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ORIGINAL ARTICLE

Efficacy of Bisacurone in Bone Fracture Healing Process: An Experimental Study on Osteoporotic Rats

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ABSTRACT

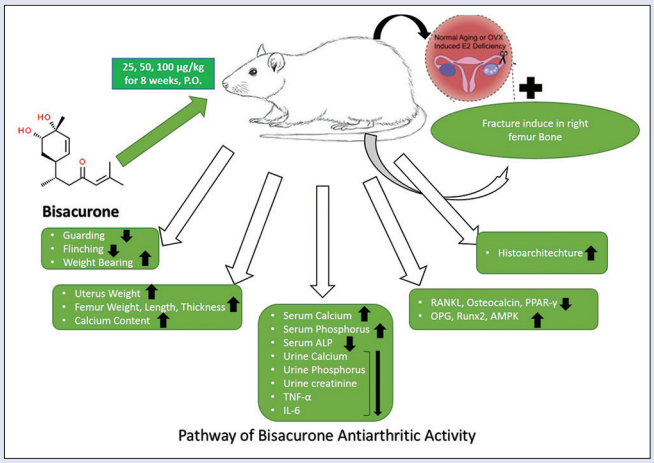
Background: Osteoporosis (OP) is a common disorder resulting in bone fragility and fracture. Due to OP, bone mass and microarchitectural bone tissues are damaged, increasing the risk of fracture. **Objectives:** To determine the efficacy of bisacurone in healing fractured bones and its inflammatory response in ovariectomy (OVX)-induced OP in female rats. **Materials and Methods:** After inducing fracture in femur bone, bisacurone (25, 50, and 100 μg/kg) and alendronate (20 mg/kg) were orally administered to rats for 8 weeks. Subsequently, its fracture healing and anti-inflammatory potential were evaluated via assessment of various biochemical and molecular parameters. **Results:** Bisacurone therapy significantly ($P < 0.05$) increased the calcium content, serum calcium, and phosphorus in OVX-induced OP rats. It significantly ($P < 0.05$) reduced the serum alkaline phosphate (ALP); urine biochemical parameters such as urinary calcium, phosphorus, and creatinine; and inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α). Moreover, bisacurone was found to be significantly ($P < 0.05$) effective in downregulating the bone turnover markers such as osteocalcin, receptor activator of nuclear factor-kappa-B ligand (RANKL), peroxisome proliferator-activated receptor gamma (PPAR-γ), and upregulating osteoprotegerin (OPG), runt-related transcription factor 2 (Runx2), and AMP-activated protein kinase (AMPK) in OVX-induced OP rats. **Conclusion:** Bisacurone effectively healed the fracture and improved the bone quality in arthritic conditions by reducing inflammatory cytokines and altering the level of bone turnover markers. Therefore, bisacurone therapy should be considered for the management of fracture healing process in the arthritic conditions.

Key words: Bisacurone, interleukin-6, OPG, osteoporosis, ovariectomy, RANKL, Runx2, tumor necrosis factor-alpha

SUMMARY

- Osteoporosis was induced in female Sprague–Dawley rats through ovariectomy (OVX).
- The right femur bone was fractured in each OVX-induced OP rat.
- Bisacurone (100 μg/kg) dose markedly reduced the inflammation and significantly restored the calcium content in the bone.
- Rats treated with bisacurone (100 μg/kg) substantially restored the bone turnover markers via upregulation of RANKL, osteocalcin, and PPAR and upregulation of OPG, Runx2, and AMPK.
- Bisacurone can be considered as an important moiety of plant origin for the healing of bone fracture in osteoporosis.

Abbreviations used: AMPK: AMP-activated protein kinase; ANOVA: Analysis of variance; Arg1: Arginase1; Bcl-xL: B-cell lymphoma-extra-large; BMPs: Bone morphogenetic proteins BMPs; C. longa Linn: Curcuma longa Linn; CD4: Cluster of differentiation 4; CD8: Cluster of differentiation 8; CMC: Carboxymethyl cellulose; DMARDs: Disease-modifying anti-rheumatic drugs; ELISA: Enzyme-linked immunosorbent assay; HIF-1α: Hypoxia-inducible factor 1 alpha; HUVECs: Umbilical vein endothelial cells; IL-1: Interleukin-1; IL-12: Interleukin-12; IL-15: Interleukin 15; IL-6: Interleukin-6; iNOS: Inducible nitric oxide synthase; IκBα: Kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; NF-κB: Nuclear factor kappa B; NSAIDs: Non-steroidal anti-inflammatory drugs; OP: Osteoporosis; OPG: Osteoprotegerin; OVX: Ovariectomy; PPAR-γ: Peroxisome proliferator- activated receptor gamma; PTH: Parathyroid hormone; RANKL: Receptor activator of nuclear factor kappa-B ligand (RANKL); ROS: Reactive oxygen species; ROP: Retro-orbital puncture; RT-PCR: Reverse transcriptase-polymerase chain reaction; Runx2: Runt-related transcription factor 2; SC: Subcutaneous; SEM: Standard error mean; Serum ALP: Serum alkaline phosphate; STZ: Streptozotocin; TGF-α: Tumor growth factor-α; TGF-β: Tumor growth factor-β; TNF-α: Tumor necrosis factor-alpha; VCAM-1: Vascular cell adhesion molecule 1.



Pathway of Bisacurone Antiarthritic Activity

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INTRODUCTION

In recent times, surgical condition has a significant impact on the general population in middle- and low-income countries.[1] Injury in musculoskeletal tissue muscle makes people disabled, deteriorate their life quality, and render them as a burden on the society. Such cases constitute a significant number of surgical procedures and are a major

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health concern worldwide. However, this burden can be significantly reduced if an affordable and long-term therapeutic strategy to speed up the healing process of fractures and osteotomies can be explored.[2] Osteoporosis (OP) is a very common bone disease that occurs during middle age or after menopause. Due to osteoporosis, bones become fragile and prone to fracture. In 2010, a total of 158 million people were at a high risk of fracture, and it is projected to be doubled by 2040 due to the demographic shifts.[3]

OP is characterized by skeletal fragility owing to reduced bone mass and micro-architecture deteriorated bone tissues, increasing the risk of bone fracture.[4] The current fracture healing scenario involves several cellular and molecular processes acting in concurrence with the biomechanical and physiological principles.[5] Fracture healing is a very common and natural process, taking place in both osteoporotic fractured and normal bones.[6] It involves three phases: reactive, reparative, and remodeling.[7] The reactive phase happens after fracture and stays for approximately 7 days and is distinguished by granulated tissue formation and injured region inflammation. The reparative phase comes after the reactive phase and involves fracture callus formation and lamellar bone deposition.[8] The reparative stage is followed by the remodeling phase, which is the final phase that occurs after 2 months of fracture and is involved in reshaping the fractured bone to its original form.[9] Due to OP, the fracture-healing process gets complicated. It has been observed in previous studies that callus maturation does not happen rapidly in osteoporosis. This further decelerates the healing process. After a fracture, an osteoporotic bone may heal completely even with low bone density, low calcium, and low estrogen; however, healing will take a longer time.[6,10]

There are several causes of postmenopausal OP, such as an imbalance between bone formation and resorption of bone calcium.[7] The number of osteoclasts increasing or decreasing after fracture in osteoporotic rats has been observed[11] After menopause, the formation of cytokines such as interleukin (ILs)-6, IL-1, tumor necrosis factor-alpha (TNF-α), and bone-resorbing hormones increase due to the estrogen deficiency. This indicates that the chemical compounds that can inhibit the production of these cytokines may be useful for managing bone loss in OP after menopause.[12,13] A study reported that improvement of bone formation leads to adequate retrieval of bone mass in patients with osteoporosis. The agents that lead to this mechanism of bone formation and further improvement of bone mass may have osteogenetic potential for fracture management in OP.[14]

Currently, various anti-osteoporotic therapeutic strategies are available in the market, such as replacement of estrogen therapy, bisphosphonate, corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying ant rheumatic drugs (DMARDs), and biological response modifiers.[15] However, these drugs are associated with severe adverse effects. These include hormonal imbalance due to the prolonged use of hormonal therapy and various other complications. Furthermore, long-term steroid use is also associated with muscular weakness, peptic ulcer, and cataract.[16] Bone marrow suppression, renal, hepatic and gastrointestinal dysfunction are the reported side effects associated with the use of DMARDs.[17] In addition, infliximab, a biological response modifier, accounts for developing secondary skin infection or skin cancer such as nonmelanoma and lymphoma skin cancers.[18] Therefore, there is a requirement for alternative therapy to manage anti-inflammatory responses.[19]

Curcuma longa Linn. (family Zingiberaceae) has been used for a long time as a traditional medicine to treat several therapeutic conditions.[20] Curcumin is a well-known bioactive phytochemical, derived from *C. longa*, and has shown both *in-vitro* and *in-vivo* anti-carcinogenic activity.[21] *C. longa* has other bioactive compounds known as curcuminoids, which have a similar structure to curcumin. In recent years, researchers have reported the non-carcinogenic and non-mutagenic effects of bisacurone.[22] Recently, another new active compound has been isolated from *C. longa*, identified as bisacurone, which has many health-promoting benefits.[23] Studies have evidenced that hot-water extract of *C. longa* exhibit anti-inflammatory and antioxidant potential due to the presence of bisacurone in *C. longa*.[24] This compound exhibits various therapeutic effects such as anti-cancer, anti-oxidant, and anti-inflammatory.[25] It has been reported previously that bisacurone shows anti-inflammatory activity through inhibition of the TNF-α expression.[26]

Based on these findings, this study was aimed to assess the impact of bisacurone on fracture healing and inflammatory response in ovariectomy (OVX)-induced OP in female rats.

MATERIALS AND METHODS

Animals

Adult female Sprague–Dawley rats 6–7 weeks old (200–250 g) were procured from the animal house of The Fourth People's Hospital of Shaanxi. Rats were maintained inhouse at 24°C ± 1°C, and normal dark-light cycle, with standard pellet feed and filtered water during the experimental time of 0800 hr and 1700 hr in a quiet laboratory environment. The animal ethics committee of The Fourth People's Hospital of Shaanxi approved all the experimental research protocols (No.: 2121-0783).

Chemicals and Kits

Reagents used during the study included rat-specific diagnostic kits for the estimation of serum and urine calcium (CARZM 50), phosphorus (PHOSM 25), creatinine (CRE 100), and alkaline phosphatase (ALP SLR 25) obtained from the Lab-Care Diagnostics (Mumbai, India). IL-6 and TNF-a ELISA assay kits were purchased from Cayman Chemical (Ann Arbor, MI, USA). Total RNA Extraction kit and one-step reverse transcription-polymerase chain reaction (RT-PCR) kit were purchased from MP Biomedicals India Private Limited, India. Other chemicals or reagents used in this study were acquired from local vendors and were of analytical grade.

Induction of Ovariectomy

All rats were considered for ovariectomy. OVX-induced OP was performed on two paravertebral skin incisions under general anesthesia and sterile conditions. On the day of surgery, rats were anesthetized with diazepam (5 mg/kg) and ketamine (50 mg/kg) intramuscular injection. Rats were administered ibuprofen 20 mg/kg preoperatively and every 8–12 h for the next 5 days after surgery.[27] Rats were cleaned and sterilized with several applications of 50% ethanol and povidone-iodine (betadine). Incisions were closed after removal of ovaries and ligation of uterine tubes.[28] Before surgery, antibiotic therapy with ceftriaxone at a dose of 50 mg/kg was injected intramuscularly into the rats and then 24 and 48 h after surgery. In the sham-operated group, only an incision was made. All animals were kept for 3.5 months after surgery in cages to develop OP.[29] Rats were then subjected to complete osteotomy in the right femur.

Induction of Femoral fracture

Induction of femoral fracture was done according to a method previously described by Farahani *et al*., 2015.[30]

Treatment protocol

After surgery, 140 (N = 140) rats were randomly selected and allocated into seven groups (n = 20 in each group) as follows: group I:

OVX-induced OP control (control): OVX-induced OP animals received oral gavage of carboxymethyl cellulose (CMC: 0.5% w/v, 10 mL/kg) water); group II: sham-operated (sham): sham-operated animals received oral gavage of CMC (10 mL/kg, orally in 0.5% w/v); group III: (A 3): OVX-induced OP rat treated with alendronate (3 mg/kg, subcutaneous, SC); group IV: (B 25): OVX-induced OP rats treated with bisacurone (25 µg/kg, orally in 0.5% w/v CMC); group V: (B 50) OVX-induced OP rats treated with bisacurone (50 µg/kg, orally in 0.5% w/v CMC); group VI: (B 100): OVX-induced OP treated with bisacurone (100 µg/kg, orally in 0.5% w/v CMC); group VII: (B 100 + A3): OVX-induced OP treated with bisacurone and alendronate (100 µg/kg, orally in 0.5% w/v CMC and alendronate 3 mg/kg, SC). The doses of bisacurone (25, 50, 100 µg/kg) were selected based on the earlier reports.[31]

Treatment using bisacurone, alendronate, and CMC to respective groups was continued for 8 weeks. The weekly body weight of each rat was measured. Rats were kept on fast the entire night, and each rat was kept in an individual metabolic cage on the 56th day to collect the urine for 24 h. The amount of urine phosphorus, creatinine, and calcium was analyzed from the urine sample. These rats were then anesthetized with ether, and blood samples were collected through a retro-orbital puncture (ROP). Serum samples were collected by the centrifugation method and stored at −80°C for further analysis of phosphorus, ALP level, and calcium with colorimetric assay kits using a semi-automatic biochemical analyzer (Prietest Touch Plus; Robotnik, Ambernath, Thane, India).[32] TNF-α and IL-6 were estimated in serum samples by using ELISA kits according to suppliers' instructions. After the collection of samples (blood and urine), the rats were euthanized, and uterine horn was isolated after removing surrounding fat and weighed immediately. The femur bone of each rat was isolated and separated. The length, thickness, and weight of the femur bone were measured using digital slide calipers (Mitutoyo South Asia, New Delhi, India). The length of the femur bone was identified by measuring the actual length between the proximal tip of the femur and the distal tip of the medial condyle.[33] For histopathological analysis, femur bone was used and calcium content was determined by the measurement of femur bone ash.

Estimation of calcium content in femoral bone Ash

The calcium content was estimated for the left femur and the 4th lumbar vertebra as described previously elsewhere.[34]

Measurement of bone turnover markers

The estimation of bone turnover markers such as RANKL, OPG, Runx2, PPAR-γ, and AMPK in femur fracture was performed according to a method previously described by Adil *et al.*[35] The levels of mRNA expression were analyzed in femur epiphysis by using reverse transcriptase-polymerase chain reaction (RT-PCR) as described previously.[36] Single-stranded cDNA was synthesized from 5 mg of total cellular RNA by using a commercially available RT-PCR kit (MP Biomedicals India Private Ltd., India).[37] Primer sequences of RANKL, OPG, Runx2, osteocalcin, PPAR-γ, AMPK, and b-actin are mentioned in Table 1.

Histopathological analysis

The femur bone was collected from each rat for the analysis of bone histomorphometry. A small bone section was dipped in neutral buffered formalin (10%), and decalcification was carried out by keeping them in ethylenediaminetetraacetic acid solution (10%). The decalcified bone pieces were fixed in Paran wax and sectioned longitudinally with an approximate bone thickness of 5 m by using HM 325 rotary microtome (SP 1600, Leica Biosystems, Nussloch, Germany). Eosin and hematoxylin were used for bone staining.[38] The sections were examined under a light microscope (Olympus CX23, Gurgaon, India).

Statistical analysis

GraphPad Prism 5.0 software (GraphPad, San Diego, CA) was used to perform the data analysis. Data were expressed as mean ± standard error mean (SEM) and analyzed using one-way ANOVA followed by Bonferroni test and Tukey's multiple range *post hoc* analysis (for parametric tests including behavioral pain test, body weight gain, uterus weight, femur bone weight, thickness and length, serum phosphorus, serum calcium, serum ALP, urine calcium, phosphorus, creatinine, bone calcium ash, mRNA expression of RANKL, OPG, Osteocalcin, Runx2, PPAR-γ AMPK, inflammatory cytokines levels, TNF-α, and IL-6) or Kruskal–Wallis test for *post hoc* analysis (non-parametric tests, i.e., histopathology score). $P < 0.05$ was considered to be statistically significant.

RESULTS

Effect of bisacurone on behavioral parameters after fracture in female OVX-induced OP rats

Spontaneous flinching, guarding, and weight-bearing capacity in the left hind limb were examined in OVX-induced OP rats. Control rats spent significantly ($P < 0.05$) higher time guarding and showed an increase in the flinching number compared to sham-operated rats. However, a marked ($P < 0.05$) reduction in weight-bearing capacity was observed in control rats with respect to the sham-operated rats. Although, after 8 weeks of therapy of bisacurone (100 µg/kg), alendronate, and combination of alendronate and bisacurone (100 µg/kg), OVX-induced OP rats showed a significant ($P < 0.05$) reduction in the time spent on guarding, a significant ($P < 0.05$) decrease in flinching number, and a significant ($P < 0.05$) increase in the weight-bearing capacity compared to the control rats. There was no substantial variation of guarding, flinching, and weight-bearing capacity between alendronate-treated OVX-induced OP rats and OVX-induced OP rats treated with the combination of alendronate and bisacurone. However, a trend of better behavioral performance was observed in the group treated with the combination of alendronate and bisacurone therapy [Figure 1].

Effect of bisacurone on the body weight and uterus weight after fracture in female OVX-induced OP rats

Our results suggested that body weight substantially ($P < 0.05$) increased in the control rats as compared to the sham-operated rats. However, there was no substantial difference in the body weight between the control rats and drug-treated rats. After surgery, there was a significant ($P < 0.05$) reduction in the uterus weight in the control rats with respect to the sham-operated rats. However, alendronate, bisacurone (100 µg/kg), and a combination of alendronate and bisacurone (100 µg/kg) treatment significantly ($P < 0.05$) increased the uterus weight in OVX-induced OP rats compared to the control rats. Furthermore, no substantial difference was found in the uterus weight between alendronate-treated rats and rats treated with the combination therapy of alendronate and bisacurone (100 µg/kg), but rats treated with combination therapy of alendronate and bisacurone (100 µg/kg) showed an increasing trend of uterus weight compared to alendronate-treated rats [Table 2].

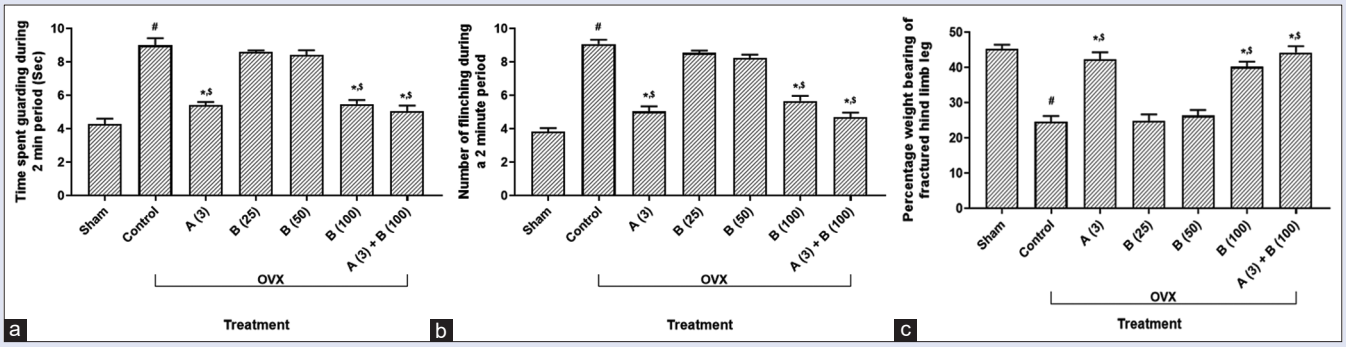

Figure 1: Effects of bisacurone on pain-related behaviors after fracture in female OVX-induced OP rats. (a) Spontaneous guarding; (b) Spontaneous flinching; (c) Hind limb weight-bearing capacity in rats. Data are expressed as mean ± S.E.M. (n = 6) and analyzed by one-way ANOVA followed by the Bonferroni test. *$P < 0.05$ as compared to the control group, #$P < 0.05$ as compared to the sham group, $$P < 0.05$ as compared to one another. Sham: Sham-operated rats; Control: OVX control rats; a (3): Alendronate (3 mg/kg, s.c.)-treated rats; B (25): Bisacurone (25 µg/kg, p.o.)-treated rats; b (50): Bisacurone (50 µg/kg, p.o.)-treated rats; B (100): Bisacurone (100 µg/kg, p.o.)-treated rats; a (3) + b (100): Alendronate (3 mg/kg, s.c.) and Bisacurone (100 µg/kg, p.o.)-treated rats

Table 1: Primer sequences of RANKL, OPG, Run×2, osteocalcin, PPAR-γ, AMPK, and b-actin

Gene	Sequence		Size (bp)
	Forward primer	Reverse primer	
RANKL	GGGAATTACAAAGTGCACCAG	GCCATCCTTCTCAAAGTTGT	69
OPG	TCAAGTGCTTGAGGGCATAC	TGGAGATCGAATTCTGCTTG	119
Runt-related transcription factor 2 (Run×2)	TGTTCTCTGATCGCCTCAGTG	CCTGGGATCTGTAATCTGACTCT	146
Osteocalcin (OCN)	AAGCAGGAGGGCAATAAGGT	CAAGCAGGGTTAAGCTCACA	240
AMPK	ATTGGATTTCCGAAGTATTGATG	CCTGGTCTTGGAGCTACGTCA	124
PPAR-γ	GCCTGCGGAAGCCCTTTGGT	AAGCCTGGGCGGTCTCCACT	136
β-actin	GTCACCCACACTGTGCCCATCT	ACAGAGTACTTGCGCTCAGGAG	764

Effect of bisacurone on the length, thickness, and weight of femur bone after fracture in female OVX-induced OP rats

The control rats showed a major ($P < 0.05$) reduction in the length, thickness, and weight of the femur bone compared to the sham-operated rats. We further observed that the bisacurone (100 µg/kg), alendronate, and combination of alendronate and bisacurone-treated (100 µg/kg) OVX-induced OP rats significantly ($P < 0.05$) restored the length, thickness, and weight of the femur bone compared to the control rats. Moreover, analysis evidenced no significant difference in the length, thickness, and weight of femur bone between the alendronate-treated group and the group treated with the combination of alendronate and bisacurone (100 µg/kg). However, a better trend of femur bone structure was observed in the rats treated with the combination of alendronate and bisacurone (100 µg/kg) [Table 2].

Effect of bisacurone on the calcium content of femur bone after fracture in female OVX-induced OP rats

The percentage of calcium content in the femur bone is reported in Table 1. The findings of this analysis revealed that the calcium content of femur bone in control rats was markedly ($P < 0.05$) lower with respect to the sham-operated rats. Eight weeks of treatment with bisacurone (100 µg/kg), alendronate, and a combination of alendronate and bisacurone (100 µg/kg) showed a substantial ($P < 0.05$) restoration of calcium content in the femur bone compared to the control rats. No substantial difference was observed in the calcium content of group of rats treated with the combination of alendronate and bisacurone (100 µg/kg) and only alendronate-treated rats, but the group of rats treated with the combination of alendronate and bisacurone (100 µg/kg) showed a trend of more calcium content in the femur bone [Table 2].

Effect of bisacurone on biochemical properties after fracture in female OVX-induced OP rats

The findings of the biochemical analysis revealed that the phosphorus levels and serum calcium were markedly ($P < 0.05$) decreased in the control rats as compared to the sham-operated group, whereas serum ALP levels showed a significant ($P < 0.05$) increase as compared to the sham-operated rats. Bisacurone (100 µg/kg), alendronate, and a combination of alendronate and bisacurone (100 µg/kg)-treated OVX-induced OP rats indicated a substantial ($P < 0.05$) rise in serum calcium and phosphorus levels compared to the control rats. Serum ALP level was found to decline substantially ($P < 0.05$) in the bisacurone (100 µg/kg), alendronate, and the group treated with the combination of alendronate and bisacurone (100 µg/kg) in comparison to the control groups. There was no significant difference in the serum biochemical levels of rats treated with alendronate and those treated with the combination therapy of alendronate and bisacurone (100 µg/kg), but rats treated with combination therapy of alendronate and bisacurone (100 µg/kg) showed an increasing trend of serum calcium and phosphorus levels and decreasing trend of serum ALP level compared to the alendronate-treated rats [Table 3].

Effect of bisacurone on urine biochemical outcome after fracture in female OVX-induced OP rats

The findings of urine biochemical records revealed a marked ($P < 0.05$) increase in the urine phosphorus, calcium, and creatinine levels in the OVX-induced OP control group compared to the sham-operated rats. Eight weeks of treatment with alendronate, bisacurone (100 µg/kg), and a combination of alendronate and

Table 2: Effect of bisacurone on physical parameters after fracture in female OVX-induced OP rats

Parameter	Sham	Control	A (3)	B (25)	B (50)	B (100)	A (3) + B (100)
Body weight gain (g)	25.19±3.12	47.06±2.73$^{\#}$	43.77±2.92$^{*\$}$	43.39±±2.43	47.03±2.11	44.8±4.30$^{*\$}$	45.08±4.47$^{*\$}$
Weight of uterus (g)	0.66±0.04	0.32±0.02$^{\#}$	0.63±0.03$^{*\$}$	0.32±0.03	0.37±0.04	0.51±0.02$^{*\$}$	0.66±0.04$^{*\$}$
Weight of femur bone (g)	0.77±0.05	0.30±0.04$^{\#}$	0.68±0.03$^{*\$}$	0.30±0.03	0.34±0.02	0.60±0.05$^{*\$}$	0.74±0.06$^{*\$}$
Length of femur bone (mm)	22.97±0.51	18.22±0.38$^{\#}$	22.93±0.42$^{*\$}$	19.12±0.53	20.05±0.49	22.88±0.36$^{*\$}$	22.93±0.65$^{*\$}$
Thickness of femur bone (mm)	5.13±0.17	4.22±0.17$^{\#}$	5.10±0.19$^{*\$}$	4.15±0.13	3.83±0.18	5.12±0.19$^{*\$}$	5.02±0.13$^{*\$}$
% Calcium in femur bone	1.29±0.04	0.44±0.03$^{\#}$	1.05±0.07$^{*\$}$	0.48±0.03	0.51±0.02	0.98±0.07$^{*\$}$	1.17±0.06$^{*\$}$

Data are expressed as mean±S.EM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple range tests. $^{*}P$<0.05 as compared to the OVX-induced OP control group, $^{\#}P$<0.05 as compared to the sham group, $^{\$}P$<0.05 as compared to one another. Sham: Sham-operated rats; Control: OVX-induced OP control rats; A (3): Alendronate (3 mg/kg, s.c.)-treated rats; B (25): Bisacurone (25 µg/kg, p.o.)-treated rats; B (50): Bisacurone (50 µg/kg, p.o.)-treated rats; B (100): Bisacurone (100 µg/kg, p.o.)-treated rats; A (3) + B (100): Alendronate (3 mg/kg, s.c.) and Bisacurone (100 µg/kg, p.o.)-treated rats

Table 3: Effect of bisacurone on serum and urine biochemical parameters and inflammatory cytokines (TNF-α and IL-6) after fracture in female OVX-induced OP rats

Parameter	Sham	Control	A (3)	B (25)	B (50)	B (100)	A (3) + B (100)
Serum Calcium (mmol/L)	9.98±0.72	5.89±0.47$^{\#}$	9.20±0.17$^{*\$}$	6.15±0.33	6.58±0.41	8.28±0.31$^{*\$}$	9.77±0.58$^{*\$}$
Serum Phosphorus (mmol/L)	13.97±0.81	6.79±0.51$^{\#}$	12.04±0.51$^{*\$}$	6.48±0.31	6.92±0.49	11.44±0.71$^{*\$}$	12.44±0.85$^{*\$}$
Serum ALP (U/L)	22.19±0.97	36.96±1.85$^{\#}$	25.15±2.12$^{*\$}$	36.17±1.47	33.71±1.04	26.71±1.34$^{*\$}$	24.16±1.83$^{*\$}$
Urinary Calcium (mmol/L)	15.16±1.45	36.96±0.69$^{\#}$	16.65±1.12$^{*\$}$	35.67±1.47	34.54±0.76	16.71±1.49$^{*\$}$	15.69±1.57$^{*\$}$
Urinary Phosphorus (mmol/L)	41.16±3.95	108.80±3.37$^{\#}$	46.4±3.58$^{*\$}$	107.9±4.14	106.30±3.96	53.37±4.69$^{*\$}$	42.89±4.63$^{*\$}$
Urinary Creatinine (mg/dL)	0.63±0.07	2.80±0.14$^{\#}$	0.91±0.10$^{*\$}$	2.17±0.37	2.03±0.26	0.94±0.15$^{*\$}$	0.78±0.08$^{*\$}$
TNF-α (pg/mL)	1.73±0.08	3.41±0.09$^{\#}$	2.12±0.10$^{*\$}$	3.27±0.10	3.15±0.09	2.52±0.10$^{*\$}$	1.90±0.05$^{*\$}$
IL-6 (pg/mL)	98.69±7.53	319.3±7.20$^{\#}$	129.5±4.53$^{*\$}$	313.2±5.33	299.4±6.70	247.3±5.15$^{*\$}$	127.2±5.80$^{*\$}$

Data are expressed as mean±S.E.M (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple range tests. $^{*}P$<0.05 as compared to the OVX-induced OP control group, $^{\#}P$<0.05 as compared to the sham group, $^{\$}P$<0.05 as compared to one another. Sham: Sham-operated rats; Control: OVX-induced OP control rats; A (3): Alendronate (3 mg/kg, s.c.)-treated rats; B (25): Bisacurone (25 µg/kg, p.o.)-treated rats; B (50): Bisacurone (50 µg/kg, p.o.)-treated rats; B (100): Bisacurone (100 µg/kg, p.o.)-treated rats; A (3) + B (100): Alendronate (3 mg/kg, s.c.) and Bisacurone (100 µg/kg, p.o.)-treated rats

bisacurone (100 µg/kg) markedly (P < 0.05) reduced the level of these parameters in OVX-induced OP rats with respect to the control rats. Moreover, OVX-induced OP rats treated with combination therapy of alendronate and bisacurone (100 µg/kg) showed a higher decreasing trend of urine biochemical parameters compared to alendronate-treated rats, but no significant difference was found between these two therapies [Table 3].

Effect of bisacurone on bone turnover markers after fracture in female OVX-induced OP rats

Figure 2 shows that mRNA expression of RANKL, osteocalcin, and PPAR-γ was significantly (P < 0.05) upregulated in the control group compared to the sham-operated rats. However, the rats treated with bisacurone (100 µg/kg), alendronate, and combination therapy of alendronate and bisacurone (100 µg/kg) showed significant (P < 0.05) downregulation of osteocalcin, PPAR-γ, and RANKL mRNA expression compared to the control rats. Similarly, OVX-induced OP control rats showed significant (P < 0.05) downregulation of Runx2, OPG, and AMPK compared to the sham-operated rats. Noticeably, rats treated with bisacurone (100 µg/kg), alendronate, and combination therapy of alendronate and bisacurone (100 µg/kg) showed a significant (P < 0.05) upregulation of Runx2, OPG, and AMPK compared to the control rats. No significant difference was found between the alendronate-treated OVX-induced OP rats and the rats treated with the combination of alendronate and bisacurone (100 µg/kg). Nevertheless, rats treated with combination therapy showed better trends of RANKL, osteocalcin, and PPAR-γ downregulation and Runx2, OPG, and AMPK upregulation [Figure 2].

Effect of bisacurone on level of TNF-α and IL-6 after fracture in female OVX-induced OP rats

The OVX-induced OP control rats showed a substantial (P < 0.05) increase in the level of IL-6 and TNF-alpha in comparison to the sham-operated rats. The bisacurone (100 µg/kg), alendronate, and combination of alendronate and bisacurone (100 µg/kg)-treated rats showed a marked (P < 0.05) attenuation in the IL-6 and TNF-alpha levels in comparison to the OVX-induced OP control rats. No significant difference was observed in the levels of IL-6 and TNF-alpha between the alendronate-treated OVX-induced OP rats and a combination of alendronate and bisacurone (100 µg/kg)-treated OVX-induced OP rats. However, a better reducing trend of cytokines was observed in OVX-induced OP rats administered with the combination of alendronate and bisacurone (100 µg/kg) [Table 3].

Histopathological changes induced by bisacurone after fracture in female OVX-induced OP rats

In the histopathological analysis, Figure 3b shows the normal structure of femur bone with many lamellae and lacuna observed in the sham-operated rats. The femur bone of control rats showed the presence of decreased intertrabecular spaces with porosity [Figure 3a]. The femur bone of OVX-induced OP rats treated with bisacurone (100 µg/kg), alendronate, and a combination of alendronate and bisacurone (100 µg/kg) were discovered to be normal with intertrabecular spaces and free of porosity or destruction [Figure 3e, 3c, and 3f, respectively]. However, no significant difference in histoarchitecture was observed between the group of rats treated with the combination of alendronate and bisacurone (100 µg/kg), and alendronate-treated rats. Combination therapy of alendronate and bisacurone (100 µg/kg) showed a better histoarchitecture than alendronate-treated OVX-induced OP rats [Figure 3].

DISCUSSION

Healing of bone fracture is a process where tissue formation and bone function gradually increase along with the recovery of the bone injury. OP plays an inhibitory role in the healing process, which leads to loss of activity and suffering from many disabilities. Bone loss is very common

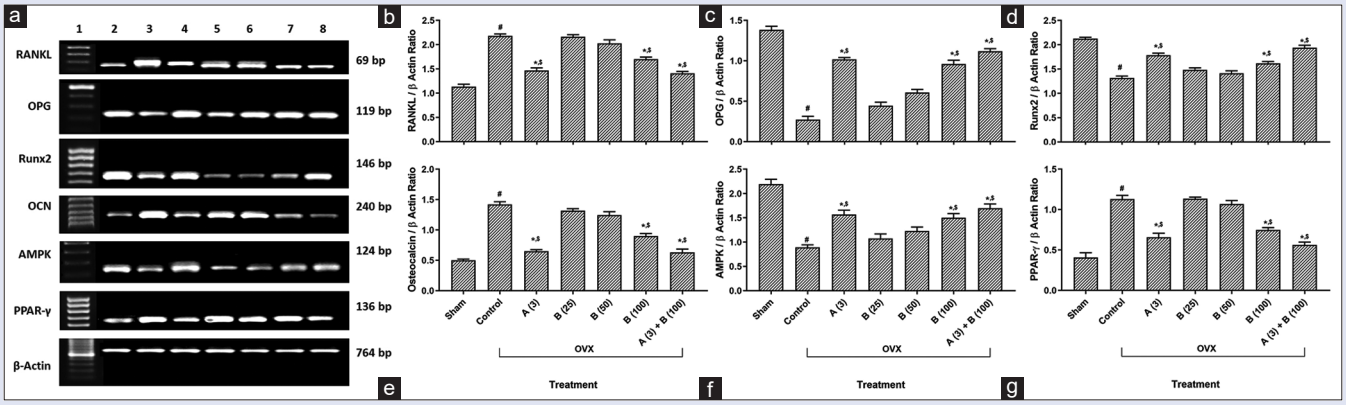

Figure 2: Effects of bisacurone on RANKL, OPG, Runx2, Osteocalcin, AMPK, and PPAR-γ after fracture in female OVX-induced OP rats. (a). Quantitative representation of the mRNA expression are as follows: (b) RANKL; (c) OPG; (d) Runx2; (e) Osteocalcin; (f) AMPK; (G) PPAR- γ. 1000 base pair ladder of mRNA expression (Lane 1); Sham-operated group mRNA expression (Lane -2); Control group mRNA expression (Lane 3); alendronate 3 mg/kg group mRNA expression (Lane 4); Bisacurone 25, 50, 100 µg/kg groups mRNA expression (Lanes 5–7); and combination of bisacurone 100 µg/kg and alendronate 3 mg/kg group mRNA expression (Lane 8). Data are expressed as mean ± S.E.M. (n = 6) and analyzed by one-way ANOVA followed by the Bonferroni test. *$P < 0.05$ as compared to the control group, #$P < 0.05$ as compared to the sham group, $$P < 0.05$ as compared to one another. Sham: Sham-operated rats; Control: OVX control rats; a (3): Alendronate (3 mg/kg, s.c.)-treated rats; b (25): Bisacurone (25 µg/kg, p.o.)-treated rats; b (50): Bisacurone (50 µg/kg, p.o.)-treated rats; b (100): Bisacurone (100 µg/kg, p.o.)-treated rats; a (3) + b (100): Alendronate (3 mg/kg, s.c.) and Bisacurone (100 µg/kg, p.o.)-treated rats

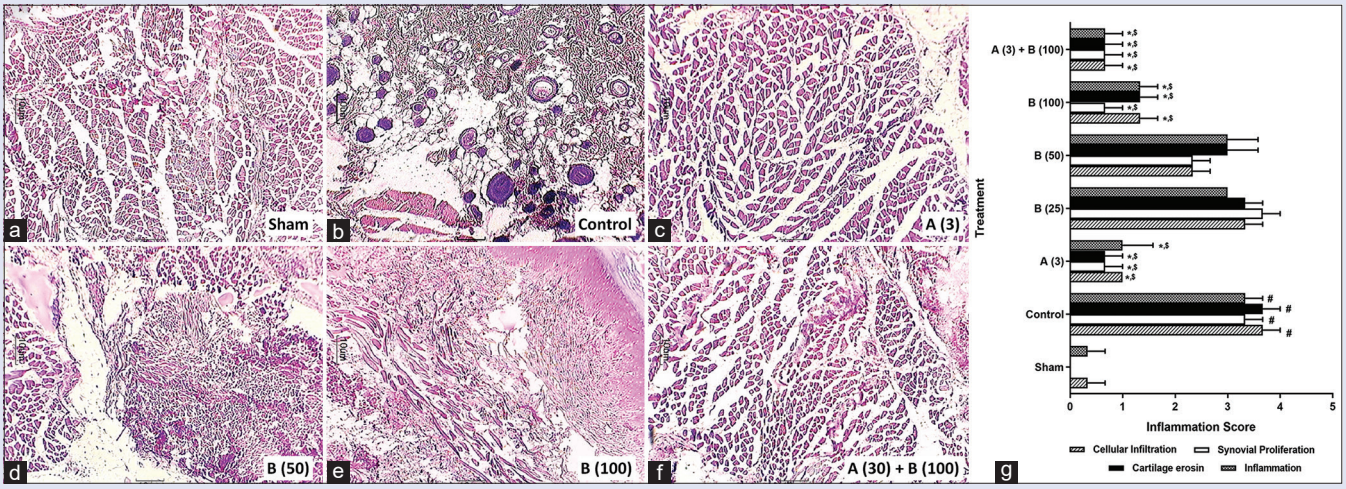

Figure 3: Effects of bisacurone on the histopathology of femur bone after fracture in female OVX-induced OP rats. Representative histological images from (a) sham; (b) control; (c) Alendronate (3 mg/kg); (d) Bisacurone (50 µg/kg); (e) Bisacurone (100 µg/kg) and (f) Alendronate + bisacurone (3 mg/kg and 100 µg/kg)-treated rats. Images stained with H and E (×100). The quantitative representation of histological score (g). Data were expressed as mean ± S.E.M. (n = 3), and one-way ANOVA followed by the Kruskal–Wallis test was applied for *post hoc* analysis. For comparison with control group: *$P < 0.05$, comparison with sham group: #$P < 0.05$ and comparison with one another: $$P < 0.05$. Sham: Sham-operated rats; Control: OVX control rats; a (3): Alendronate (3 mg/kg, s.c.)-treated rats; b (25): Bisacurone (25 µg/kg, p.o.)-treated rats; b (50): Bisacurone (50 µg/kg, p.o.)-treated rats; b (100): Bisacurone (100 µg/kg, p.o.)-treated rats; A (3) + B (100): Alendronate (3 mg/kg, s.c.) and Bisacurone (100 µg/kg, p.o.)-treated rats

for osteoporotic patients because the metaphyseal region of bone mass is damaged and requires treatment. Therefore, exploring a therapeutic molecule with osteogenic potential, bone formation capability, and anti-bone loss potential is the need of the hour.[39] Women are more prone to pathological fractures due to postmenopausal OP.[39] Hormone replacement therapy is the most recommended therapy, but it results in several adverse events such as breast cancer, ovarian cancer, and cardiac disease.[40] It is crucial to explore alternative treatments thoroughly according to the general population health concern, keeping in view the burden of fractures related to OP.[41] Available therapy has been deemed inadequate to control the complications related to fracturing and the prevalence of OP. Therefore, studies have been undertaken to find a more potential therapeutic strategy for the management of OP. *Curcuma longa* is used as a traditional medicine to treat a variety of illnesses, including arthritis. It has a broad spectrum of therapeutic properties and pharmacological effects such as antioxidant, anticancer, antihypertensive, anti-inflammatory, anti-hyperlipidemia, antidiabetic, and anti-arthritic potentials. *Curcuma longa* contains several phytoconstituents, such as demethoxycurcumin, curcumin, ar-turmerone, bisdemethoxycurcumin, and volatile oils, accountable for its various pharmacological properties.[42] Along with these compounds, researchers have identified an active compound from *C. longa*, which is found to be safe and effective against malignancy and hepatitis.[22,43] Evidence from research demonstrated that a decoction of *C. longa* possesses anti-inflammatory and antioxidant potential due to the existence of this compound known as bisacurone.[23] Moreover, an in-vitro analysis reported that

bisacurone shows its activity against inflammation through inhibition of TNF- α in the endothelial cells.[23] Furthermore, recent studies reported its cardioprotective effect in streptozotocin (STZ)-induced diabetic animal model via the downregulation of apoptotic and inflammatory pathways.[44]

The behavioral pain model of C57BL/6J and C3H/HeJ mice has been previously discussed.[45] In our experiment, we included female rats based on various previous studies conducted on pain and bone-related research using this animal model.[46] Here, we have directly observed behavioral parameters such as flinching, weight-bearing, and guarding of the fractured femur bone.[47] These parameters have a notable utility in assessing the effect of new analgesic therapy on fracture pain or fracture rehabilitation. The patient's ability to bear weight on the injured extremity voluntarily is one of the indicators of successful bone repair after therapy.[48]

Previous studies reported in the preclinical model of acute and chronic pain that there was a significant variation in spontaneous pain-related activities (lifting/licking, guarding) across time in rats versus mice.[49] Researchers observed that flinching and guarding behaviors were unconstrained and unpredictable pain behaviors because the animal used to guard their paw (guarding) and withdraw their paw (flinching) to reduce the use of their fractured hind limb.[45] Weight-bearing measurement was performed using a capacitance meter, where the meter measured the load borne by the hind limb that was fractured compared to the hind limb of the non-fractured rats.[50] In our experiment, the rats treated with bisacurone showed a significant reduction in guarding and flinching compared to the control OVX-induced OP rats. Additionally, bisacurone-treated rats were able to bear more weight compared to the control OVX-induced OP rats.

For bone resorption, biochemical parameters are widely used to examine the effect of different drugs on the restoration of bone in arthritic bone fractures.[51] Menopause is the leading cause of calcium elimination through the renal system and excretion of calcium from the intestine, resulting in a negative balance of calcium.[35] Evidence from previous studies reported that OVX-induced OP showed a marked increase in phosphorous and urinary calcium levels due to bone turnover.[52] In our experiment, it was observed that OVX-induced OP rats had decreased phosphorus and serum calcium levels. It has been reported that phosphorus and calcium endocytic absorption is stimulated by estrogen receptors, which also help in the transport of calcium and phosphorus into the duodenal cells.[53] After being treated with bisacurone, absorption of calcium increased, which led to increased levels of phosphorus and calcium in the serum of OVX-induced OP rats. Similarly, a large amount of phosphorus and calcium elimination through urine indicated a higher bone loss in the OVX-induced OP rats. The elimination of phosphorus and calcium was substantially reduced with the administration of bisacurone and alendronate. The estrogenic effect of bisacurone can explain these findings. In OP, the serum ALP level is found to be high;[54] however, OVX-induced OP rats showed reduced serum ALP after being treated with bisacurone. These findings indicate that bisacurone helps in enhancing the resorption of bone and increasing the mobilization of bone minerals. It has been shown in a previous study that kidney disease is the most common risk factor in OVX-induced OP rats.[44] In our experiment, bisacurone was also found to be effective against kidney damage caused by ovariectomy. The bone fracture was classified by reduced bone thickness, weight, and length of the femur bone.[55] The observation of our experiment notified that consumption of bisacurone for 56 days significantly increased bone thickness, weight, and length. The mechanism behind this is almost similar to the mechanism of alendronate for the same parameters in the femur bone. Moreover, consumption of bisacurone enabled the restoration of femoral calcium ash content to a normal level. Therefore, bisacurone was found to be effective in calcium absorption in OVX-induced OP rats.

The main reason for bone loss in postmenopausal females is the persistent inflammatory immune level.[56] Moreover, previous studies have shown that T-lymphocyte cells get considerably affected due to the estrogen-deficiency, leading to an altered number of T-lymphocyte cells in the spleen and bone marrow.[57] In accordance with our findings, estrogen-deficient animals frequently showed variation in the ratio of CD4+ and CD8+ cells in the bone marrow.[58] Earlier studies have reported that T-lymphocyte cells regulate the environment of bone marrow in an osteoclastogenic and anti-osteoclastogenic manner. This regulation is dependent on the subset of T cells and has a significant impact on bone homeostasis.[59] After the withdrawal of the estrogen, activated T-lymphocytes help to produce a large amount of TNF-α, resulting in increased bone resorption. Studies have shown that Th-17 cells, in particular, have a link with T-cell activation and osteoclast activation.[60] The role of CD8+ and CD4+ cells has been widely explored in post-menopausal condition.[59] Depending on how these cells are triggered, osteoclastogenic or anti-osteoclastogenic effects can be mediated through both subsets.[59] However, much is still unexplored about the role of T-lymphocyte cells in the inflammatory process of postmenopausal bone loss. Even the role of bone marrow neutrophils is still unclear in the osteoporotic bone loss. Several phenomenological studies reported unaltered,[61] decreased,[62] or increased[63] neutrophil numbers after the withdrawal of estrogen, but no mechanistic studies are available for these observations. In reviews, various pro-inflammatory cytokines are mentioned, which are found to be involved in increasing the extremity of inflammatory disorders in estrogen-deficient subjects. IL-6 is one of the cytokines, which plays a crucial role in the process of inflammatory cells recruitment.[64] Several studies have reported that after estrogen-deficiency, IL-6 expression increases due to the tissue injury.[65] In our experiment as well, we observed that IL-6 expression increased in the periosteal cells of OVX-induced OP rats. Indeed, it has been previously observed that increased IL-6 expression is related to higher neutrophil numbers in the callus of the fracture after trauma.[66] Therefore, we can suggest that higher IL-6 expression after menopause is responsible for the increased neutrophil numbers in callus of the fracture in OVX-induced OP rats.

The immunomodulatory effect and vast clinical application of bisacurone have been widely discussed in various studies. Osteoblast differentiation was found to be enhanced by bisacurone through the suppression of nuclear factor kappa B (NF-κB) and TNF-α in the arthritic bone loss. TNF-α has also been reported to inhibit the differentiation of osteoblasts by activating NF-κB.[57] A previous study reported that bisacurone suppresses the vascular cell adhesion molecule 1 (VCAM-1) expression via TNF-α-induction, which potentially suppresses the generation of reactive oxygen species (ROS) in TNF-α-stimulated human umbilical vein endothelial cells (HUVECs). Moreover, bisacurone-mediated suppression of TNF-α results in the downregulation of transcription factor NF-κB.[67] This was attributed to the inhibition of phosphorylation of the IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha).[26] Thus, we can suggest that bisacurone has an anti-inflammatory and bone/cartilage protective effect via the inhibition of proinflammatory cytokines.

The bone remodeling maintenance process is very important for the normalization of bone mass. A previous examination evidenced that imbalance of bone turnover markers in arthritis lead to a reduction in the capacity of the bone remodeling process and an increase in the risk of bone fracture.[68] In arthritis, bone turnover markers are altered due to the activation of PPAR-γ, which assists in increasing the chances of bone fragility.[35] RANKL acts as a regulator in the bone development process. The expression of RANKL occurs in the activated T cells, where it targets

the immune system through mature dendritic cells and produces many RANKL receptors (RANK). In an in-vitro analysis, the survival of mature dendritic cells was observed to be promoted by RANKL through the upregulation of B-cell lymphoma-extra-large (Bcl-xL) expression and subsequently influencing the secretion of proinflammatory cytokines like IL-6 and IL-1, IL-12, and IL-15.[69] Therefore, RANKL was found to be a regulator with positive feedback during the generative T-cell–dendritic cell interactions.[70] OPG is a glycoprotein containing 380 amino-acids and seven distinct structural domains. It is generated by several tissues of the body, including the cardiovascular (CV) system, and behaves as a cytokine receptor[53] OPG synthesis is promoted by tumor growth factor−α (TGF−α), tumor growth factor−β (TGF−β), and bone morphogenetic proteins (BMPs), while factors such as parathyroid hormone (PTH), cyclosporine A, and prostaglandin E2 inhibit the synthesis of OPG.[53] Osteoclast maturation and differentiation has been found to be inhibited by OPG.[36] It helps to promote apoptosis in the endothelial cells and plays a crucial role in the survival of cells through its TNF-related apoptosis-inducing ligand interaction.[70] OPG and RANKL present a similar mechanism of action.[53] RANKL inhibits the activation and formation of osteoclasts by RANK deactivation. RANK is a 616 amino-acid transmembrane protein located in the osteoclasts.[53] In healthy condition, RANKL is generated from osteoblasts and promotes the activation and synthesis of osteoclast, which leads to the loss and reabsorption of bone calcium. OPG can block RANKL and inhibit the interaction between RANK and RANKL, and osteoclast maturation.[53] Therefore, it can prevent the release of phosphorus and calcium from bone and prevent the bone remodeling disorder.[70] Animal cell studies and preclinical studies have reported elevated osteoclast differentiation in response to increased mRNA expression of RANKL and decreased OPG mRNA expression in arthritic rats.[53] Similarly, preclinical studies reported that arthritic rats are more prone to upregulation of RANKL and downregulation of OPG mRNA.[54] Moreover, in our experiment, arthritic condition also influenced the abnormal OPG and RANKL mRNA expression. Noticeably, bisacurone improved the mRNA expression of OPG and RANKL in the OVX-induced OP rats. Therefore, we can state that bisacurone enhances the differentiation of osteoblast and osteoclast through the alteration of OPG and RANKL mRNA expression in experimental OP rats and inhibits the effect of osteoclast formation, which is mediated through RANKL.

Runx2 is a major transcription factor that helps in the development of mesenchymal stem cells (MSC) into the lineage of osteoblast. It is also responsible for bone formation by modulation of extracellular matrix protein osteocalcin expression in the osteoblasts. Osteocalcin is a protein linked with a specific osteoblast and is a crucial factor for the bone formation process. Runx2 can also downregulate the Wingless-related integration site (Wnt) signaling pathway, resulting in reduced bone resorption in the osteoblasts.[71] The present investigation revealed that OVX-induced OP rats have upregulated osteocalcin and downregulated Runx2 mRNA expression. These findings are consistent with the previous preclinical research.[44] However, bisacurone and alendronate significantly improved bone loss by enhancing Runx2 and attenuating mRNA expression of osteocalcin in OVX-induced OP rats. Therefore, it can be inferred that bisacurone has a potential effect on the management of mRNA expression of osteocalcin and Runx2 level and helps to promote the osteoblast differentiation by Runx2 transcriptor gene activation.

A previous study reported that PPAR-γ plays a key role in the modulation of skeletal remodeling. The activation of PPAR-γ activates osteoclastogenesis and suppresses osteoblastogenesis, leading to a decrease in bone formation and an increase in bone resorption.[72] In OVX-induced OP control rats, the level of PPAR-γ was found to be increased, which significantly reduced after the treatment with bisacurone and alendronate. AMPK, a serine and threonine kinase, is a cellular energy metabolism regulator. It has been reported that deficiency of AMPK stimulates an M1- phenotype.[72] The increased ratio of inducible nitric oxide synthase and arginase1 (iNOS/Arg1) represents M1- phenotype resulting in the increased glucose intake. Furthermore, activated macrophages were found to induce activation of hypoxia-inducible factor 1α (HIF-1α) and glycolysis under the normoxic conditions. Previous study explored that HIF-1α helps in controlling the metabolic switch along with promoting adaptation of cellular hypoxia in the inflammatory cells. Deactivating HIF-1α inhibits the polarization and glycation of M1-macrophage and suppresses the inflammatory response in skin and joints.[73] OVX-induced OP rats have a low level of AMPK and a high level of PPAR-γ, but bisacurone therapy increased the AMPK level and decreased the PPAR-γ level. Thus, it can be inferred that bisacurone has AMPK activation and PPAR-γ inhibition properties.

Histopathological examinations in our study revealed that there was porosity and low intertrabecular spaces in OVX-induced OP rats. However, the OVX-induced OP rats treated with bisacurone showed very little porosity with intertrabecular spaces, which was similar to the alendronate-treated group. These findings indicate that bisacurone has a bone protective effect due to its potential for inducing higher bone mineralization with decreased bone loss.

CONCLUSION

Bisacurone therapy significantly reduced bone loss, resorption, and pain after a fracture. The behavioral and biochemical measurements provided supportive evidence for bisacurone therapy. Moreover, histoarchitecture of bisacurone treated rats showed normal structure of bone. Bisacurone demonstrated good antiosteoporotic activity, like the standard drug alendronate. In addition, the current findings indicate that antiosteoporotic activity of bisacurone is mediated through its anti-inflammatory effect and the RANKL and OPG mRNA expression pathway. It can be concluded that our study substantially showed the efficacy of bisacurone for the treatment of postmenopausal osteoporosis which if explored further could be of profound clinical implication (Pictorial Abstract).

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ozgediz D, Chu K, Ford N, Dubowitz G, Bedada AG, Azzie G, *et al*. Surgery in global health delivery. Mt Sinai J Med 2011;78:327-41.
2. Mock C, Cherian MN. The global burden of musculoskeletal injuries: Challenges and solutions. Clin Orthop Relat Res 2008;466:2306-16.
3. Oden A, McCloskey EV, Kanis JA, Harvey NC, Johansson H. Burden of high fracture probability worldwide: Secular increases 2010–2040. Osteoporos Int 2015;26:2243-8.
4. Qian G, Zhang L, Wang G, Zhao Z, Peng S, Shuai C. 3D Printed Zn-doped mesoporous silica-incorporated Poly-Llactic acid scaffolds for bone repair. Int J Bioprint 2021;7:346.
5. Bozo IY, Deev RV, Smirnov IV, Fedotov AY, Popov VK, Mironov AV, *et al*. 3D Printed Gene-activated Octacalcium Phosphate Implants for Large Bone Defects Engineering. Int J Bioprint 2020;6:275.
6. Zheng C, Attarilar S, Li K, Wang C, Liu J, Wang L, *et al*. 3D-printed HA15-loaded beta-Tricalcium Phosphate/Poly (Lactic-co-glycolic acid) bone tissue scaffold promotes bone regeneration in rabbit radial defects. Int J Bioprint 2021;7:317.
7. Cruess RL, Dumont J. Healing of bone, tendon, and ligament. Fractures 1975;97:32-4.
8. Daskalakis E, Liu F, Huang B, Acar AA, Cooper G, Weightman A, *et al*. Investigating the influence of architecture and material composition of 3D printed anatomical design scaffolds

for large bone defects. Int J Bioprint 2021;7:268.
9. Dai KR, Hao YQ, Quality of healing compared between osteoporotic fracture and normal traumatic fracture. In: Advanced Bioimaging Technologies in Assessment of the Quality of Bone and Scaffold Materials, Springer; 2007. p. 531-41.
10. Mei Q, Rao J, Bei HP, Liu Y, Zhao X. 3D Bioprinting photo-crosslinkable hydrogels for bone and cartilage repair. Int J Bioprint 2021;7:367.
11. Manolagas SC. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 2000;21:115-37.
12. Ralston SH. Analysis of gene expression in human bone biopsies by polymerase chain reaction: Evidence for enhanced cytokine expression in postmenopausal osteoporosis. J Bone Miner Res 1994;9:883-90.
13. Chauhan R. Cancer and obesity: A biological perspective. Cancer Plus 2020;3:31-7.
14. Kinoshita T, Kobayashi S, Ebara S, Yoshimura Y, Horiuchi H, Tsutsumimoto T, *et al.* Phosphodiesterase inhibitors, pentoxifylline and rolipram, increase bone mass mainly by promoting bone formation in normal mice. Bone 2000;27:811-7.
15. Schaffer D, Florin T, Eagle C, Marschner I, Singh G, Grobler M, *et al.* Risk of serious NSAID-related gastrointestinal events during long-term exposure: A systematic review. Med J Aust 2006;185:501-6.
16. Ravindran V, Rachapalli S, Choy EH. Safety of medium- to long-term glucocorticoid therapy in rheumatoid arthritis: A meta-analysis. Rheumatology (Oxford) 2009;48:807-11.
17. Grazio S, Balen D. Complementary and alternative treatment of musculoskeletal pain. Acta Clin Croat 2011;50:513-30.
18. Elalouf O, Elkayam O. Long-term safety and efficacy of infliximab for the treatment of ankylosing spondylitis. Ther Clin Risk Manag 2015;11:1719-26.
19. Singh GB, Atal CK. Pharmacology of an extract of salai guggal ex-Boswellia serrata, a new non-steroidal anti-inflammatory agent. Agents Actions 1986;18:407-12.
20. Lodha R, Bagga A. Traditional Indian systems of medicine. Ann Acad Med Singap 2000;29:37-41.
21. Aoki H, Takada Y, Kondo S, Sawaya R, Aggarwal BB, Kondo Y. Evidence that curcumin suppresses the growth of malignant gliomas *in vitro* and *in vivo* through induction of autophagy: Role of Akt and extracellular signal-regulated kinase signaling pathways. Mol Pharmacol 2007;72:29-39.
22. Kawasaki K, Muroyama K, Fukuhara I, Murosaki S. Effect of a combination of hot water extract of curcuma longa and curcumin on serum liver enzymes, inflammatory markers, and emotional states in healthy participants with moderately high body mass index. Japanese J Clin Pharmacol Ther 2017;5:5-10.
23. Ohshiro M, Kuroyanagi M, Ueno A. Structures of sesquiterpenes from Curcuma longa. Phytochemistry 1990;29:2201-05.
24. Uchio R, Higashi Y, Kohama Y, Kawasaki K, Hirao T, Muroyama K, *et al.* A hot water extract of turmeric (Curcuma longa) suppresses acute ethanol-induced liver injury in mice by inhibiting hepatic oxidative stress and inflammatory cytokine production. J Nutr Sci 2017;6:e3.
25. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Lett 1995;94:79-83.
26. Sun DI, Nizamutdinova IT, Kim YM, Cai XF, Lee JJ, Kang SS, *et al.* Bisacurone inhibits adhesion of inflammatory monocytes or cancer cells to endothelial cells through down-regulation of VCAM-1 expression. Int Immunopharmacol 2008;8:1272-81.
27. Caro AC, Tucker JJ, Yannascoli SM, Dunkman AA, Thomas SJ, Soslowsky LJ. Efficacy of various analgesics on shoulder function and rotator cuff tendon-to-bone healing in a rat (Rattus norvegicus) model. J Am Assoc Lab Anim Sci 2014;53:185-92.
28. Little DG, Ramachandran M, Schindeler A. The anabolic and catabolic responses in bone repair. J Bone Joint Surg Br 2007;89:425-33.
29. Freidouni M, Nejati H, Salimi M, Bayat M, Amini A, Noruzian M, *et al.* Evaluating glucocorticoid administration on biomechanical properties of rats' tibial diaphysis. Iran Red Crescent Med J 2015;17:e19389.
30. Farahani MM, Farahani RM, Mostafavinia A, Abbasian MR, Pouriran R, Noruzian M, *et al.* Effect of pentoxifylline administration on an experimental rat model of femur fracture healing with intramedullary fixation. Iran Red Crescent Med J 2015;17:e29513.
31. Cui Y, Chi J, Wang L. Protective efficacy of bisacurone in diabetic cardiotoxicity in experimental animals via downregulation of inflammatory and apoptotic pathways. Lat Am J Pharm 2020;39:2507-19.
32. Nair AB, Kaushik A, Attimarad M, Al-Dhubiab BE. Enhanced oral bioavailability of calcium using bovine serum albumin microspheres. Drug Deliv 2012;19:277-85.
33. Reddy NP, Lakshmana M, Udupa UV. Antiosteoporotic activity of OST-6 (Osteocare), a herbomineral preparation in calcium deficient ovariectomized rats. Phytother Res 2004;18:25-9.
34. Washburn ML, Shear MJ. Composition of bone. XIII. Direct gravimetric determination of Ca, Mg, and PO4. Proc Soc Exp Biol Med 1932;29:625-7.
35. Adil M, Khan RA, Kalam A, Venkata SK, Kandhare AD, Ghosh P, *et al.* Effect of anti-diabetic drugs on bone metabolism: Evidence from preclinical and clinical studies. Pharmacol Rep 2017;69:1328-40.
36. Wei W, Wang X, Yang M, Smith LC, Dechow PC, Wan Y. PGC1β mediates PPARγ activation of osteoclastogenesis and rosiglitazone-induced bone loss. Cell Metab 2010;11:503-16.
37. Smith SY, Samadfam R, Chouinard L, Awori M, Benardeau A, Bauss F, *et al.* Effects of pioglitazone and fenofibrate co-administration on bone biomechanics and histomorphometry in ovariectomized rats. J Bone Miner Metab 2015;33:625-41.
38. Jiang Z, Li Z, Zhang W, Yang Y, Han B, Liu W, *et al.* Dietary Natural N-Acetyl-d-Glucosamine prevents bone loss in ovariectomized rat model of postmenopausal osteoporosis. Molecules 2018;23:2302.
39. Rengarajan T, Keerthiga S, Duraikannu S, Periyannan V. Exploring the anticancer and anti-inflammatory activities of ferruginol in mcf-7 breast cancer cells. Cancer Plus 2020;1:1-12.
40. Izzah Ibrahim N, Mohamed N, Nazrun Shuid A. Update on statins: Hope for osteoporotic fracture healing treatment. Curr Drug Targets 2013;14:1524-32.
41. Helmers R, Milstein DM, Straat NF, Rodermond HM, Franken NA, Savci-Heijink CD, *et al.* Outcome of a rabbit model for late irradiation effects in mandibular oral mucosa and bone: A pilot study. J Clin Transl Res 2020;6:225-35.
42. Akbar S. Curcuma longa L.(Zingiberaceae). In: Handbook of 200 Medicinal Plants. Springer; 2020. p. 781-807.
43. Poles J, Karhu E, McGill M, McDaniel HR, Lewis JE. The effects of twenty-four nutrients and phytonutrients on immune system function and inflammation: A narrative review. J Clin Transl Res 2021;7:333-76.
44. Cui M, Yu LZ, Zhang N, Wang LJ, Sun J, Cong J. Zoledronic acid improves bone quality in the streptozotocin-induced diabetes rat through affecting the expression of the osteoblast-regulating transcription factors. Exp Clin Endocrinol 2019;6:68-75.
45. Jimenez-Andrade JM, Martin CD, Koewler NJ, Freeman KT, Sullivan LJ, Halvorson KG, *et al.* Nerve growth factor sequestering therapy attenuates non-malignant skeletal pain following fracture. Pain 2007;133:183-96.
46. Walker K, Fox AJ, Urban LA. Animal models for pain research. Mol Med Today 1999;5:319-21.
47. Gerstenfeld LC, Al-Ghawas M, Alkhiary YM, Cullinane DM, Krall EA, Fitch JL, *et al.* Selective and nonselective cyclooxygenase-2 inhibitors and experimental fracture-healing. Reversibility of effects after short-term treatment. J Bone Joint Surg Am 2007;89:114-25.
48. Skak SV, Jensen TT. Femoral shaft fracture in 265 children. Log-normal correlation with age of speed of healing. Acta Orthop Scand 1988;59:704-7.
49. Banik RK, Woo YC, Park SS, Brennan TJ. Strain and sex influence on pain sensitivity after plantar incision in the mouse. Anesthesiology 2006;105:1246-53.
50. Medhurst SJ, Walker K, Bowes M, Kidd BL, Glatt M, Muller M, *et al.* A rat model of bone cancer pain. Pain 2002;96:129-40.
51. Vohra M, Sharma A, Bagga R, Arora SK. Human umbilical cord-derived mesenchymal stem cells induce tissue repair and regeneration in collagen-induced arthritis in rats. J Clin Transl Res 2020;6:203-16.
52. Meema HE, Meema S. The relationship of diabetes mellitus and body weight to osteoporosis in elderly females. Can Med Assoc J 1967;96:132-9.
53. Ferreira EC, Bortolin RH, Freire-Neto FP, Souza KS, Bezerra JF, Ururahy MA, *et al.* Zinc supplementation reduces RANKL/OPG ratio and prevents bone architecture alterations in ovariectomized and type 1 diabetic rats. Nutr Res 2017;40:48-56.
54. Syversen U, Stunes AK, Gustafsson BI, Obrant KJ, Nordsletten L, Berge R, *et al.* Different skeletal effects of the peroxisome proliferator activated receptor (PPAR) alpha agonist fenofibrate and the PPARgamma agonist pioglitazone. BMC Endocr Disord 2009;9:10.
55. Bai F, Chen X, Yang H, Xu HG. Acetyl-11-Keto-β-Boswellic Acid Promotes Osteoblast Differentiation by Inhibiting Tumor Necrosis Factor-α and Nuclear Factor-κB Activity. J Craniomaxillofac Surg 2018;29:1996-2002.
56. Gusain N, Butola LK. The cross-sectional population-based study conducted to elucidate the prevalence of Vitamin D deficiency, bone turnover marker's variation among adolcents of Haryana. Int J Recent Sci Res 2020;11:39631-4.

57. Roggia C, Gao Y, Cenci S, Weitzmann MN, Toraldo G, Isaia G, *et al.* Up-regulation of TNF-producing T cells in the bone marrow: A key mechanism by which estrogen deficiency induces bone loss in vivo. Proc Natl Acad Sci 2001;98:13960-65.
58. Tyagi AM, Srivastava K, Mansoori MN, Trivedi R, Chattopadhyay N, Singh D. Estrogen deficiency induces the differentiation of IL-17 secreting Th17 cells: A new candidate in the pathogenesis of osteoporosis. PLoS One 2012;7:e44552.
59. Zhao W, Liu Y, Cahill CM, Yang W, Rogers JT, Huang X. The role of T cells in osteoporosis, an update. Int J Clin Exp Pathol 2009;2:544-52.
60. Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, *et al.* Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. J Clin Invest 2000;106:1229-37.
61. Szabó A, Hartmann P, Varga R, Jánvári K, Lendvai Z, Szalai I, *et al.* Periosteal microcirculatory action of chronic estrogen supplementation in osteoporotic rats challenged with tourniquet ischemia. Life Sci 2011;88:156-62.
62. Figueroa-Vega N, Moreno-Frias C, Malacara JM. Alterations in adhesion molecules, pro-inflammatory cytokines and cell-derived microparticles contribute to intima-media thickness and symptoms in postmenopausal women. PLoS One 2015;10:e0120990.
63. Jilka RL, Passeri G, Girasole G, Cooper S, Abrams J, Broxmeyer H, *et al.* Estrogen loss upregulates hematopoiesis in the mouse: A mediating role of IL-6. Exp Hematol 1995;23:500-6.
64. Dunn SL, Vera DL, Weaver KF, Garcia JV. Relationships between inflammatory and metabolic markers, exercise, and body composition in young individuals. J Clin Transl Res 2021;7:289-96.
65. Aydin A, Halici Z, Albayrak A, Polat B, Karakus E, Yildirim OS, *et al.* Treatment with carnitine enhances bone fracture healing under osteoporotic and/or inflammatory conditions. Basic Clin Pharmacol Toxicol 2015;117:173-9.
66. Recknagel S, Bindl R, Brochhausen C, Gockelmann M, Wehner T, Schoengraf P, *et al.* Systemic inflammation induced by a thoracic trauma alters the cellular composition of the early fracture callus. J Trauma Acute Care Surg 2013;74:531-7.
67. Farrugia M, Baron B. The role of TNF-alpha in rheumatoid arthritis: A focus on regulatory T cells. J Clin Transl Res 2016;2:84-90.
68. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL, *et al.* Mechanisms of diabetes mellitus-induced bone fragility. Nat Rev Endocrinol 2017;13:208-19.
69. Rengarajan T, Keerthiga S, Duraikannu S, Periyannan V. Exploring the anticancer and anti-inflammatory activities of ferruginol in mcf-7 breast cancer cells. Cancer Plus 2020;1:1-12.
70. Yoneda T, Ishimaru N, Arakaki R, Kobayashi M, Izawa T, Moriyama K, *et al.* Estrogen deficiency accelerates murine autoimmune arthritis associated with receptor activator of nuclear factor-κB ligand-mediated osteoclastogenesis. Endocrinology 2004;145:2384-91.
71. Haxaire C, Haÿ E, Geoffroy V. Runx2 controls bone resorption through the down-regulation of the Wnt pathway in osteoblasts. Am J Pathol 2016;186:1598-609.
72. Sorocéanu MA, Miao D, Bai XY, Su H, Goltzman D, Karaplis AC. Rosiglitazone impacts negatively on bone by promoting osteoblast/osteocyte apoptosis. J Endocrinol 2004;183:203-16.
73. Yu Y, Cai W, Zhou J, Lu H, Wang Y, Song Y, *et al.* Anti-arthritis effect of berberine associated with regulating energy metabolism of macrophages through AMPK/HIF-1α pathway. Int Immunopharmacol 2020;87:106830.