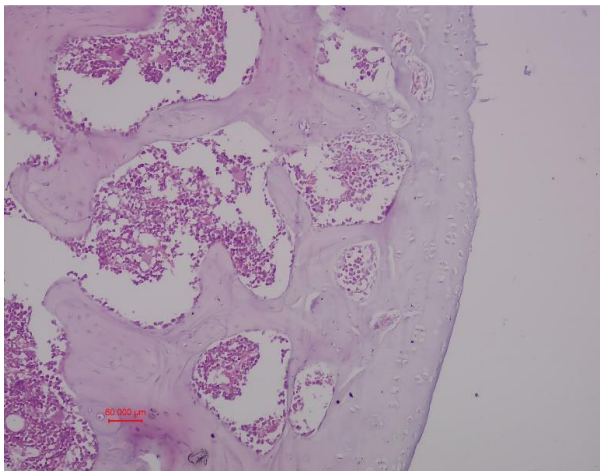

Figure 7c) Positive control

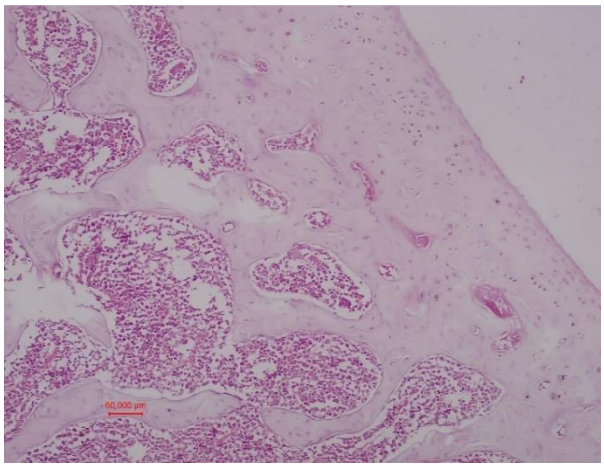

Figure 7d) Low dose

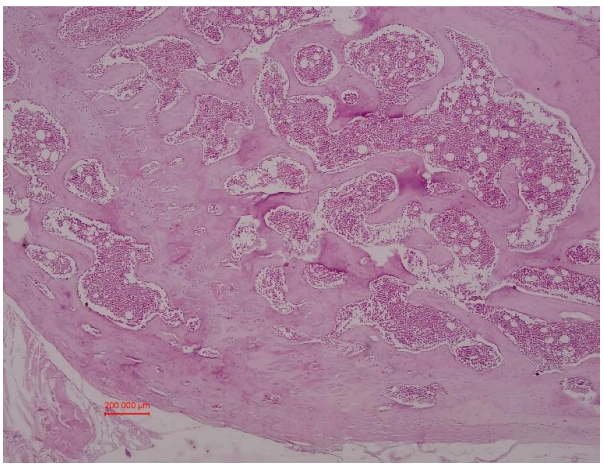

Figure 7e) High dose

Figure 7a, b, c, d, e: Histopathological examination of femur bone sections stained with H&E showing structural variations among groups at 10× magnifications.