A Histo-Physiological Study of The Effect of Resveratrol on Osteoporosis-Induced in Rats

Mohammad Abdulazeez Ali[1], Zahraa Mohammed Abdulazeez[2]

[1]*PhD, Collage of Health and Medical Technique, Sawa Private University, Iraq.,*
[2]*Collage of Dentistry, University of AL Muthanna, Iraq*

KEYWORDS
Osteoporosis, D-galactose, Resveratrol, ALP and OC.

ABSTRACT
Using an animal model of D-galactose-induced osteoporosis (OP), the researchers examined the effects of resveratrol supplementation on body weight, alkaline phosphatase (ALP) levels and bone calcin (OC) levels and classified mice randomized into the following groups: Subgroup C (n=10): within 60 days participants will receive 1 ml of distilled water. In the D-galactose group (n=10), osteoporosis will be induced by intraperitoneal administration of 200 mg/kg/day of D-galactose for 60 consecutive days. Group R of 10 subjects will receive a dose of 25 mg/kg of oral resveratrol for 60 days.The DR group of the group of 10 subjects will receive D-galactose 200 mg/kg/day intraperitoneally in for osteoporosis and oral resveratrol at 25 mg/kg/day for 60 days. There was a distinct difference in body weight before and after OP infusion in each group in the study. The D-galactose group (D) showed significantly higher levels of ALP and OC compared with the control group. In contrast, the resveratrol group (R) showed significantly lower levels compared with the control group and the D group. Finally, the group treated with the combination of D-galactose and resveratrol (DR) showed significantly higher levels compared to the control group and the group treated with resveratrol alone (R). the. and more microfractures On the other hand, the resveratrol group (R) had thicker bone trabeculae, reduced intertrabecular space, and fewer microfractures. In addition, a protective effect against D-galactose-induced changes was observed in the resveratrol group, and these effects were dose-dependent The findings suggested resveratrol treatment in an animal model of osteoarthritis induced by D-galactose improved their weight, bone turnover markers (ALP and OC), and histological parameters were dependent on resveratrol levels.

1. Introduction

Osteoporosis (OP) is a systemic osteoporosis characterized by decreased bone density, altered skeletal muscle structure, and increased susceptibility to fracture [1], OP [2, 3] leads to osteoporosis and the risk of collapse increases, imposing a significant financial burden on individuals and society. The complex and varied etiology of OP is influenced by interactions between endocrine, nutritional, genetic, physiological, and immunological factors [4]. Estrogen deficiency is believed to be associated with osteoporosis postmenopausal syndrome (PMOP) is closely associated [5]. There is a strong association between persistent OP and impaired bone homeostasis, characterized by enhanced bone resorption and decreased bone formation [6].

Based on current epidemiological data, the worldwide prevalence of OP according to the survey set by the World Health Organization is estimated at 19.7 percent [OP] prevalence varies greatly across 7 continents and countries on, from 8.0% in Oceania to 26.9% in Africa, 4.1 in the Netherlands]. The rates ranged from % to 52.0% in Turkey [8] OP representing an immediate public health problem becomes more pronounced as the population ages [7]. Therefore, there is a need to investigate and develop new therapies for OP that provide better clinical outcomes and fewer side effects.” Herbs such as Reynoutria japonica Houtt., Veratrum nigrum L., and Catsiaatora Linn contain resveratrol, a Natural polyphenol compound of estrogen diethylstilbestrol [8]. It holds the resemblance. Like phytoestrogens, resveratrol has been found to competitively bind estrogen receptors in the laboratory, with subsequent anti-osteoporotic effects [9]. Another study showed resveratrol to have regulatory effects on bone turnover and alter bone metabolism Thanks to its antioxidant properties, it can protect the body from oxidative stress, a factor that can lead to bone loss [10]. Evidence from research studies suggests that resveratrol has the potential to reduce bone loss and fracture risk in

postmenopausal women and individuals with diabetes [11,12]. Unfortunately, the potential benefits of resveratrol have not been established in the treatment of OP, existing research was thus inconclusive. The results of preclinical animal tests can provide vital information for therapeutic purposes and for scientific and medical researchers to gain a deeper understanding of the causes of disease.

2. Methodology

All animal procedures were conducted according to the protocols approved by the college of veterinary medicine, Al-Qadisiyah University. Male Sprague-Dawley (SD) rats (3-month old, weighing 280±10 gm). Animals were kept at 26–28°C with free access to water and chow. Rats were randomized into the following groups: Control group (C) (n=10): will give 1ml of distilled water daily for 60 days. D-galactose group (D) (n=10): will inject of D-galactose (200 mg/kg/day) intraperitoneal for 60 consecutive days for osteoporosis induction. Resveratrol group (R) (n=10): will gavage with resveratrol (25 mg/kg/day) for 60 days. D-galactose+ Resveratrol group (DR) (n=10): will inject of D-galactose (200 mg/kg/day) intraperitoneal for osteoporosis induction and gavage with resveratrol (25 mg/kg/day) for 60 consecutive days for osteoporosis induction for 60 days.

ELISA assay

ELISA Kits provided by the manufacturer, the detection kit (BIKW, Beijing, China) was used to assess serum levels of alkaline phosphatase (ALP) and osteocalcin (OC) using colorimetric analysis with a spectrophotometer operating at a wavelength of 450 nm.

Histological staining

After removing the femoral condyle from each rat, it was fixed in 4% paraformaldehyde for 24 hours and decalcified in a 10% EDTA buffer (pH 7.0) for two weeks. After dehydration, the samples were embedded in paraffin and sliced to a thickness of approximately 4 mm. A stain called hematoxylin-eosin (HE) was applied to the slices. Photographs were taken of the slices after they were examined under an optical microscope (Leica, Wetzlar, Germany).

Statistical analysis

Software developed and maintained by SPSS Inc. in Chicago, USA (version 15.0) was used to conduct all statistical analyses. The mean ± standard deviation is used to express all data. Using one-way analysis of variance and the Newman-Keuls posttest, we compared the various groups statistically. A significance level of $P \leq 0.05$ was use.

3. Results and discussion

Body Weights

The results of the current study showed that there are significant differences between all study groups at ($P \leq 0.05$) in body weights before and after OP induced and Resveratrol as shown in Fig 1.

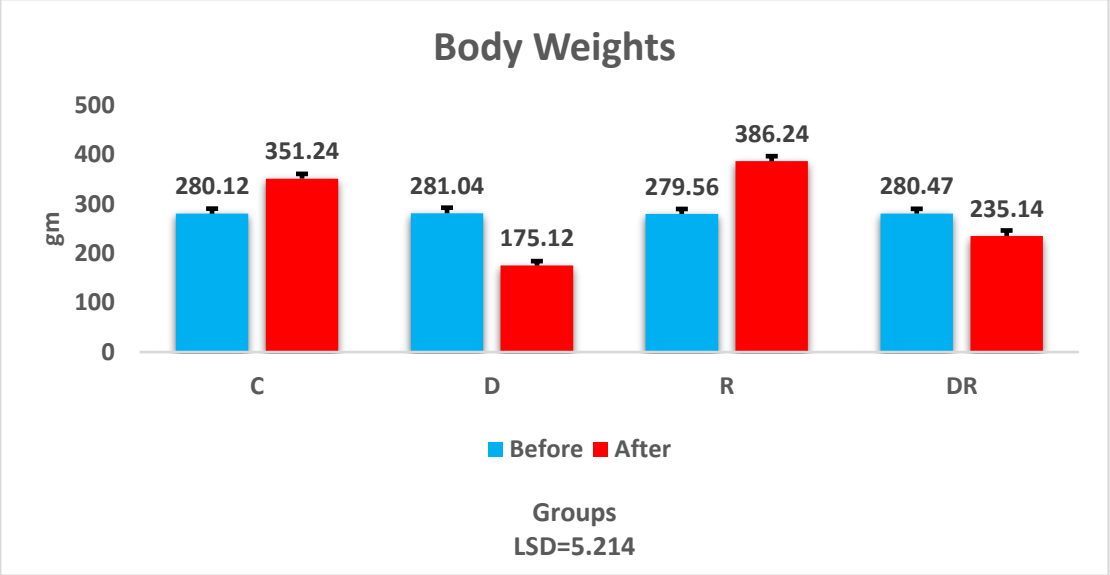

Fig 1. Effects of resveratrol treatment on body weights

The levels of ALP and OC

Figures 2 and 3 display the results of the present study, which indicate a statistically significant difference in the levels of ALP and OC across all research groups (P≤0.05). Specifically, the results demonstrated a statistically significant increase in the levels of ALP and OC in the D group when compared to the control group. The current study found that the levels of ALP and OC were significantly lower in the R group when compared to the control and D groups, while the DR group had significantly higher levels of ALP and OC when compared to the control and R groups, with a probability level of P≤0.05.

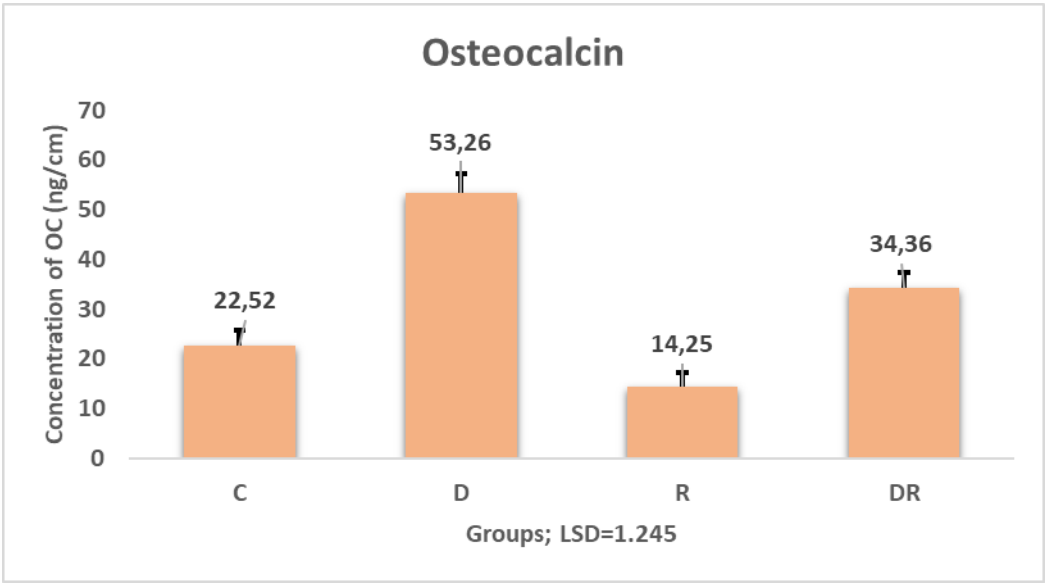

Fig 2. Effects of resveratrol treatment on serum Osteocalcin (OC) Group.

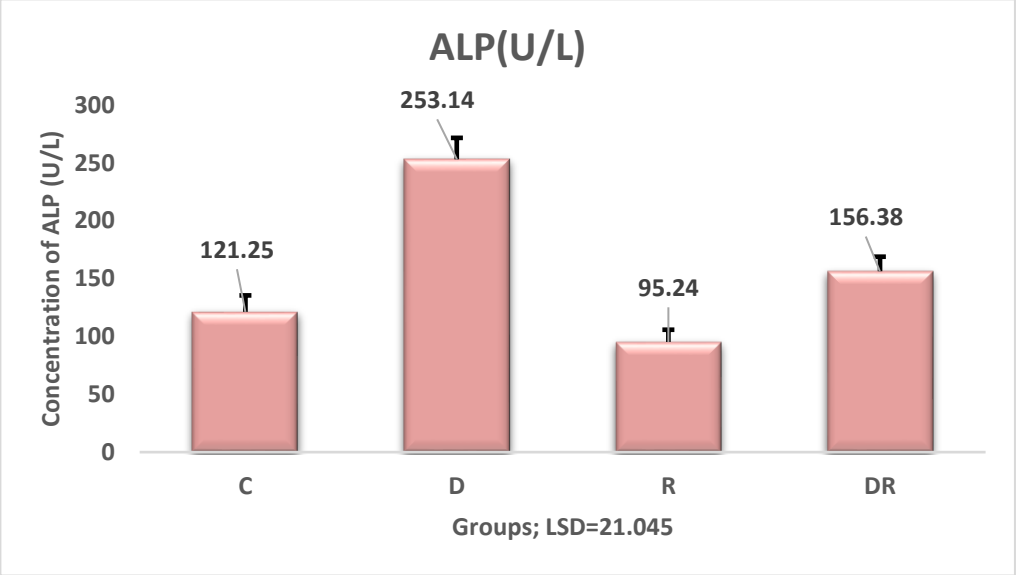

Fig 3. Effects of resveratrol treatment on serum alkaline phosphatase (ALP) Group.

Histological Changes in bone

Model Histological examination with HE staining verified osteoporotic alterations in the bones of rats with osteoporosis (Fig. 4). Microfractures of bone trabeculae, as measured by the production of connective tissue in the border zone of broken trabeculae, decreased intertrabecular space, and thinning of bone trabeculae were all detected in D-galactose rats. There were fewer microfractures of bone trabeculae and better bone trabeculae and intertrabecular space in the group of rats that received resveratrol. Additionally, these safeguards against D-galactose alterations were dose-dependent.

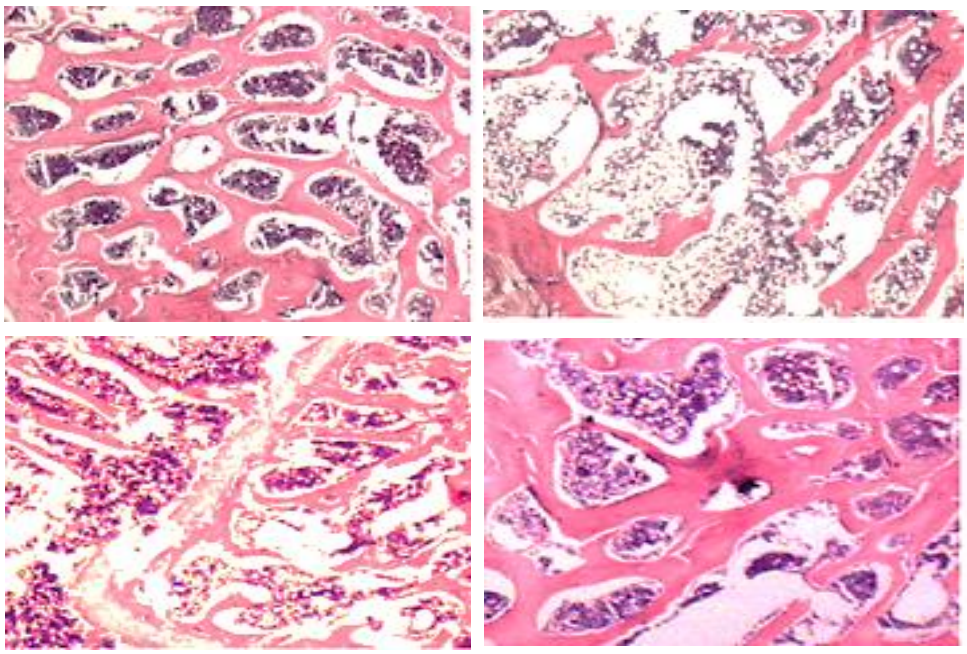

Figure 4. Morphological picture of proximal metaphysis of femoral bone (A) Control rats. (B) The rat after D-galactose treatment for 60 days. (C) The rat after treated with 25 mg/kg/day resveratrol for 60 days. (D) The rat after D-galactose treatment and 25 mg/kg/day resveratrol together for 60 days.

Discussion

In this study, we established that D-galactose treatment at a dose of 200 mg/kg/day could effectively correct microarchitectural damage and osteoporosis in rats. seen in individuals with reduced bone density, microarchitectural degradation, and greater fracture susceptibility [13,14] When oxidative

stress occurs, it can damage osteoblasts and osteoclasts, causing bone resorption activity to exceed osteolysis [15 ,16]. It can prevent bone loss due to oxidative stress by inhibiting the effect of resveratrol [17]. Furthermore, resveratrol can reduce bone loss due to estrogen deficiency through estrogen receptor binding and estrogen-like effect [18]. Furthermore, this meta-analysis showed that resveratrol showed positive effect on biochemical markers increasing variety. In us in vitro studies, we did not observe statistically significant changes in ALP activity, type 1 collagen, or osteopontin expression when using moderate doses of resveratrol (20 mM) and observed changes that lie notably at resveratrol concentrations of 25 and 30 mM. ALP plays an important role in mineralization and osteoclast formation. There is a positive correlation between protein OC levels and bone formation rates [19]. Bone resorption, formation, and mineralization are interrelated processes, ALP (alkaline phosphatase) and OC (osteocalcin) are two proteins that can be used as indicators of this process Osteoporosis and alkaline phosphatase (ALP) and elevated osteocalcin (OC) levels are associated [20] no Changes in the ratio of osteoclast to osteoclast activity lead to corresponding changes in ALP levels, blood tests for bone formation This finding suggests that inhibition of osteoclast activity by resveratrol may explain this phenomenon. One study showed that resveratrol effectively reduced bone loss, while another study reported its ability to inhibit bone differentiation [21,22]. Rats administered resveratrol exhibited reduced in vivo ALP levels compared to rats administered D-galactose, attributed to the inhibitory effect of resveratrol on osteoclastogenesis. Resveratrol effectively mitigated osteoporosis generated by D-galactose in living organisms, as evidenced by the decreased levels of serum ALP. Nevertheless, our research revealed that resveratrol increased ALP levels in cells that were exposed to the substance in a laboratory setting. This effect was observed due to resveratrol's ability to promote the growth of osteoblasts and the creation of bone. Collectively, our study and those of other scientists have demonstrated that resveratrol has the ability to enhance the formation of osteoblasts and inhibit the formation of osteoclasts. Researchers have hypothesized that resveratrol may function as an oestrogen receptor agonist because of its structural similarity to oestrogen. The work of Zhao et al. [23] provided no explanation for the specific mechanism by which long-term resveratrol treatment protects against ovariectomy-induced bone loss in rats In order to gain a deeper understanding of the mechanism of resveratrol is function, we performed in vitro studies. Osteoporosis development is positively associated with estrogenic activity, osteoblasts are the main beneficial cells regulating bone formation in this disease According to a study resveratrol has the ability to promote differentiation of bone marrow stem cells (BMSCs) and adipose derived stromal cells (ADSCs) between weak) [24] work we do to enhance bone marrow formation. of resveratrol A new emphasis was obtained. In our in vitro work, injections of resveratrol increased both ALP levels and activity. Additional studies were performed on the expression of genes exclusive to osteoblasts, such as osteopontin and type I collagen. Resveratrol-induced activation of these genes provides further evidence that it stimulates differentiation of BMSCs into bone marrow. Osteoblast differentiation and bone mineral formation are regulated by complex mechanisms. Further studies were conducted to investigate the molecular mechanisms by which resveratrol exerts its beneficial effects. However, the study did have a few flaws. Osteoblasts and osteoclasts are important cells in the progression of osteoporosis. The only finding in this in vitro study was the effect of resveratrol on osteoblasts. Importantly, the benefit of resveratrol in preventing osteoporosis is dose-dependent; A dose of 25 mg/kg/day was insufficient to produce observable changes in the organism. Our study highlighted the need for optimal doses of resveratrol in the treatment of osteoporosis and provided supporting evidence and therapeutic potential in this area.

4. Conclusion and future scope

The results show that resveratrol supplementation significantly reduces D-galactose-induced bone toxicity, as improvements in body weight, bone turnover markers, and bone mineral content these findings suggest that osteoporosis Resveratrol may have potential in managing or preventing

Reference

[1] .Sabri SA, Chavarria JC, Ackert-Bicknell C, Swanson C, Burger E. Osteoporosis: an update on screening, diagnosis, evaluation, and treatment. Orthopedics. (2023) 46:e20–6. doi: 10.3928/01477447-20220719-03.

[2] Li N, Cornelissen D, Silverman S, Pinto D, Si L, Kremer I, et al. An updated systematic review of cost-effectiveness analyses of drugs for osteoporosis. PharmacoEconomics. (2020) 39:181–209. doi: 10.1007/s40273-020-00965-9.

[3] Yu G, Tong S, Liu J, Wan Y, Wan M, Li S, et al. A systematic review of costeffectiveness analyses of sequential treatment for osteoporosis. Osteoporos Int. (2023) 34:641–58. doi: 10.1007/s00198-022-06626-1.

[4] Aibar-Almazán A, Voltes-Martínez A, Castellote-Caballero Y, Afanador-Restrepo DF, Carcelén-Fraile MDC, López-Ruiz E. Current status of the diagnosis and management of osteoporosis. Int J Mol Sci. (2022) 23:9465. doi: 10.3390/ijms23169465.

[5] Arceo-Mendoza RM, Camacho PM. Postmenopausal osteoporosis. Endocrinol Metab Clin N Am. (2021) 50:167–78. doi: 10.1016/j.ecl.2021.03.009.

[6] .Patel D, Wairkar S. Bone regeneration in osteoporosis: opportunities and challenges. Drug Deliv Transl Res. (2023) 13:419–32. doi: 10.1007/s13346-022-01222-6.

[7] Jiang Y, Zhang P, Zhang X, Lv L, Zhou Y. Advances in mesenchymal stem cell transplantation for the treatment of osteoporosis. Cell Prolif. (2021) 54:e12956. doi: 10.1111/cpr.12956.

[8] .Xiao PL, Cui AY, Hsu CJ, Peng R, Jiang N, Xu XH, et al. Global, regional prevalence, and risk factors of osteoporosis according to the World Health Organization diagnostic criteria: a systematic review and meta-analysis. Osteoporos Int. (2022) 33:2137–53. doi: 10.1007/s00198-022-06454-3.

[9] Salari N, Ghasemi H, Mohammadi L, Behzadi MH, Rabieenia E, Shohaimi S, et al. The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis. J Orthop Surg Res. (2021) 16:609. doi: 10.1186/s13018-021-02772-0.

[10] Gennari L, Bilezikian JP. New and developing pharmacotherapy for osteoporosis in men. Expert Opin Pharmacother. (2018) 19:253–64. doi: 10.1080/14656566.2018.1428559.

[11] .Fontalis A, Kenanidis E, Kotronias RA, Papachristou A, Anagnostis P, Potoupnis M, et al. Current and emerging osteoporosis pharmacotherapy for women: state of the art therapies for preventing bone loss. Expert Opin Pharmacother. (2019) 20:1123–34. doi: 10.1080/14656566.2019.1594772.

[12] El-Ghazaly MA, Fadel NA, Abdel-Naby DH, Abd EH, Zaki HF, Kenawy SA. Amelioration of adjuvant-induced arthritis by exposure to low dose gamma radiation and resveratrol administration in rats. Int J Radiat Biol. (2020) 96:857–67. doi: 10.1080/09553002.2020.1748911.

[13] .French L, Smith M and Shimp L. Prevention and treatment of osteoporosis in postmenopausal women. J Fam Pract 2002, 51: 875– 882.

[14] Selby P. Postmenopausal osteoporosis. Curr Osteoporos Rep 2004, 2: 101 – 106.

[15] .Kimball JS, Johnson JP, Carlson DA. Oxidative stress and osteoporosis. J Bone Joint Surg. (2021) 103:1451–61. doi: 10.2106/JBJS.20.00989.

[16] León-Reyes G, Argoty-Pantoja AD, Becerra-Cervera A, López-Montoya P, RiveraParedez B, Velázquez-Cruz R. Oxidative-stress-related genes in osteoporosis: a systematic review. Antioxidants (Basel). (2023) 12:915. doi: 10.3390/antiox12040915

[17] Mizutani K, Ikeda K, Kawai Y, Yamori Y. Resveratrol stimulates the proliferation and differentiation of osteoblastic MC3T3-E1 cells. Biochem Biophys Res Commun. (1998) 253:859–63. doi: 10.1006/bbrc.1998.9870.

[18] .Zhang Y, Liu M, He Y, Deng N, Chen Y, Huang J, et al. Protective effect of resveratrol on estrogen deficiency-induced osteoporosis though attenuating NADPH oxidase 4/nuclear factor kappa B pathway by increasing miR-92b-3p

expression. Int J Immunopathol Pharmacol. (2020) 34:1120272288. doi: 10.1177/2058738420941762

[19] Mizutani K, Ikeda K, Kawai Y and Yamori Y. Resveratrol attenuates ovariectomy-induced hypertension and bone loss in stroke-prone spontaneously hypertensive rats. J Nutr Sci Vitaminol (Tokyo) 2000, 46: 78 – 83.

[20] .Lin Q, Huang YM, Xiao BX and Ren GF. Effects of resveratrol on bone mineral density in ovarectomized rats. Int J Biomed Sci 2005, 1: 76 – 81.

[21] .He X, Andersson G, Lindgren U and Li Y. Resveratrol prevents RANKL-induced osteoclast differentiation of murine osteoclast progenitor RAW 264.7 cells through inhibition of ROS production. Biochem Biophys Res Commun 2010, 401: 356 –362.

[22] .Shakibaei M, Buhrmann C and Mobasheri A. Resveratrol-mediated SIRT-1 interactions with p300 modulate receptor activator of NF-kappaB ligand (RANKL) activation of NF-kappaB signaling and inhibit osteoclastogenesis in bone-derived cells. J Biol Chem 2011, 286: 11492 –11505.

[23] Zhao H, Li X, Li N, Liu T, Liu J, Li Z and Xiao H, et al. Long-term resveratrol treatment prevents ovariectomy-induced osteopenia in rats without hyperplastic effects on the uterus. Br J Nutr 2014, 111: 836 – 846.

[24] Dosier CR, Erdman CP, Park JH, Schwartz Z, Boyan BD and Guldberg RE. Resveratrol effect on osteogenic differentiation of rat and human adipose derived stem cells in a 3-D culture environment. J Mech Behav Biomed Mater 2012, 11: 112– 122.