IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2024 Vol.:30, Issue:5

Evaluation of Anti-Osteoporotic Activity of *Solanum torvum* Fruits Using Glucocorticoid Induced Osteoporosis in Wistar Albino Rats

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Mythili Priya M[1]*, Indumathy R[2], Karthickeyan G[3]

[1]*Department of Pharmacology, Student of pharmacy, College of Pharmacy, Madras Medical College, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, India.*

[2]*Department of Pharmacology, Faculty of pharmacy, College of Pharmacy, Madras Medical College, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, India.*

[3]*Department of Pharmacology, Student of pharmacy, College of Pharmacy, Madras Medical College, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, India.*

Submitted: 27 April 2024
Accepted: 02 May 2024
Published: 30 May 2024

HUMAN JOURNALS
ijppr.humanjournals.com

Keywords: *Solanum torvum*, Anti-osteoporosis, Glucocorticoid induced osteoporosis, osteoblast, osteoclast.

ABSTRACT

This study was conducted utilising glucocorticoid-induced osteoporosis in wistar albino rats to assess the anti-osteoporotic effect of *Solanum torvum* fruits. Acute oral toxicity studies were performed for ethanolic extract of *Solanum torvum* (EEST) fruits and their *invivo* anti-osteoporotic activity at a dose of 200mg/kg and 400mg/kg was performed using glucocorticoid induced rat model. The parameters evaluated in order to demonstrate the effect of EEST on osteoporosis were biochemical, biomechanical evaluation, radiological observation and histopathological examination. The results were analyzed using one-way ANOVA and p value were calculated to find the significance of the results. There were no signs of morbidity or mortality in the acute toxicity study data. *Invivo* anti-osteoporotic results showed that treatment with EEST has been effective in reducing osteoporosis by increase in the bone mineral content and reduction in alkaline phosphatase and improving the hardness of the bone. The beneficial effects of EEST on bone production and density were validated by radiological observation and histological analysis, which showed elevated osteoblast activity and trabecular thickness in the treated group.

INTRODUCTION

Osteoporosis is a dynamic systemic skeletal disease characterised by diminished bone mass/density and micro-architectural disintegration of bone tissue. Bone formation initially surpasses bone resorption, but by the third decade this has reversed resulting in a net loss of bone mass. This leads to an increased bone fragility and susceptibility to fracture[(1)]. The homeostasis of a typical bone is absolutely adjusted between the bone resorption by osteoclast and the bone formation by osteoblast[(2)].

Osteoporosis influences more than 200 million individuals worldwide and its predominance rises as individuals get older. Over 70% of people over the age of 80 endure from this condition. Females are more likely than males to endure from this condition. Around 2% to 8% of males and 9% to 38% of females in the developed world are afflicted. Osteoporosis causes over 9 million fractures each year worldwide. An osteoporotic fracture influences one in every three females and one in every five males over 50. In comparison to people living at lower latitudes, people living in areas of the world with less vitamin D from sunlight had higher fracture rates [(3)].

Age and sex are the primary risk factors for osteoporosis; risk seems to be higher during infancy and adolescence, while risk lowers in adulthood due to peak bone mass [(4)]. However, the risk doubles again after the age of 45, especially for women and it increases even more for men beyond the age of 60. Genetic factors also contribute significantly to variations in bone density. Increasing longevity, ethnicity and genetic predisposition are among the non-modifiable risk factors. Risk factors that can be changed include low calcium and vitamin D intake due to insufficient calcium intake, sociocultural factors that cause less sunlight exposure, inadequate vitamin D fortification of foods, highly pigmented skin, higher levels of phytates and oxalates (particularly in the Indian diet), early menopause, sedentary lifestyles, low physical activity, ignorance of bone health and a history of fractures. Osteoporosis is largely caused by a lifetime deficiency in calcium, oestrogen, and vitamin D as well as increased alcohol and nicotine use[(5)]. Certain medications, such as prednisone, dexamethasone, methotrexate and heparin can seriously harm bones over time and eventually result in bone loss. The chance of developing osteoporosis is also increased by certain gastrointestinal and endocrine conditions [(6)].

In clinical practice, synthetic glucocorticoids (GCs) have been used for decades to treat autoimmune illnesses, rheumatism, gastrointestinal disorders, tumours and organ

transplantation. Even though GC's therapeutic benefits have been amply demonstrated, prolonged usage of the drug unavoidably resulted in a large number of negative effects. The most prevalent secondary osteoporosis in adults is called glucocorticoid-induced osteoporosis (GIO), which is one of the major side effects. According to preliminary data, 30% of patients who used GC for more than six months developed osteoporosis[7]. Adult patients with GIO typically have back pain, height loss, humpback and even fractures as a result of a fast, dose-dependent bone loss. Reduced bone mineral density has also been reported in a number of paediatric conditions treated with GC, such as organ transplantation, rheumatoid arthritis, asthma and systemic lupus erythematosus. A population-based study showed that children who got more than four sessions of glucocorticoids had a higher risk of fracture[8].

Reduced bone formation was linked to increased osteoblast apoptosis and impaired osteogenesis in GIO [9]. Current osteoporosis medications include body building supplements, raloxifene, teriparatide and bisphosphonates. These medications have side effects that include nausea, dizziness, gastrointestinal disturbances and peptic ulcers. Herbal remedies are being researched as a potential remedy for these side effects[10].

The goal of current research is to prevent and treat osteoporosis with low or no side effects by using nature's gifts or herbs. *Solanum torvum*, also referred to as "sundai," is a member of the solanaceae family. Traditional medicine frequently uses *Solanum torvum* fruits as an antihypertensive. It possesses sedative, digestive, hemostatic, cardiovascular, anti-platelet aggregation, antioxidant, anti-inflammatory and diuretic properties [11]. Calcium, the most prevalent mineral for healthy bones, is abundant in *Solanum torvum*. According to earlier research, plants that contain quercetin increase the small intestine's ability to absorb calcium, boost vitamin D activity and prevent osteoclastogenesis and osteoblast apoptosis [12]. The goal of the current study is to assess the anti-osteoporotic effect of *Solanum torvum* fruits on glucocorticoid-induced osteoporosis in wistar albino rats.

2. MATERIALS AND METHODS

2.1 Collection, authentication and extraction of plant material

Powder of *Solanum torvum* fruits were purchased from Palayamkottai, Tamil Nadu. The fruits were authenticated by Dr. S. Mutheeswaran, Scientist, Xavier Research Foundation, St. Xavier’s College, Palayamkottai, Tamil Nadu. The powdered material were passed through a 40-mesh sieve. The powder was extracted with ethanol (99.9%) using a Soxhlet extractor.

The percentage yield of the ethanolic extract of *Solanum torvum* was calculated using the following formula.

$$\text{Yield value} = \frac{\text{Weight of the residue}}{\text{Weight of the dried plant}} \times 100$$

2.2 Experimental animals

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA/IAEC approval no: 1917/GO/ReBi/S/16/CPCSEA/20.09.2021 and 6/AEL/IAEC/MMC, Date:26/12/2023.

A total of 33 Female wistar albino rats used for this study was procured from Animal house, Madras Medical College, Chennai, India. The animals were housed in the animal house in sanitized polypropylene cages containing paddy husk bedding. The bed material was changed twice a week. The animals were maintained under controlled conditions of temperature (23±2° C), humidity (50±5%) and 12 hr light-dark cycle. Water and commercial diet were freely available for these animals. All animal cages used in the study had a proper identification i.e., labels. Each animal in the cage was marked on tail with picric acid for their appropriate identification.

2.3 Acute oral toxicity

Three healthy adult Wistar albino rats weighing between 150-250gm was selected for the study. For all the three animals food, but not water was withheld overnight prior to dosing. Being a traditional herbal extract, the mortality was unlikely at the highest starting dose level (2000mg/kg b.w). Hence a limit test one dose level of 2000mg/kg was conducted in all the three animals as per the OECD guidelines 423[13].

2.4 Experimental procedure

Thirty healthy female wistar albino rats were weighed and grouped into six groups (n=6). Group I kept as normal control received normal saline orally. Group II was treated with dexamethasone 7mg/kg in the intramuscular route once a week for 4 weeks. Group III kept as standard control group was treated with dexamethasone 7mg/kg once a week for 4 weeks intramuscularly and standard drug alendronate 0.2 mg/kg p.o daily from day 15 to 28. Group

IV and Group V were considered as treatment groups that received *Solanum torvum* at a doses of 200 mg/kg and 400 mg/kg orally from day 15 to 28 along with dexamethasone 7mg/kg once a week for 4 weeks intramuscularly.

2.5 Evaluation parameters

2.5.1.Body weight changes

The body weight was measured every week till the end of the study and the body weight changes were observed.

2.5.2.Biochemical parameters

The animals were given anaesthesia with isoflurane 2-3% on the 29th day and the blood was collected by cardiac puncture. The blood was collected in a rapid clot activator tube, the blood was centrifuged at 3000rpm for 15 mins and the serum was collected and stored at 20°C. Using an automatic analyzer, the biochemical analysis was performed to determine the levels of serum calcium[(14)], serum phosphorus[(15)] and serum alkaline phosphatase[(16)] (ALP).

2.5.3.Biomechanical parameters

After euthanizing the animal by overdose of isoflurane, the animal was placed on a dissecting board and the hip ball and socket joint was reached by invasion and retraction. The bone was dislocated and cleansed by removing the tissues around it and femoral bone was stored in 10% neutralized buffer formalin solution. The processed femur bone was used to analyse the biomechanical strength of bone. The weight of each bone was measured using digital balance. The length was measured by using ruler and the thickness was measured using Vernier Caliper. The hardness of the bone was measured in order to determine the periosteal and endosteal arrangement of cortical bone. The hardness was measured by determining the fracture point. The fracture point is measure of the point at which the bone breaks when weight was applied. In the present study hardness tester was used. This bone breaking strength is indicated in (kg/m) N as unit[(17)].

2.5.4.Radiological observation

At the end of the study, radiographs was taken using table top procedure (44kVp and 2 mAs) with exposure time of 10ms and working distance of 1m in all animals[(18)].

2.5.5.Histopathological examination

The isolated femur bone was defatted by treating the bone with 5% nitric acid for the period of 24 hours. Then the bone samples dehydrated using automated vacuum tissue processor. Dehydrated samples are then embedded in paraffin wax and then sectioned. The sectioned bone was stained with haematoxylin and eosin (H&E) and observed under light microscope[19,20].

2.6 Statistical analysis

All the values were expressed as mean ± SEM. The data was statistically analyzed by means of one-way ANOVA followed by Dunnett's multiple comparison test using Graphic pad prism software version 10.2.2. One way ANOVA was used to correlate the statistical difference between the variables P values ($P<0.01$, $P<0.0001$) was considered as statistically significant.

3 RESULTS AND DISCUSSION

3.1. Extraction

The percentage yield of ethanolic extract of *Solanum torvum* was found to be 5.71% w/w.

3.2. Acute oral toxicity study

The results of the Acute toxicity studies revealed no indications of morbidity or mortality. The subjects exposed to the test substance did not exhibit any adverse health effects or signs of illness that could be attributed to toxicity. These findings indicate that, at the tested dose levels, the substance does not pose an immediate threat to the well-being or survival of the subjects. Hence $1/5^{th}$ and $1/10^{th}$ dose of 2000mg/kg was selected as low and high dose respectively.

3.3. Body weight changes

The body weight of the animals were taken for every week for 4 weeks and were represented in the following **table 1.** There was a marginal change in body weight throughout the experiment with normal rats. The disease control rats showed decrease in body weight. There was a progressive increase in the weight of rats which were treated with 200mg/kg and 400mg/kg of ethanolic extract of *Solanum torvum* **(Figure 1)**.

Table 1: Effect of *Solanum torvum* on body weight changes

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Control	156.96±1.57	158.69±1.50	161.16±1.90	163±1.92	164.81±1.72
Disease control	166.28±1.08	165±1.06	163.14±1.23	158.52±1.94	155.79±2.19
Standard control	162.58±1.43	162.14±1.07	161.88±1.33	165.25±1.08	167.91±1.36
200 mg/kg of EEST	143.88±1.92	144.13±2.16	147.80±1.97	149.94±1.90	152.50±1.99
400 mg/kg of EEST	153.89±1.10	154.44±1.14	157.87±1.12	159.80±1.15	163.33±1.40

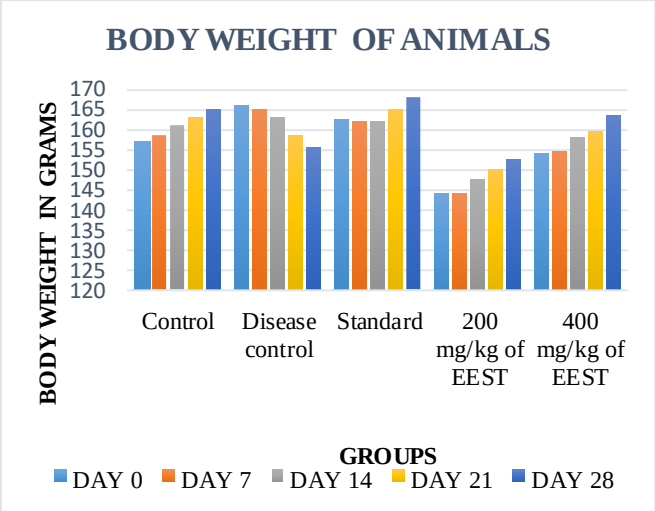


Figure 1: Effect of *Solanum torvum* on body weight changes

3.4. Biochemical parameters

On 29th day, blood samples were collected aseptically for performing the anti-osteoporotic effect of EEST on animals. Biochemical parameters include serum calcium, serum phosphorous, serum alkaline phosphatase levels were measured. The changes in the biochemical parameters in animals in all the groups were assessed and represented in the following **table no. 2**.

Table 2: Effect of *Solanum torvum* on Biochemical parameters

Groups	Serum calcium level (mg/dl)	Serum phosphorous level (mg/dl)	Serum alkaline phosphatase level (IU/L)
Normal control	9.00±0.09	8.44±0.09	144.50±4.50
Disease control	6.27±0.25####	4.49±0.13####	250.29±7.02##
Standard control	8.11±0.11****	7.43±0.10***	144.96±6.09**
200 mg/kg of EEST	7.79±0.24****	6.62±0.21**	185.10±8.47*
400 mg/kg of EEST	8.79±0.06****	8.03±0.4****	149.96±9.96**

All the values are expressed as mean±SEM (n=6).

Analysed by One-way analysis of variance followed by multiple comparison Dunnet 't' test.

Serum calcium: #### $p < 0.0001$ compared with normal control. ****$p < 0.0001$ compared with disease control.

Serum phosphorous: #### $p < 0.0001$ compared with normal control. ****$p < 0.0001$ compared with disease control. ***$p < 0.001$ compared with disease control.

Serum alkaline phosphatase: ## $p < 0.01$ compared with normal control. **$p < 0.01$ compared with disease control. *$p < 0.05$ compared with disease control.

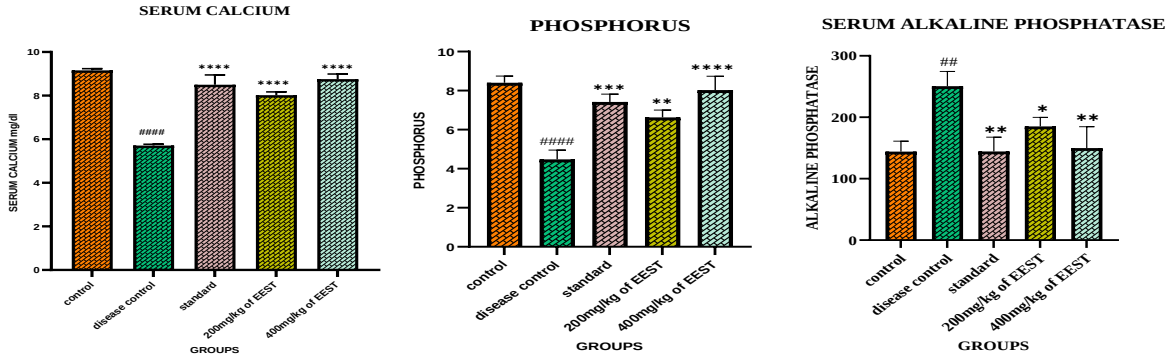


Figure 2: Graphical representation of serum calcium, phosphorous, alkaline phosphatase in various groups

It was seen from the above data that the serum calcium, serum phosphorous levels were decreased significantly in disease control group compared with normal control group. The standard and treatment group such as low dose 200 mg/kg and high dose 400 mg/kg of EEST group has significantly increased levels compared with disease control group.

The serum ALP level of disease control is increased significantly as compared with the normal group. In the standard group the serum ALP level is decreased significantly as compared with the disease control group. The treatment group such as low dose 200mg/kg and high dose 400mg/kg of EEST has significantly decreased serum ALP levels as compared with disease control.

3.5. Biomechanical parameters

On 29th day, after euthanizing the animal femur bone was isolated and femur bone was subjected for biomechanical evaluation. Bone biomechanical parameters include bone weight, bone length, bone thickness and bone hardness were measured. The changes in the biochemical parameters in animals in all the groups were assessed and represented in the following **table no. 3.**

Table 3: Effect of *Solanum torvum* on biomechanical changes

Groups	**Bone weight in gram**	**Bone length in mm**	**Bone thickness in mm**	**Bone hardness in (kg/m)N**
Normal control	0.42±0.004	33.35±0.17	4.13±0.04	200.5±1.06
Disease control	0.26±0.07####	31.55±0.13	3.38±0.05####	153.16±0.89####
Standard control	0.39±0.005****	32.44±0.06	3.9±0.03****	171.5±0.89****
200 mg/kg of EEST	0.37±0.006****	32.03±0.12	3.8±0.04****	163.66±0.80****
400 mg/kg of EEST	0.41±0.005****	31.72±0.18	3.8±0.03****	165±0.68****

All the values are expressed as mean±SEM (n=6).

Analysed by One-way analysis of variance followed by multiple comparison Dunnet ‘t’ test.

Bone weight, Bone thickness, Bone hardness: #### $p < 0.0001$ compared with normal control. ****$p < 0.0001$ compared with disease control.

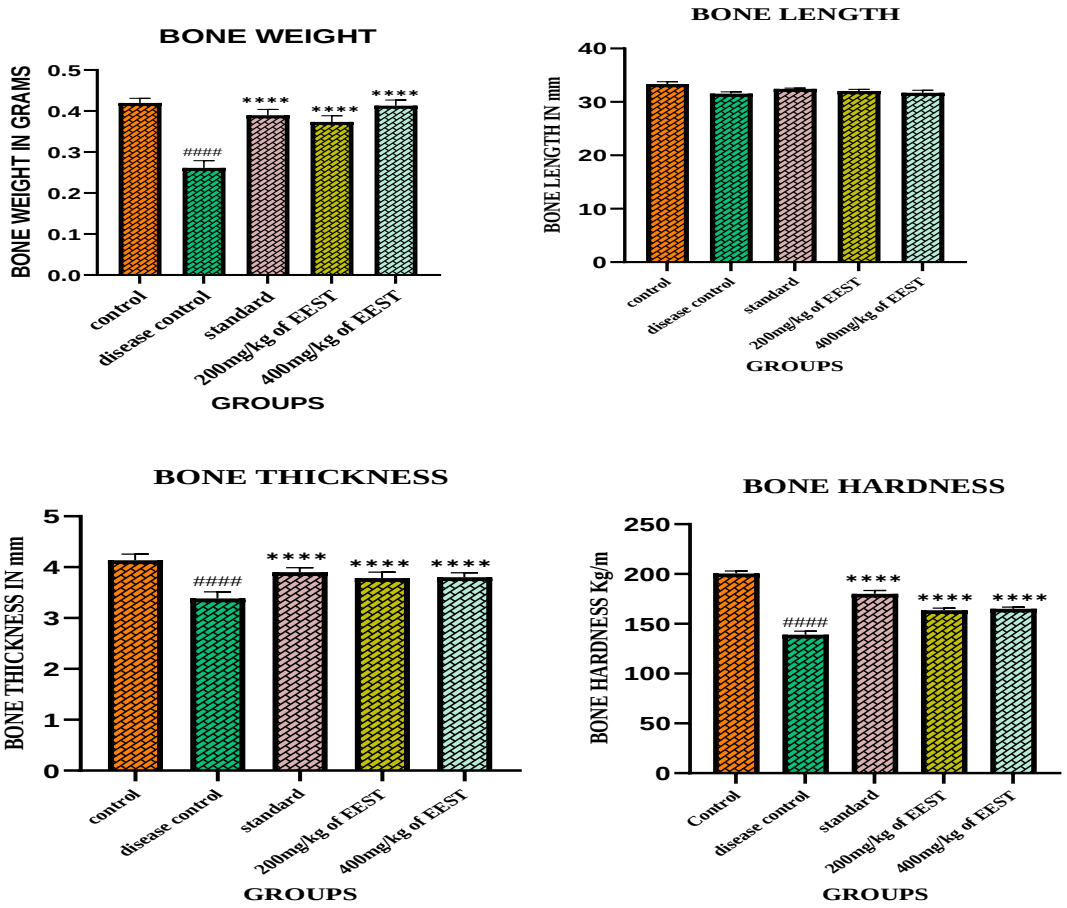


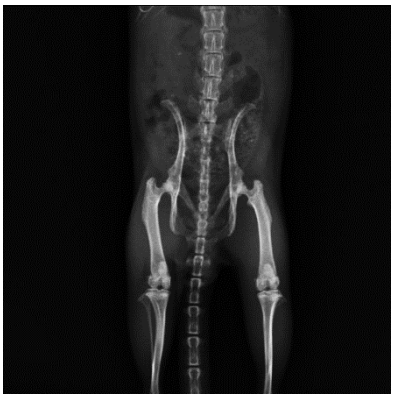
Figure 3: Graphical representation of Bone weight, Bone length, Bone thickness, Bone hardness in various groups

As shown in the **figure 3**, the bone weight, bone thickness, bone hardness of disease control is reduced significantly as compared with the normal group. In the standard group and the treatment group such as low dose 200mg/kg and high dose 400mg/kg of EEST has significant increase in the bone weight, bone thickness, bone hardness compared with disease control group. The bone length of disease control has no changes significantly as compared with the normal group. In the standard group and the treatment group such as low dose 200mg/kg and high dose 400mg/kg also has no significant changes in bone length as compared with disease control.

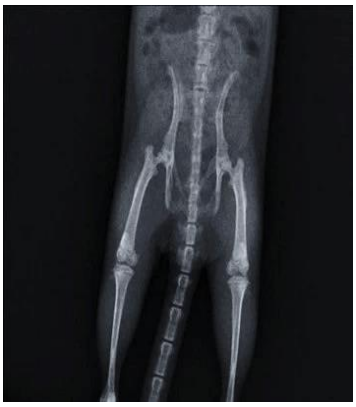
3.6. Radiological observation

The x-ray examination of femur bone of the animals were taken. Normal control rat showed normal bone structure, thick and radiopaque cortex with normal cavity density **(Figure 4a).** Disease control group showed completely radiolucent view with very thin cortex with abnormal marrow cavity density **(Figure 4b).** Treatment with alendronate and the test drug

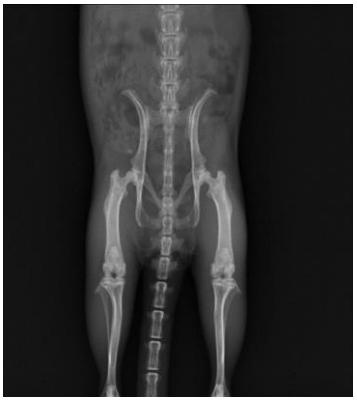
showed marked improvement in bone formation and thickening of cortical bone which confirmed that the test drug has an effect in reducing porous bone and improving bone formation **(Figure 4c, 4d, 4e).**



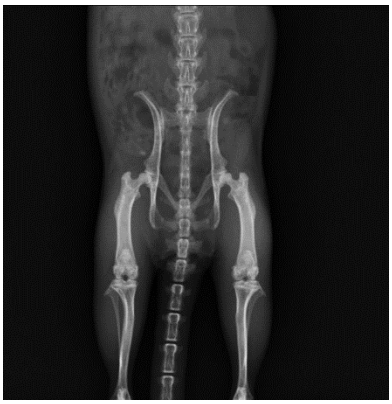
4a



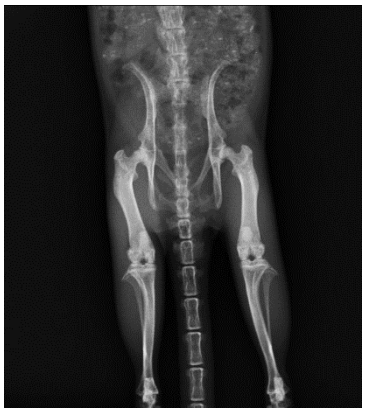
4b



4c



4d

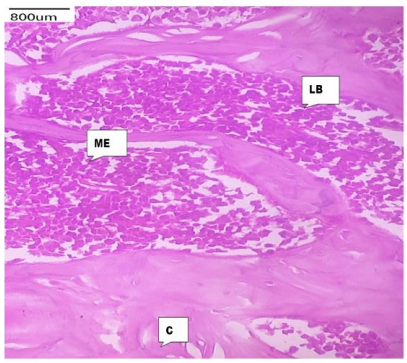


4e

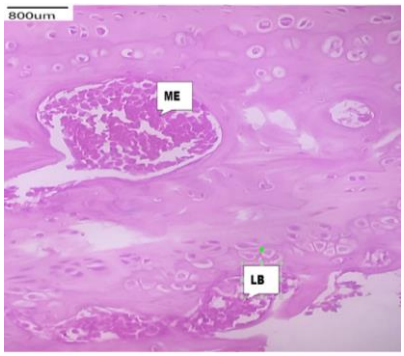
Figure 4: X-ray imaging of normal control (4a), disease control (4b), standard control (4c), 200 mg/kg of EEST (4d), 400 mg/kg of EEST (4e).

3.7. Histopathological examination

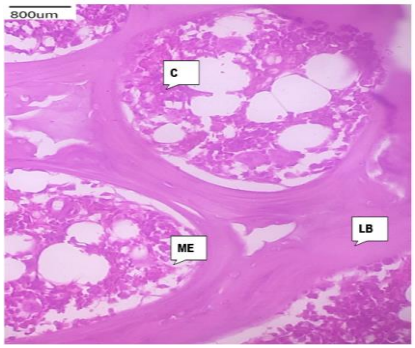
On 29th day, after euthanizing the animal, femur bone was isolated. The sectioned femur bone was stained with haematoxylin and eosin and it was examined for any histological changes.



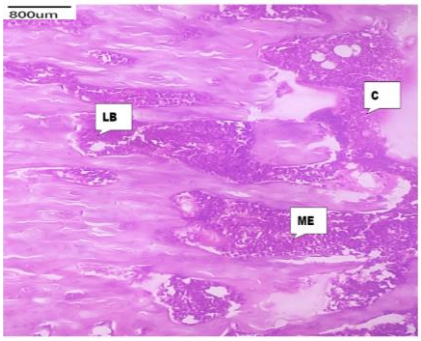
5a



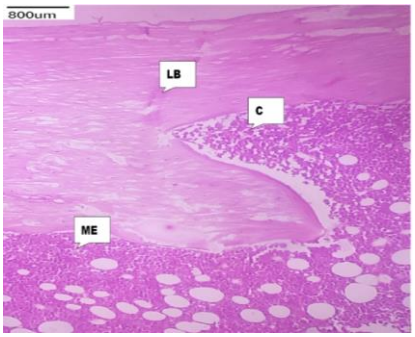
5b



5c



5d



5e

Figure 5: Histopathological images of normal control (5a), disease control (5b), standard control (5c), 200 mg/kg of EEST (5d), 400 mg/kg of EEST (5e).

Control group animals showed normal architecture and normal bone compactness **(figure 5a).** The rats in disease control group showed thinning of trabeculae, increase in osteoclast number and decreased osteoblast number **(figure 5b)**. Alendronate and treatment group 200 mg/kg of EEST and 400 mg/kg of EEST exhibited significant restorative progress with increased osteoblast, trabecular thickness **(figure 5c, 5d, 5e).**

Therefore, from the above histopathological examination of the treated group of rats showed that the ethanolic extract of *Solanum torvum* is active in bone formation and has beneficial effects in the treatment of osteoporosis.

4. Conclusion

Solanum torvum has been chosen as the plant to perform the research on its anti-osteoporotic action based on the literature review. The results of the Acute toxicity studies revealed no indications of morbidity or mortality. Various parameters were evaluated such as biochemical, biomechanical, radiological observation and histopathological examination. Results has shown that treatment with EEST has been effective in reducing osteoporosis by increase in the bone mineral content and reduction in alkaline phosphatase and improving the hardness of the bone. It was further confirmed by the radiological observation and histopathological examination. From the study it is concluded that the ethanolic extract of *Solanum torvum* fruits possess beneficial effect against glucocorticoid induced osteoporosis proved by the valid data obtained from the *in-vivo* evaluation which includes serum markers, biomechanical properties, radiological observation and histopathological examination. The present study provided basic evidence that the *Solanum torvum* has the beneficial effect for the treatment of osteoporosis. Further studies are required to elucidate the molecular mechanism of action of phytoconstituents of plant extract and its therapeutic potential in the treatment of osteoporosis.

ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to God for guiding and supporting me throughout the completion of my project. I am immensely grateful to my parents, Mr. Muthuvedi and Mrs. Chitra, for their unwavering love, unwavering support and unwavering belief in me. I owe my deep gratitude to my project guide Dr. R. Indumathy, M. Pharm., Ph.D., Assistant Professor, Department of Pharmacology, Madras Medical College, Chennai 03. I would like to express my heartfelt gratitude to my guide for the unwavering support and guidance provided throughout my project. I wholeheartedly extend my gratitude to my friends for their support and kind help during my project work at every stage.

Conflict of interest statement: The authors declared no conflict of interest.

References

1. Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. *Molecules.* 2016; 21 :559.
2. Pouresmaeili F, Kamalidehghan B, Kamarehei M et al. Comprehensive overview on osteoporosis and its risk factors. Therapeutics and Clinical Risk Management. 2018; 14: 2029-2049.
3. Laura Masi *et al*. Epidemiology of osteoporosis. Clin Cases Miner Bone Metab.2008;5(1):11-13.
4. Oden, A, *et al*. Burden of high fracture probability worldwide: Secular increases 2010-2040. Osteoporos Int. 2015; 26(9): 2243-2248.

5. Sozen T, Osizik L, Basaran N. An overview and management of osteoporosis. European journal of rheumatology. 2017;4(1):46.
6. Ahmed SF, Elmantaser M. Secondary osteoporosis: Calcium and Bone Disorders in Children and Adolescents. 2009;16(5):170-90.
7. Gudbjornsson B, Juliusson UI, Gudjonsson FV. Prevalence of long term steroid treatment and the frequency of decision making to prevent steroid induced osteoporosis in daily clinical practice. Ann Rheum Dis. 2002; 61(4):32–36
8. Leonard MB *et al.* Glucocorticoid-induced osteoporosis in children: impact of the underlying disease. Pediatrics.2007; 119(7):166-174
9. Jia J, Yao W, Guan M, *et al*. Glucocorticoid dose determines osteocyte cell fate. FASEB. 2011; 25(10):3366–3376.
10. Khosla S, Hofbauer LC. Osteoporosis treatment: recent developments and ongoing challenges. Lancet Diabetes Endocrinol. 2017 ;5(11):898-907.
11. Agrawal A. D, Bajpei P S, Patil A *et al*. *Solanum torvum* Sw- A phytopharmacological review. 2008; 45: 223-226
12. Wong SK, Chin KY, Ima-Nirwana S. Quercetin as an Agent for Protecting the Bone: A Review of the Current Evidence. Int J Mol Sci. 2020 Sep 3;21(17):6448.
13. OECD., Acute oral toxicity 423., [INTERNET]., 2001,[01]., https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf
14. Gitelman, H. J. Anal. Biochem. Calcium kit.1967;18:521.
15. Dick C F, Dos-Santos A L, Meyer-Fernandes J R. Phosphate assay kit. 2011;56:1-7.
16. Alkaine phosphatase kit pNNP kinetic method. Available from www.tulipgroup.com>html >pack-inserts
17. Merlin Mary M. Evaluation of ethanolic root extract of Daucus carota on steroid induced osteoporosis in rats. RJPT. 2019;2(11):5461-5466.
18. Mohana Priya G V, Sakthi Abirami, Logeswari. Evaluation of Anti-Osteoporotic Activity of Rasna Saptaka Kwatham – A Siddha Formulation Using Corticosteroid Induced Osteoporosis in Wistar Albino Rats. IJPPR. 2023; 28(1): 678-699.
19. Shi J, Sun J, Zhang W, Liang H, *et al.* Demineralized Bone Matrix Scaffolds Modified by CBD-SDF-1α Promote Bone Regeneration via Recruiting Endogenous Stem Cells. ACS Appl Mater Interfaces. 2016 Oct 19;8(41):27511-27522.
20. Yu Z, Dang X, Wang K, *et al*. Lithium chloride attenuates the abnormal osteogenic/adipogenic differentiation of bone marrow-derived mesenchymal stem cells obtained from rats with steroid-related osteonecrosis by activating the β-catenin pathway. Int J Mol Med. 2015 Nov;36(5):1264-72.