

Histomorphometric Evaluation of Bone Tissue Exposed to Experimental Osteoporosis and Treated with *Retama Raetam* Extract

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Abstract: Plant-derived phenolic compounds manifested beneficial effects and potential inhibition of several stages of carcinogenesis. In the present study, we studied the efficacy of plant polyphenol compounds in the treatment of osteoporosis and their modulatory effects on dexamethasone-induced osteoporosis in rats. Thirty six female Sprague-Dawely rats (150 gm) were used in this study and divided into 6 groups (for each 6). Group1 was used as a control group. Group 2 received intraperitoneal injection (i.p.) of dexamethasone in a dose of (20 mg / kg. B.W). Group 3: received hydroalcoholic extract of *Retama raetam* seeds (30 mg / kg b.w.). Group 4 received similar dose of dexamethasone together with plant extract at the same time. Group 5 received plant extract alone for two month and then dexamethasone for one month, while group 6, received Dexa alone for one month and then the plant extract for two months. All these groups were treated for three months. Bone samples were stained with Hx & E for morphological study and histochemical stain (alkaline phosphatase) for studying the regeneration of diseased bone tissue. All the samples were analyzed using a computer –assisted quantitative system. Our results revealed that dexamethasone can induce hisopathological and histochemical changes in bone tissue. The morphometric parameters showed marked decrease in mid-diaphyseal cortical bone thickness and increased bone resorption in treated groups compared with controls. Results of histochemical study revealed that ALP activity was decreased in dexamethasone treated-rats, and there was improvement in this activity accompanying the treatment with plant extract. Also, results of present study revealed that the plant extract used has a protective effect against osteoporosis more powerful than it has as a treatment measure from the disease in concerning with tissue thickness and size of osteocytes. Treatment with plant extract results in amelioration of the imbalance between bone resorption and formation.

Key word: Dexamethasone, *Retama raetam*, bone, osteoporosis, histology and histomorphometry.

INTRODUCTION

Osteoporosis has become a major health hazard in the recent years afflicting over 2000 million people worldwide. Osteoporosis is a systematic skeletal disease characterized by low bone mass due to micro architectural deterioration of bone tissue, with consequent increase in bone fragility and susceptibility to fractures. This loss and deterioration of the structure of bone tissue is caused by a net imbalance in bone remodeling, due either to an increase activity or number of osteoclasts and / or a reduced number or activity of osteoblasts^[1,5]. Estrogen deficiency occurring at menopause plays a major role in the development of osteoporosis in postmenopausal women^[8,20]. In order to prevent adverse effects of estrogen loss, estrogen replacement therapy has been proposed and the use of selective estrogen receptor modulators (SERM) has

been developed^[26].

Phytoestrogens are plant compounds with estrogen-like biological activity. The main classes of phytoestrogens are isoflavones, flavonoids, coumestans and lignans. Particularly after consumption of isoflavones and lignans, heterocyclic phenols are formed, which in stereochemical structure are close estrogen, and have the capacity to bind to the estrogen receptors^[21,25].

Alkaline phosphatase (ALP) is an enzyme that is expressed by osteoblasts, especially those adjacent to blood vessels. It is also expressed in the endothelial cells of these vessels as a combination of reactions from endothelial cells and perivascular cells^[9]. The ALP produced by osteoblasts is a non-specific type of this enzyme. Its activity and localization are a valuable index for tissue development and differentiation. It is another marker of osteoblast activity and bone

formation^[22]. Previous studies have shown that bone alkaline phosphatase ALP can be considered a marker of bone formation in *vitro*^[11] and in *vivo*^[32].

In the present study, we aim to investigate the efficacy of the plant-derived phenolic compounds present in the seeds of *Retama raetam* in the protection or treatment of osteoporosis and their modulatory effects on dexamethasone-induced osteoporosis in rats.

MATERIALS AND METHODS

Plant Material: Seeds of *R. raetam* were collected in April 2005 approximately at 30 km along the Cairo-Suez Desert. Identification of the plants was confirmed by the Department of Flora Agricultural, Museum, Ministry of Agricultural and Herbarium of the Department of Botany, Faculty of Science, and Cairo University. Voucher Specimens were kept in herbarium, National Research Center, El-Tahrir Str. Dokki, Cairo, Egypt.

Extraction and Isolation: Powder of the air-dried seeds of *Retama raetam* (500 g) was defatted with CHCl_3 (3 x 1 L) and extracted with $\text{CH}_3\text{OH} : \text{H}_2\text{O}$ (7:3, 5 x 3 L) at room temperature. The combined extracts were filtered, evaporated under reduced pressure and lyophilized (200 g). Ten grams of the dry residue was used for antiosteoporosis study. Weighed samples of *Retama raetam* extract were used to prepare the solutions, which were diluted with distilled H_2O to the appropriate concentration for experiment. The rest of the dry extract was redissolved in 2 L H_2O and extracted with EtOAc (5 x 2 L). After evaporation of solvents, the EtOAc extract and the remaining H_2O phase gave dark brown solids 25 and 105 g, respectively. The EtOAc extract was loaded on polyamide 6S column (50 x 3 cm). The column was eluted with H_2O , and then H_2O -EtOH mixtures of decreasing polarity and 10 fractions (1 L, each) were collected. The major phenolic fractions obtained were combined into four fractions after TLC analysis. Fraction 1 (3.5 g) was fractionated by column chromatography on Sephadex LH-20 with aqueous EtOH (0- 70%) for elution to give compounds **1** (20 mg) and **3** (38 mg). Fraction 2 (3 g) was subjected to column chromatography on cellulose and n-BuOH saturated with H_2O as an eluent to give two major subfractions, then each of them was separately fractionated on a Sephadex LH-20 to yield pure samples **4** (18 mg) and **5** (18 mg). Using the same procedure fraction 3 (2 g) gave chromatographically pure samples **2** (22 mg), and **6** (19 mg).

Animals and Experimental Design: Thirty six female

of Sprague-Dawley rats, weighing about 150 g, were obtained from (National Research Centre, Egypt). The experimental animals were housed in an air condition room with 12h / 12h light – dark illumination cycles and given distilled water to drink and fed a standard diet *ad libitum*. The research was conducted in accordance with the internationally accepted principles for laboratory animals use and care as found in for example the European Community guideline (EEC Directive of 1986; 86/ 609/ EEC).The animals were divided into 6 groups as follows:

Group 1: was used as a control group.

Group 2: received intraperitoneal injection of dexamethasone in a dose of (20 mg / kg. b.w.).

Group 3: received aqueous methanol extract of *Retama raetam* seeds (30 mg / kg b.w.).

Group 4: received (20 mg / kg. b.w.) in dose of dexamethasone together with (30 mg / kg b.w.) of *Retama raetam* seeds extract at the same time.

Group 5: received plant extract for two months and then dexamethazone for one month.

Group 6: received dexamethasone alone for one month and then the plant extract for two months. All these groups were treated for three months.

Histopathological Studies in Bone: Histopathological analysis was carried out on femur of rats. After the rats were sacrificed the left femur were removed, dissected free of soft tissue and fixed in 10 % buffered formal saline. The specimens were then decalcified in EDTA solution for 10-14 days at 5°C. Tissues were dehydrated in graded alcohols and embedded in paraffin. 5µm sections were cut and stained with hematoxylin and eosin (H & E) (Drury and Wallington, 1980) for morphological study and histochemical stain for alkaline phosphatase.

Quantitative Measurement: Quantitative analysis measurement was achieved by using computerized image analyzer (Leica Qwin 500 image) in Image Analyzer Unit, Pathology Department, National Research Center, and Cairo, Egypt. Average measurements from each tissue section (10 fields per section at low power magnification x100) were pooled to determine means.

RESULTS AND DISCUSSIONS

Results: Our results revealed that dexamethasone can induce hisopathological and histochemical damage in bone tissue. The histological sections of the middle shaft of the femur bone of a control rat showing the normal architecture of the bone tissue. The bone tissue

is covered by periosteum from the outside and from the inside by a similar layer called the endosteum. Within the bone tissue, osteocytes are embedded in matrix within lacunae that appear ovoid in shape in old ones and more rounded in appearance in younger cells. A Number of canals are observed carrying the blood vessels and nerves called Haversian canals. (Fig. 1 a and b).

Histological section of femur revealed that dexamethazone causes marked deformity in the bone tissue structure in the form of widening of the medullary cavity and Haversian canals at the expense of the bone tissue thickness. Both the outer and the inner surfaces of the bone tissue show irregularities containing multinucleated cells. The endosteum shows marked increase in its thickness. The bone tissue under the periosteum is destroyed and replaced by fibrous tissue. Lacunae with spindle-shaped osteocytes and flattened oval nuclei are observed (Fig. 1 c and d). The section of the middle shaft of the femur bone of a rat treated with the plant extract only showing normal bone tissue microstructure denoting safety of this plant extract (Fig. 2 a and b).

Examination of the middle shaft of the femur bone of a rat treated with dexamethazone and the plant extract showing improvement of bone tissue architecture in the form of regularity of both inner and outer surfaces of the bone tissue although the Haversian canals are still widened. The number of oosteocytes is increased in comparison with that in group 2 (control positive group). Most of the oosteocytes are of the young type denoting increased rate of formation of osteocytes and bone tissue regeneration (Fig. 2 c and d).

Histologically, the section of the middle shaft of the femur bone of a rat treated with the plant extract for two months and then injected with dexamethazone for one month, showing regular both inner and outer surfaces of bone tissue The lamellae of the matrix under the periosteum are regular in arrangement enclosing between them old spindle shaped oosteocytes while the young ones are located more centrally in a non-regular manner denoting a good protective measure of this extract (Fig. 3 a and b).

The section of the middle shaft of the femur bone of a rat treated with dexamethazone for a month and then with the plant extract, showing mild amelioration as inner and outer surfaces of bone tissue are still irregular with points of invasion. The old oosteocytes are located in the center of the bone tissue, while the young ones are located more peripherally but in an abnormal arrangement denoting incomplete regeneration of bone tissue (Fig. 3 c and d).

In bone, ALP (alkaline phosphatase) activity was detected widely in osteoblasts, osteocytes, and

endosteal cells. In areas of new bone formation, ALP activity was detected in the young osteocytes in osteoid tissue. However, no ALP activity could be detected in calcified bone matrix.

Figures (4 a, b, c and d) showed that the activity of alkaline phosphatase in femur of animals following the administration of dexamethazone is decreased. Treatment with the plant extract showed increased enzyme activities throughout the experimental period when compared to their respective dexamethazone-treated groups.

The morphometric parameters showed decrease in mid-diaphyseal cortical bone thickness and increased bone resorption in dexamethazone- treated groups compared with controls. These findings are much improved in the animals treated with plant extract.

In histogram 1, the mid-diaphyseal cortical bone thickness is greatly increased in the group of animals received the plant extract alone more than that in both groups of animals received dexamethazone only (control +ve group) and those received dexamethazone for a month and then the plant extract (Group 6).

This increase in bone tissue thickness is slightly higher in the group of animals received the plant extract only than that in both groups of animals received dexamethazone with plant extract (Group 4) and animals received plant extract only and then dexamethazone (group 5).

On the other hand, in histogram 2, revealed that the area occupied by osteocytes it was clear that osteocytes had the maximum area in the normal case and this area is greatly decreased in the group of animals received dexamethazone only (control +ve group). By using the plant extract (Groups 4, 5, 6) these results were improved although they did not return to the normal levels. Using the plant extract alone (Group 3) also decreases the area occupied by osteocytes.

Discussion: The present study was conducted to evaluate the effect of aqueous methanole extract of *Retama raetam* on osteoporosis-induced by dexamethazone. The elucidation of the cellular and molecular mechanisms that lead to glucocorticoids (GC)-induced osteoporosis and the development of improved means of identifying those at risk remain important challenges^[19,29]. In human subjects receiving long-term treatment of GC, bone loss is associated with decrease in the overall formation rate and in the mean thickness of the walls of newly synthesized trabecular packets^[7], a pattern of loss consistent with a decrease in the number and/or activity of osteoblasts rather than an increase in the number and/or activity of osteoclasts^[30].

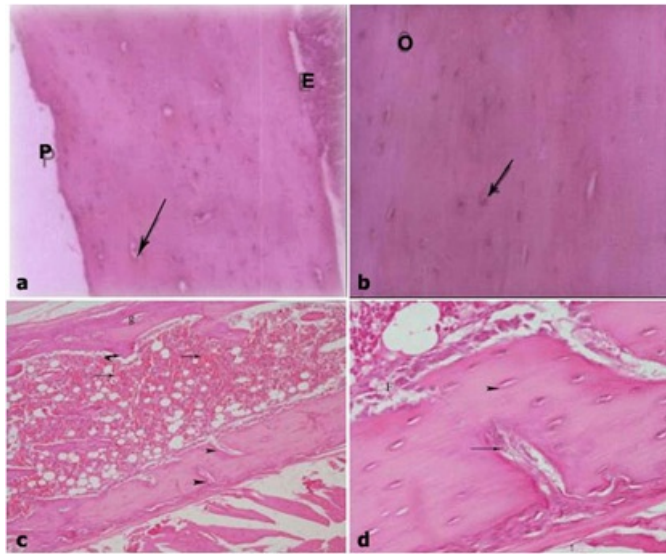


Fig. 1: (a) A photomicrograph of the middle shaft of the femur bone of a control rat showing the normal architecture of the bone tissue. The periosteum (P), the endosteum (E) and a number of canals are observed carrying the blood vessels and nerves, Haversian canals (arrow). (b): A higher magnification of the previous section showing the bone cells (osteocytes) in lacunae, the old cells appear spindle in shape with oval flattened nuclei (O) while the young cells appear more rounded with rounded nuclei (arrow). (c) A photomicrograph of the middle shaft of the femur bone of a rat treated with dexamethazone, showing widening of the medullary cavity at the expense of the bone tissue thickness and Haversian canals forming gaps (g) within the bone tissue. Both the outer and the inner surfaces of the bone tissue show areas of invasion (arrow head) and irregularities (wavy arrow) especially at the inner surface. Spots of hemorrhage are observed in the bone marrow (straight arrow). (d): A higher magnification of the previous section showing areas of invasion of bone tissue containing multinucleated cells (arrow). The endosteum shows marked increase in its fibrous content leading to increase in its thickness (F). (Hx. & E. X 100 & 200)

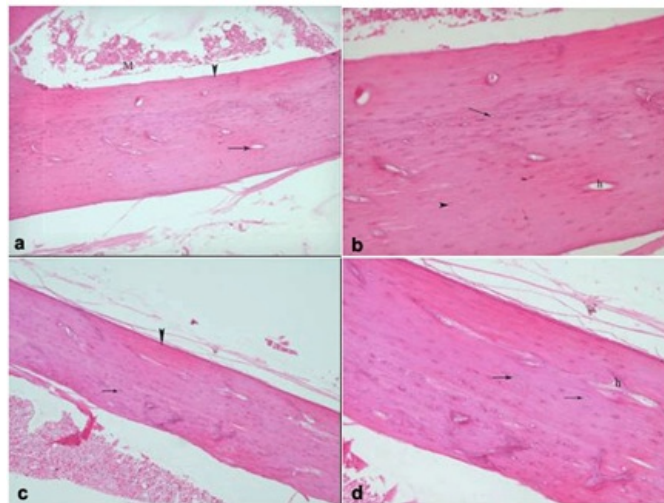


Fig. 2: (a) A photomicrograph of the middle shaft of the femur bone of a rat treated with the plant extract only showing that both surfaces of bone tissue are regular (arrow head). The Haversian canals (arrow) are normal in size. Bone marrow (M) is observed. (b): A higher magnification of the previous section showing normal sized Haversian canals (h) and regular arrangement of the lamellae of the matrix. Old osteocytes (arrow) are located at the center of the bone tissue, while the young ones are located more

peripherally (arrow head). **(c):** A photomicrograph of the middle shaft of the femur bone of a rat treated with dexamethazone and plant extract at the same time, showing both the inner and the outer surfaces of the bone tissue become regular (arrow head) especially the outermost one. The number of oosteocytes is increased in comparison with that in the group 2 (arrow). **(d):** A higher magnification of the previous section showing the Haversian canals (h) are still widened. Most of the oosteocytes are of the young type having a more or less rounded shape denoting increased rate of formation of oosteocytes (arrow). (Hx. & E. X 100 & 200)

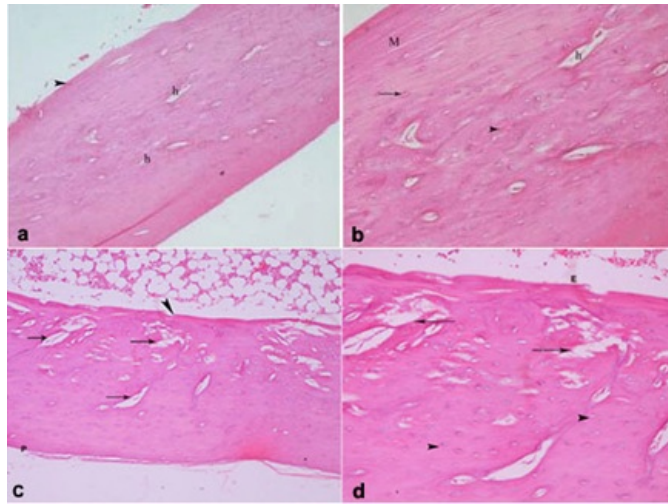


Fig. 3: (a) A photomicrograph of the middle shaft of the femur bone of a rat treated with the plant extract for two months and then injected with dexamethazone for one month, showing regular both inner and outer surfaces of bone tissue (arrow head). The Haversian canals (h) are slightly dilated. **(b):** A higher magnification of the previous section showing that the lamellae of the matrix (M) under the periosteum are regular in arrangement enclosing between them old spindle shaped oosteocytes (arrow), while the young ones (arrow head) are located more centrally in a non-regular manner. **(c):** A photomicrograph of the middle shaft of the femur bone of a rat treated with dexamethazone for one month and then with the plant extract for two months, showing irregular inner and outer surfaces of bone tissue with points of invasion (arrow head). Some of the Haversian canals are normal in size while others are markedly dilated (arrows). **(d):** The old oosteocytes are located in the center of the bone tissue (arrow heads), while the young ones are located more peripherally but in an abnormal arrangement denoting incomplete regeneration of bone tissue. Gaps in bone matrix are still observed (arrows). (Hx. & E. X 100 & 200)

The rat model is useful in studying osteoporosis and the dexamethasone rat was judged to be the standard animal for the study of bone loss caused by estrogen deficiency. The rats treated with dexamethazone (20 mg/ kg. b.w.) showed sign of bone loss represented in marked deterioration of bone architecture, deformity in the bone tissue structure, widening in the medullary cavity at the expense of bone tissue thickness and in Haversian canals forming gaps within the bone tissue. Both the outer and the inner surfaces of the bone tissue show areas of invasion and irregularities (especially at the inner surface). Spots of hemorrhage are observed in the bone marrow and presence of a number of perforations Fig (1 C). Our results are in agreement with^[36]. The quantitative loss of bone, as well as the changes of its internal structure, is considered responsible for the

increased fracture risk in postmenopausal osteopenia. Dexamethasone inhibit gastrointestinal absorption and renal tubular reabsorption of calcium, resulting in secondary hyperparathyroidism^[16], which is consistent with the increase in PTH expected with decreased calcium absorption and lower plasma osteocalcin, suggested that dexamethazone treatment suppressed both osteoblastic activity and bone resorption, indicating an overall suppression of bone turnover^[3].

Examination of the femur bone of a rat treated with dexamethazone in dose (20 mg / Kg. b. w.) and the plant extract (30 mg / Kg. b. w.) showing improvement of bone tissue architecture in the form of regularity of both inner and outer surfaces of the bone tissue although the Haversian canals are still widened. The number of oosteocytes is increased in comparison with that in group treated with Dexa. Most of the

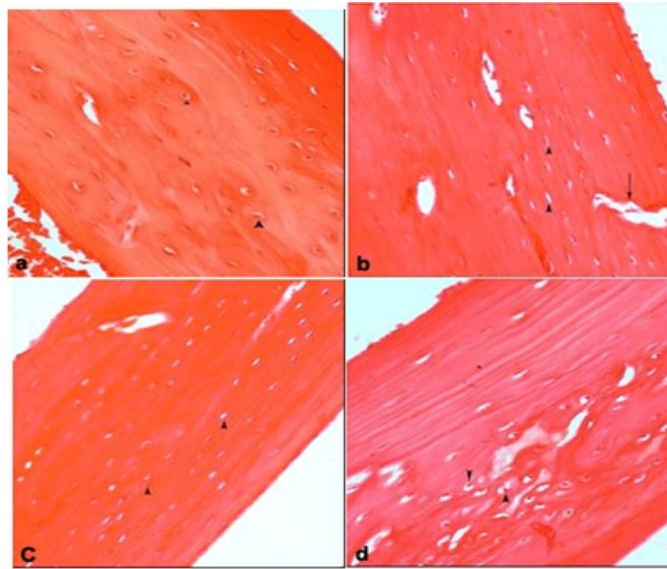
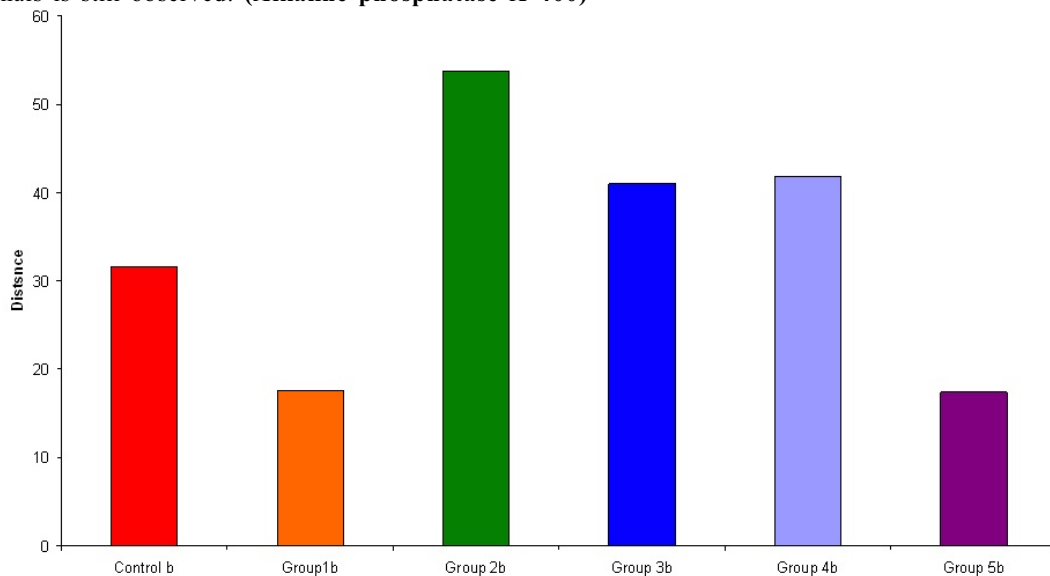
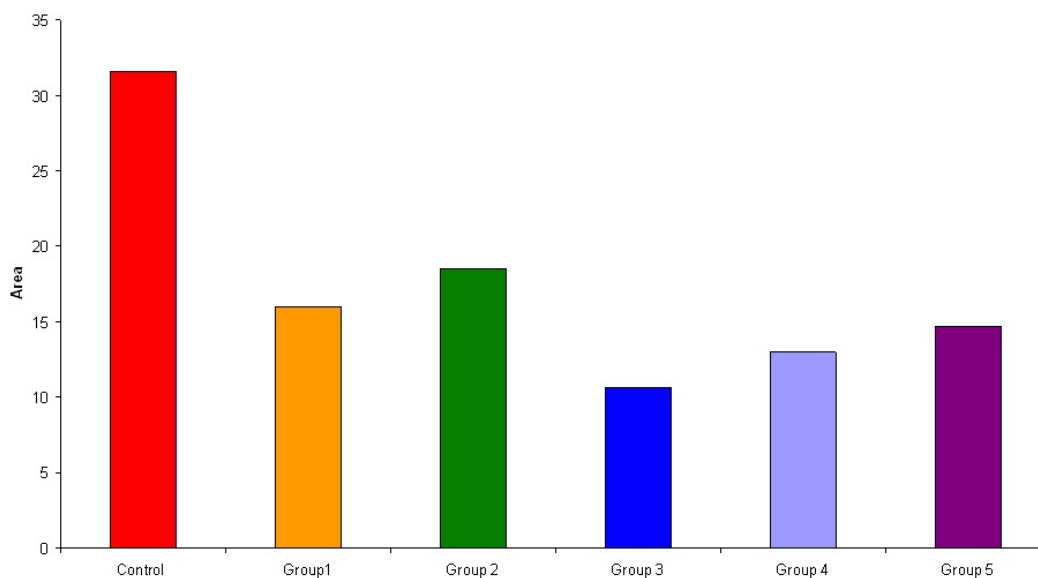


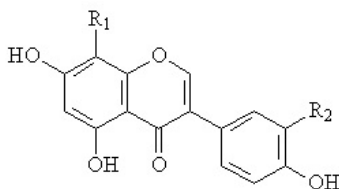
Fig. 4: (a) A photomicrograph of a section of control bone tissue showing the lacunae containing the osteocytes taking the dark brown color of the stain. They are of two types: the old ones (oval) and the young ones (rounded). (b): A photomicrograph of a section of bone tissue from a dexamethazone treated rat showing decrease in the positivity of the reaction as the lacunae appear either empty or containing atrophied osteocytes (arrow head). Irregularities as invasion sites (arrow) and widening of the Haversian canals are observed. (c): A photomicrograph of a section of bone tissue from a rat treated with plant extract for two months and then with dexamethazone for one month, showing a good protective effect of the plant extract in the form of preserving the number of active osteocytes (arrow head) and the regularity of both surfaces of bone tissue. (d): A photomicrograph of a section of bone tissue from a rat treated with dexamethazone for one month and then with plant extract for two months showing formation of new osteoid tissue and young active osteocytes (arrow heads). However, this new osteoid is irregular and widening of Haversian canals is still observed. (Alkaline phosphatase X 400)



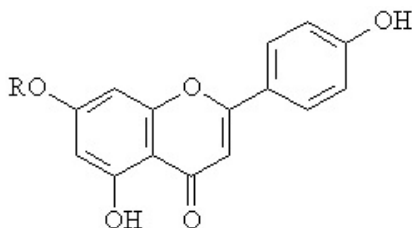
Histogram (1): The effect of dexamethasone and plant extract on the thickness of bone tissue. Control b= control, Group 1b= group injection of dexamethasone, Group 2b = group received extract of *Retama raetam* seeds, Group 3b= group received dexamethasone together with *Retama raetam* seeds extract at the same time. , Group 4b = group received plant extract and then dexamethasone, Group 5b= group received dexamethasone alone and then the plant extract



Histogram (2): The effect of dexamethasone and plant extract on the area of osteocytes. Control = control, Group 1 = group injection of dexamethasone, Group 2 = group received extract of *Retama raetam* seeds, Group 3 = group received dexamethasone together with *Retama raetam* seeds extract at the same time. , Group 4 = group received plant extract and then dexamethazone, Group 5 = group received dexamethasone alone and then the plant extract



- | | | |
|---|--------------|---------|
| 1 | R1 = glucose | R2 = H |
| 2 | R1 = glucose | R2 = OH |
| 3 | R1 = R2 = H | |
| 4 | R1 = H | R2 = OH |



- | | |
|---|-------------|
| 5 | R = glucose |
| 6 | R = H |

osteocytes are of the young type denoting increased rate of formation of osteocytes and bone tissue regeneration (Fig. 2 c and d). Zhang and coworkers^[37] reported that in rats, administration of dexamethasone showed bone resorption due to estrogen deficiency resulting in a marked bone loss. The estrogen deficiency leads to the increased rate of bone remodeling (bone resorption and formation), because estrogen decreases a number of remodeling cycles by attenuating the birth rate of osteoclasts and osteoblasts from their prospective progenitors. The imbalance between bone resorption and formation in favor of the former observed in osteoporosis is due to changes in the working life-span of osteoclasts and osteoblasts. The flavonoid extract has been found to be effective in preventing osteoporosis induced by ovariectomy in rats (which indicate that flavonoids may enhance the development of osteoblasts through their active metabolites^[4]). Also soy yoghurt, fermented with *E. faecium* and *L. jugurti* and supplemented with isoflavones, was effective in preventing bone mass loss in mature ovariectomized rats and of increasing bone mass in intact rats^[27]. Phytoestrogens have been suggested as having the potential to reduce bone loss due to their similarity to estrogen.

Histologically, the section of the femur bone of a rat treated with the plant extract (30 mg / Kg. b. w.) for two months and then injected with dexamethasone in dose (20 mg / Kg. b. w.) for one month, showing regular both inner and outer surfaces of bone tissue. The lamellae of the matrix under the periosteum are regular in arrangement enclosing between them old spindle shaped osteocytes while the young ones are located more centrally in a non-regular manner denoting a good protective measure of this extract (Fig. 3 a and b). Weinstein and coworkers^[35] showed that glucocorticoids inhibit osteoblastogenesis and promote apoptosis in osteoblasts and osteocytes in mice. Osteocytes transduce mechanical stimuli in bone, and glucocorticoids have been shown to reduce bone loading-bearing capacity in rats^[12]. The deleterious effects of pharmacological doses of glucocorticoids on bone may be mediated by osteocytes, both in cortical and cancellous bone. These effects may be prevented by *Retama raetam*, which may act as an estrogen agonist as estrogen exerts pro-apoptotic effects on osteoclasts and anti-apoptotic effects on osteoblasts and osteocytes. These results indicate that estrogen may exert an inhibitory effect on resorption activity of mature osteoclasts due to inhibition of organic matrix degradation by cysteine proteinases^[24].

The section of the femur bone of a rat treated with dexamethasone in dose (20 mg / Kg. b. w.) for one month and then with the plant extract (30 mg / Kg. b. w.) for two months, showing mild amelioration as inner and outer surfaces of bone tissue are still irregular with points of invasion. The old osteocytes are located in the center of the bone tissue, while the

young ones are located more peripherally but in an abnormal arrangement denoting incomplete regeneration of bone tissue (Fig. 3 c and d). Dexamethasone has deleterious effects on bone and inhibits the synthesis of collagen and proteins, through binding with their receptors (GR) inside the cell and form a glucocorticoid-GR complex which enters the nucleus and causes changes that alter the synthesis of mRNA from the DNA molecule, altering the production of different proteins and decreases the production of osteoid in bone. The bone mineral density and longitudinal bone growth were disturbed^[3,34]. *In vitro* and *in vivo* experimental studies have shown that the isoflavones found in soy products have an anabolic effect on the bone metabolism of intact rats, increasing the osteoblastic activity and consequently bone neoformation, and inhibiting osteoclastic activity and consequently bone resorption^[14,23].

In the present study, we found that the effects of dexamethasone-induced estrogen deficiency and the effects of *Retama raetam* treatment on bones depended on given plant as prevention or treatment. The effects of dexamethasone on bone tissues was found to be much high on bone tissue. The improvement in bone tissue was greater in the group of bone treated with plant and then dexamethasone than that in the group treated with dexamethasone and the plant.

The phytochemical investigation of the aqueous methanol extracts *Retama raetam* seeds resulted in, genistein-8-C-glucoside, orobol-8-C-glucoside, genistein, orobol, apigenin-8-O-glucoside and apigenin. The structures of the isolated compounds were established through chromatography, as well as conventional chemical and spectroscopic methods of analysis (UV, ¹/₂ D NMR).

Soy protein and the isoflavone genistein have been shown to help maintain bone mass and prevent osteoporosis in rats, which simulated the post-menopausal period in women and prevent both *in vivo* and *in vitro* bone loss and increase the bone mass of femur in ovariectomized rats^[2]. Phytoestrogens including daidzein^[25] and genistein^[17] were proven to have an anabolic effect on bone metabolism, prevented bone loss and prevent the decrease of bone density and strength, and increase the activity of osteoblasts and enhance bone mineralization^[18]. Osteoblasts are the key cells involved in bone matrix formation and calcification and have been shown to differentiate into mature cells following treatment with 17 β -estradiol. In cortical bone culture of female rats, genistein and daidzein induced an increase of calcium and phosphorus content and alkaline phosphatase activity in bone tissues^[14].

The morphometric parameters showed marked decrease in mid-diaphyseal cortical bone thickness and increased bone resorption in dexamethasone-treated groups compared with controls. These findings are much improved in the animals treated with plant

extract.

The mechanism of cortical bone loss is thought to be an increased activation of Haversian remodeling systems accompanied by an increased Haversian canal diameter^[28]. Cortical bone loss markedly occurs in Haversian remodeling in humans. Osteoprotegerin is a protein produced by osteoblasts that strongly inhibits osteoclast differentiation, which leads to decreased bone resorption. Dexamethasone inhibit the production of osteoprotegerin, leading to increased osteoclast differentiation, which in turn leads to increased bone resorption. Genistein has been shown to partially prevent such negative action of dexamethasone on osteoprotegerin^[33]. Such protective action by genistein should lead to a lower level of bone loss caused by the administration of dexamethasone. Our results in the present work are in coincidence with these previous data as our results of morphometric analysis showed that using the plant extract lead to great increase in bone thickness that might be due to an increase in proliferation, differentiation and activity of osteoblasts (bone matrix-forming cells), while its direct effect on osteocytes was much less.

ALP activity was done to study the effect of *Retama raetam* on osteoblast differentiation. Our results showed that dexamethasone decreased the activity levels of ALP in bone tissue, and this effect was elevated by *Retama raetam* treatment (Fig. 4 a, b, c and d). A higher osteoblastic activity and minimal osteoclastic activity account for bone formation which in turn prevents osteoporosis. The histopathological data adds a confirmatory note to the findings. Based on the findings of the present study, we can hypothesize that *Retama raetam* prevents bone loss in a manner similar to soy isoflavones, which have been shown to effectively lower the number of mature osteoclasts by inducing osteoclast apoptosis^[14] and suppressing osteoclastogenesis, thereby reducing bone resorption^[5]. Thus, the antiosteoporotic activity of *Retama raetam* may justifiably be attributed to the steroids present which probably act as phyto-estrogens to effectively prevent or reduce bone loss. These results were also observed in similar studies with animal models^[31,6] and with postmenopausal women^[23] which it was concluded that the isoflavones were responsible for bone mass maintenance, indicating that phytoestrogens reduce bone resorption (osteoclastic activity) and increase bone formation (osteoblastic activity), thus supporting the results obtained in our study. Other findings suggest that dried plum protects against bone loss by increasing bone formation, serum bonespecific ALP and Ovx rats^[13]. Total flavonoids and flavonol glucosides exhibited an improvement in the development of osteoblasts by promoting the alkaline phosphatase^[37]. Meanwhile, the crude extract, flavonoids and isovlavone have been found effective in preventing osteoprosis caused by the administration of dexamethasone in animals studies^[4]. In vitro and

clinical studies have shown that genistein induces an elevation of bone-specific alkaline phosphatase activity^[1].

In conclouision, the present study clearly demonstrates the efficacy of using *Retama raetam* extract to prevent effect of dexamethasone-induced increase in bone turnover rate and to restore the loss of trabecular bone mass in rats. Moreover, the data indicated that the observed decrease in ALP activity of bone cells and incerase in ALP acivity by *Retama raetam* extract in osteoprotic rats might be mediated by its direct action on bone cells. This study demonstrates the beneficial effects of *Retama raetama* on osteoblastic cells and other bone-specific parameters in an animal model of dexamethasone-induced osteoporosis. Our results clearly demonstrate that *Retama raetam* could be considered as a natural alternative to hormone replacement therapy for the prevention of bone loss in postmenopausal women.

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