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Original Research Article

Boeravinone B promotes fracture healing in ovariectomy-induced osteoporotic rats via the regulation of NF-κB p65/IκB-α/SIRT-1 signaling pathway

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Abstract

Purpose: *To investigate the fracture-healing effect of boeravinone B in ovariectomy-induced (OVX) osteoporotic rats.*
Methods: *Adult female Wistar rats (n = 30) were ovariectomized and after three months, the unilateral cross-tibial fractures were fixed with intramedullary nails. The rats were then randomly assigned to three groups of 10 rats each: normal control group, OVX group and 100 mg/kg body weight boeravinone B group. Boeravinone B was orally administered for a period of 5 weeks. The effect of boeravinone B on indices of bone formation and resorption was assessed. Levels of inflammatory cytokines including tumor necrosis factor- α (TNF-α) and interleukin-1β (IL-1β) were determined using enzyme-linked immunosorbent assay (ELISA). Western blotting was used to determine the expression levels of NF-κB p65, IκB-α and SIRT1 proteins.*
Results: *There were significant increases in the activities of tartrate-resistant acid phosphatase (TRAP) and alkaline phosphatase (ALP), and collagen type I fragment (CTX) level and serum osteocalcin (OC) of OVX group, when compared with normal control group ($p < 0.05$). However, treatment with boeravinone B significantly reduced the activities and levels of these parameters, relative to OVX group ($p < 0.05$). The levels of TNF-α and IL-1β significantly increased in OVX group, relative normal control group, but were significantly lower following treatment with boeravinone B ($p < 0.05$). Bone mineral content (BMC) was not significantly altered in OVX and boeravinone B-treated groups, when compared with normal control group ($p > 0.05$). There was significant reduction in bone mineral density (BMD) of OVX group relative to normal control group ($p < 0.05$). However, treatment with boeravinone B significantly increased the BMD, when compared with OVX group ($p < 0.05$). After Week 5 of treatment, boeravinone B significantly enhanced bone remodeling and formation of callus. Treatment with boeravinone B significantly reduced the expression levels of NF-κB p65 and IκB-α proteins, and significantly upregulated the expression of SIRT-1 ($p < 0.05$).*
Conclusion: *The results obtained in this study suggest that boeravinone B promotes the healing of fracture caused by osteoporosis via a mechanism involving NF-κB p65/IκB-α/SIRT-1 signaling pathway.*

Keywords: *Osteoporosis, Boeravinone B, Bone fracture, Ovariectomy, Inflammatory cytokines*

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INTRODUCTION

Osteoporosis is a bone disorder characterized by loss of bone mass which leads to increased susceptibility to fractures and bone frailty due to disturbance in the micro-architecture of bone tissue [1]. Osteoporosis is a major cause of fracture and approximately 9 million people suffer from it globally [2]. Post-menopausal osteoporosis is characterized by deficiency of estrogen triggered by inflammation [3]. Bone mineral density (BMD) decreases in patients with osteoporosis due to deficiency of some hormones and this leads to impairment of cancellous metaphyseal bone [4]. Menopause is regulated by hormone replacement therapy (HRT), but HRT is limited by several factors and promotes ovarian, breast and endometrial cancers [5]. The use of alternative medicine has proved effective in the management of several disorders including osteoporosis. *Boerhavia diffusa* Linn. belongs to *Nyctaginaceae* family and grows in various parts of Asia, Africa and south America [7]. It is traditionally used for the treatment of inflammation, stress, convulsion, dyspepsia and jaundice [8]. The antidiabetic, antidepressant, immunomodulatory, anti-inflammatory, antitumor and antioxidant activities of extracts of this plant have been reported [8–14]. Boeravinone B is one of several compounds present in *Boerhavia diffusa* Linn, and its inflammatory activity occurs via inhibition of the activities of cyclooxygenases 1 and 2 (COX1 and COX2) [15]. The present study investigated the fracture-healing effect of boeravinone B in ovariectomy-induced osteoporotic rats.

EXPERIMENTAL

Rats

Adult female Wistar rats were obtained from Peking University Health Science Center, China. The rats were housed in iron cages under optimum conditions: 12 h day/12 h night cycle, 24 °C and 60 - 65 % humidity. They were allowed free access to standard rat feed and clean water. The study protocol was approved by the Institutional Ethics Committee of Yan'an University Affiliated Hospital, China (approval no. IACUC/YUH/2017/22). The guidelines for the proper use and care of animals as prepared by the National Academy of Sciences, National Institute of Health, were followed to provide humane care to all the rats [16].

Induction of osteoporosis and grouping

The rats were anesthetized by intraperitoneal injection of 50 mg/kg bwt pentobarbitone and subjected to bilateral ovariectomy. Unilateral cross-tibial fracture was produced in the rats after 3 months and fixed with intramedullary nails. The rats were then randomly assigned to three groups of ten rats each: normal control group, OVX group and 100 mg/kg bwt boeravinone B group. Boeravinone B was orally administered for a period of 5 weeks.

Blood sample collection and biochemical analysis

At the end of the treatment period, blood sample was collected from the retro-orbital plexus of rats. The blood was centrifuged at 3000 rpm for 10 min to obtain serum which was used for biochemical analysis. The rats were then sacrificed and their femurs excised, homogenized and also used for biochemical analysis.

Determination of indices of bone formation and resorption

The activities of ALP and TRAP, and levels of OC and CTX were estimated using their respective ELISA kits.

Determination of levels of inflammatory cytokines

The serum levels of TNF-α and IL-1β were estimated using ELISA.

Determination of BMD and x-ray analysis

Lunar Prodigy Advance was used for the determination of BMC and BMD in isolated femurs. The X-ray analysis of rat tibia was performed after 3rd and 5th weeks of treatment to assess remodeling of bone and formation of callus.

Western blotting

The cells were washed with PBS and ice-cold radio-immunoprecipitation assay buffer (RIPA) containing protease inhibitor was used to lyse them. The resultant lysate was centrifuged at 12, 000 rpm for 10 min at 4 °C, and the protein concentration of the supernatant was determined using BCA assay kit. A portion of total cell protein (20 - 30 µg) from each sample was separated on a 12 % sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and transferred to a fixed polyvinylidene fluoride membrane at 110 V and 90 ° C for 120 min. Subsequently, non-fat milk powder (3 %) in Tris-buffered saline containing 0.2 % Tween-20 (TBS-T) was added with gentle shaking at 37 ^{o}C and

incubated to block non-specific binding of the blot. Incubation of the blots was performed overnight at 4 ◦C with primary antibodies for SIRT1, NF-κB p65, IκB-α and β-actin, each diluted 1 to 500. Then, the membrane was washed thrice with TBS-T and further incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody for 1 h at room temperature. The blot was developed using an X-ray film. Grayscale analysis of the bands was performed using ImageJ analysis software (4.6.2). Respective protein expression levels were normalized to that of β-actin which was used as a standard reference.

Statistical analysis

Data are expressed as mean ± SEM, and the statistical analysis was performed using Graph Pad Prism (5.0). Groups were compared using Dunnett's post hoc test, and values of $p < 0.05$ were considered statistically significant.

RESULTS

Effect of boeravinone B on indices of bone formation and resorption

As shown in Figure 1, there were significant increases in the activities of TRAP and ALP, and levels of CTX and OC in the serum of OVX group, when compared with normal control group ($p < 0.05$). However, treatment with boeravinone B significantly reduced the activities and levels of these parameters, relative to OVX group ($p < 0.05$).

Effect of boeravinone B on levels of TNF-α and IL-1β

The serum levels of TNF-α and IL-1β were significantly increased in OVX group, when compared with normal control group, but were significantly reduced after treatment with boeravinone B ($p < 0.05$; Figure 2).

Effect of boeravinone B on NF-κB signaling pathway

As shown in Figure 5, while the expressions of IκB-α and NF-κB p65 were significantly upregulated, the expression of SIRT-1 protein was significantly down-regulated in OVX group relative to normal control group ($p < 0.05$). However, treatment with boeravinone B significantly reduced the expression levels of NF-κB p65 and IκB-α proteins, and significantly upregulated the expression of SIRT-1 ($p < 0.05$).

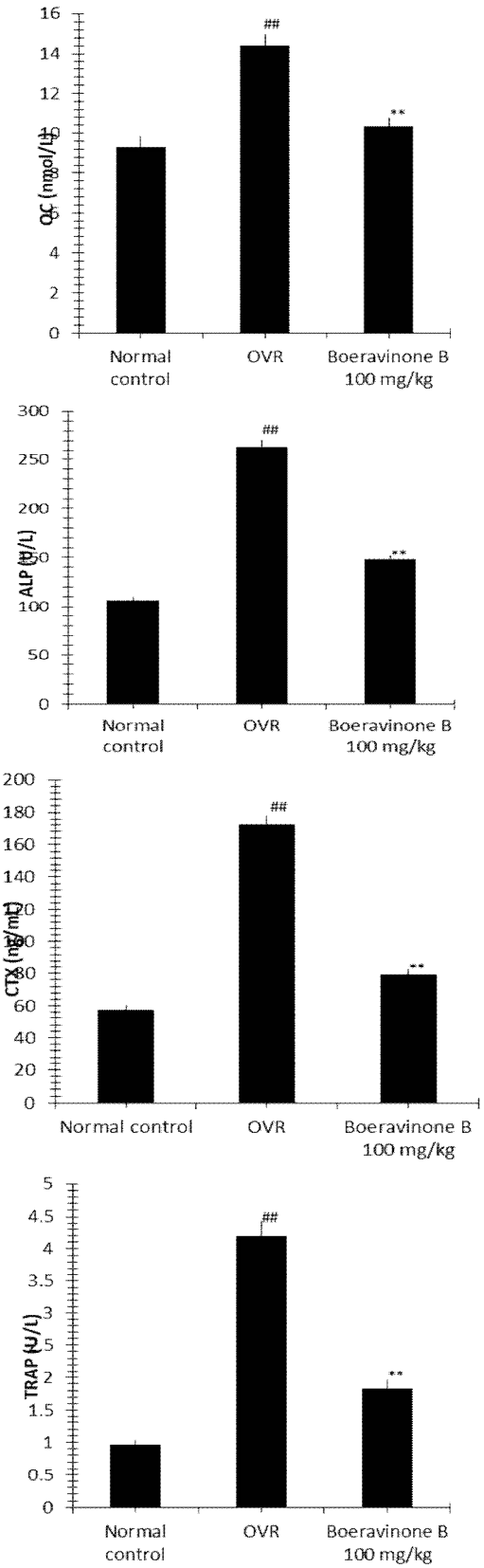

Figure 1: Effect of boeravinone B on indices of bone formation and resorption. $^{##}p < 0.05$, when compared with normal control group; $^{**}p < 0.05$, when compared with OVX group

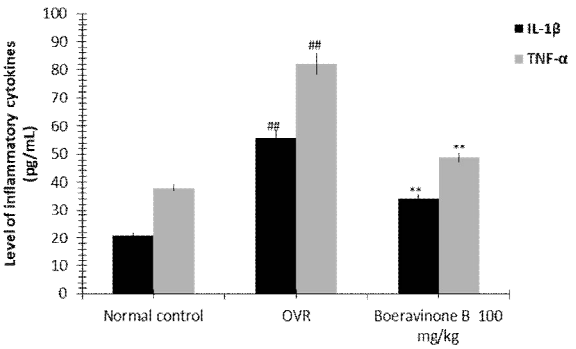

Figure 2: Effect of boeravinone B on the levels of inflammatory cytokines. $^{\#\#}p < 0.05$, when compared with normal control group; $^{**}p < 0.05$ when compared with OVX group

Effect of boeravinone B on BMD

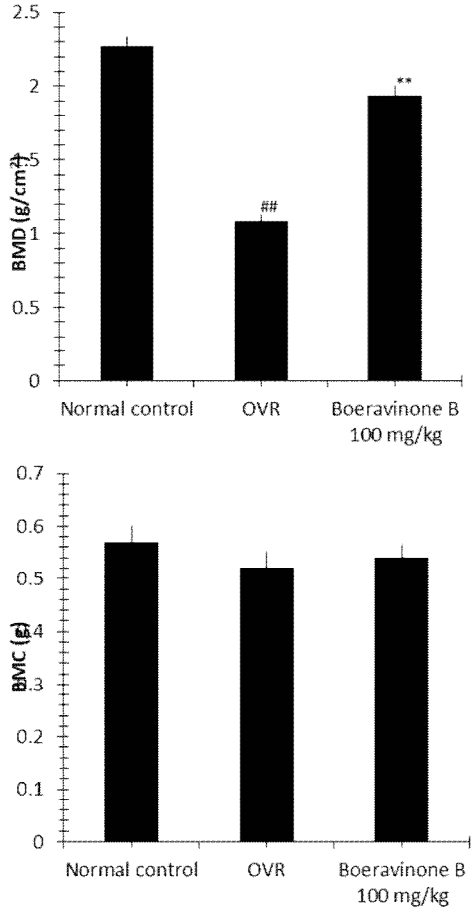

Figure 3: Effect of boeravinone B on BMD. $^{\#\#}P < 0.05$, when compared with normal control group; $^{**}p < 0.05$ when compared with OVX group

Bone mineral content was not significantly altered in OVX and boeravinone B-treated groups, when compared with normal control group ($p > 0.05$). There was significant reduction in BMD of OVX group relative to normal control group ($p < 0.05$). However, treatment with boeravinone B significantly increased the BMD, when compared with OVX group ($p < 0.05$). These results are shown in Figure 3.

Outcome of x-ray analysis

As shown in Figure 4, after the 5th week of treatment, boeravinone B significantly enhanced bone remodeling and formation of callus.

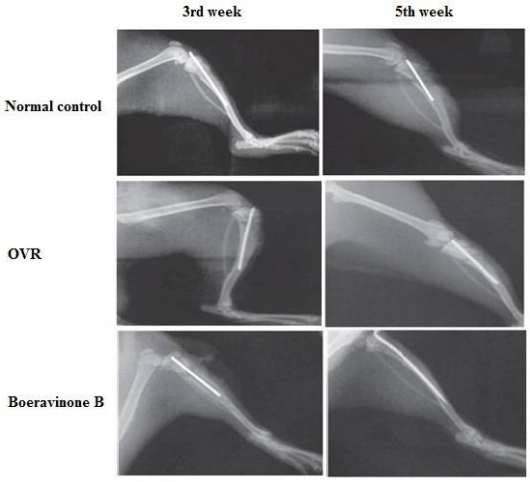

Figure 4: Effect of boeravinone B on remodeling of tibia as revealed by x-ray analysis

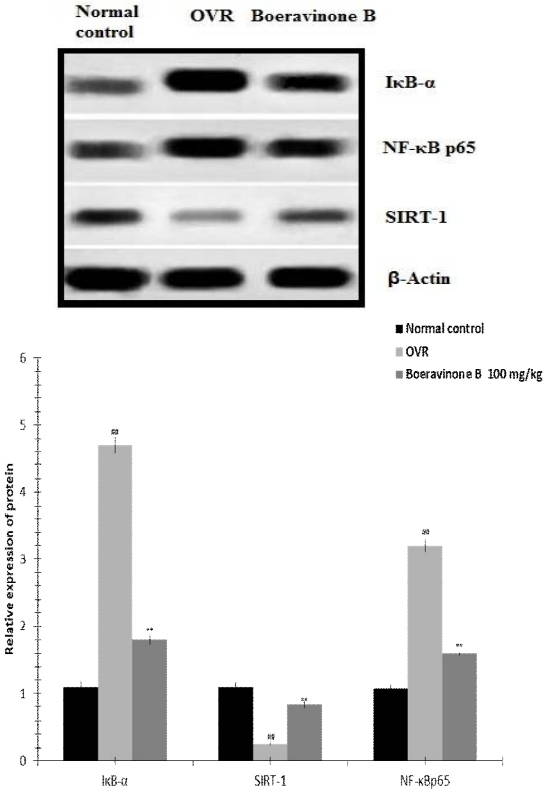

Figure 5: Effect of boeravinone B on the expressions of NF-κB p65, SIRT-1 and IκB-α proteins. $^{\#\#}P < 0.05$, when compared with normal control group; $^{**}p < 0.05$ when compared with OVX group

DISCUSSION

Osteoporosis is common among post-menopausal women, and it increases the risk of fracture due to loss of microstructure of bone protein and mass [17]. The use of HRT in the treatment of osteoporosis is limited, since it causes endometrial and ovarian cancers. The present study investigated the fracture-healing effect of boeravinone B in ovariectomy-induced osteoporotic rats.

In osteoporosis, several compounds play important roles in the remodeling of bone [18]. The results of this study showed that boeravinone B significantly reversed the altered activities of ALP and TRAP, and levels of CTX and OC in the serum of osteoporotic rats.

Bone mineral density is the most important index for determining the severity of osteoporosis and for predicting the risk of fracture. In post-menopause condition, BMD is reduced due to decrease in estrogen level, thereby resulting in permanent loss of trabecular bone. In this study, there was significant reduction in BMD of OVX group relative to normal control group. However, treatment with boeravinone B significantly increased the BMD, when compared with OVX group, an indication that BMD in the femur of osteoporotic rats may be significantly enhanced by boeravinone B. Bone resorption is regulated by inflammatory cytokines and significant increases in their levels lead to osteoporosis [19]. In this study, serum levels of TNF-α and IL-1β were significantly reduced in boeravinone B-treated group, when compared with OVX group. Fracture is common in postmenopausal women and studies have shown that osteoporosis leads to alteration in bone remodeling, which leads to delay in the healing of fracture [20]. It appears that boeravinone B may ameliorate the effect of osteoporosis on bone remodeling, thereby promoting the healing of fracture.

Studies have shown that the function of osteoblast cells is regulated by NF-κB, p65 and osteoclasts, and is inhibited via stimulation of interferon β (IFN-β) [21]. Activation of IFN-β is modulated by the STAT1/JAK pathway [22]. In this study, treatment with boeravinone B significantly reduced the expression levels of NF-κB p65 and IκB-α proteins, and significantly upregulated the expression of SIRT-1.

CONCLUSION

The results obtained in this study suggest that boeravinone B promotes the healing of fracture in rats caused by osteoporosis via a mechanism involving the NF-κB p65/IκB-α/SIRT-1 signaling pathway.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

1. *Pisani P, Renna MD, Conversano F, Casciaro E, Di Paola M, Quarta E, Muratore M, Casciaro S. Major osteoporotic fragility fractures: Risk factor updates and societal impact. World J Orthop. 2016; 7(3): 171-181.*
2. *Sözen T, Özışık L, Başaran NÇ. An overview and management of osteoporosis. Eur J Rheumatol. 2016; 4(1): 46-56.*
3. *Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. J Clin Invest. 2006; 116(5): 1186-1194.*
4. *Osterhoff G, Morgan EF, Shefelbine SJ, Karim L, McNamara LM, Augat P. Bone mechanical properties and changes with osteoporosis. Injury. 2016; 47: S11-20.*
5. *Postmenopausal hormonal therapy: Current status. Indian J Endocrinol Metab. 2013; 17: S45-49.*
6. *Struwig M, Siebert SJ. A taxonomic revision of Boerhavia (Nyctaginaceae) in southern Africa. South African Journal of Botany 2013; 86: 116–134.*

7. Agrawal B, Das S, Pandey A. Boerhaavia diffusa Linn: A Review on its Phytochemical and Pharmacological Profile. Asian journal of applied sciences 2011; 4: 663-684.
8. Pari L, Satheesh MA. Antidiabetic activity of Boerhaavia diffusa L.: effect on hepatic key enzymes in experimental diabetes. J. Ethenopharmacol., 2004; 91: 109-113.
9. Chude MA, Orisakwe OE, Aponue OJ, Gamaniel KS, Vongtau OH, Oki E, Hypoglycaemic effect of the aqueous extract of Boerhaavia diffusa leaves. Ind J Pharmacol., 2001; 33(3): 215-216.
10. Dhingra D, Valecha R., Evidence for involvement of the monoaminergic system in antidepressant-like activity of an ethanol extract of Boerhaavia diffusa and its isolated constituent, punarnavine, in mice, Pharm Biol., 2014; 52(6): 767-774
11. Pandey R, Maurya R, Singh G, Sathiamoorthy B, Naik S, Immunosuppressive properties of flavonoids isolated from Boerhaavia diffusa Linn. Int Immunopharmacol., 2005; 5: 541-553.
12. Hiruma-Lima CA, Gracioso JS, Bighetti EJB, Germonsen Robineou L, Souza Brito ARM, The juice of fresh leaves of Boerhaavia diffusa L. (Nyctaginaceae) markedly reduces pain in mice. J Ethnopharmacol., 2000; 71: 267-274.
13. Satheesh MA, Pari L, Antioxidant effect of Boerhaavia diffusa L. in tissues of Alloxan induced diabetic rats. Ind J Exp Biol., 2004; 42(10): 989-992.
14. Leyon PV, Lini CC, Kuttan G, Inhibitory effect of Boerhaavia diffusa on experimental metastasis by B16F10 melanoma in C57BL/6 mice. Life Sci., 2005; 76: 1339-1349.
15. Murthi K, Mayank AP and Lambole V. Pharmacological properties of Boerhaavia diffusa-A Review, In editor. Indian J Exp Biol., 2010; 5(2): 107-110.
16. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011.
17. Ji MX, Yu Q. Primary osteoporosis in postmenopausal women. Chronic Dis Transl Med. 2015; 1(1): 9-13.
18. Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. Biomark Res. 2017; 5: 18.
19. Amarasekara DS, Yu J, Rho J. Bone Loss Triggered by the Cytokine Network in Inflammatory Autoimmune Diseases. J Immunol Res. 2015:832127.
20. Fischer V, Haffner-Luntzer M, Prystaz K, Vom Scheidt A, Busse B, Schinke T, Amling M, Ignatius A. Calcium and vitamin-D deficiency marginally impairs fracture healing but aggravates posttraumatic bone loss in osteoporotic mice. Sci Rep. 2017; 7(1): 7223.
21. Boyce BF, Xiu Y, Li J, Xing L, Yao Z. NF-κB-Mediated Regulation of Osteoclastogenesis. Endocrinol Metab (Seoul). 2015; 30(1): 35-44.
22. Schindler C, Levy DE, Decker T., JAK-STAT signaling: from interferons to cytokines. J Biol Chem. 2007; 282(28): 20059-20063.