THE POTENTIAL PROTECTIVE EFFECTS OF *VIGNA RADIATA* AND *LEPIDIUM SATIVUM* AGAINST BONE LOSS INDUCED BY PREDNISOLONE ACETATE IN MALE AND FEMALE RATS

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Abstract

A common side effect of prolonged glucocorticoid therapy is osteoporosis which is the most common cause of secondary osteoporosis. The aim of our study was to investigate the potential anti-osteoporotic effect of Vigna radiata (V. radiata) and Lepidium sativum (L. sativum) extracts in prednisolone acetate (PA)-induced osteoporotic rats. Rats were divided into 2 main groups (30 males and 30 females). Each main group was randomly divided into 5 equal subgroups. Rats of subgroup I were served as normal control and received the vehicle. Rats of subgroup II (osteoporotic control) were co-administered PA at 5 mg/kg and the vehicle. Subgroup III was administered with alendronate sodium (3 mg/kg) and served as a reference control. Subgroups IV and V were administered with V. radiata and L. sativum extracts (500 mg/kg), respectively. Body weights of all rats were measured at 3, 6, 9 and 12 weeks after PA, reference and extracts administration. At the end of the experimental period, serum samples and femur bones were examined. PA administration significantly reduced body weight gain, serum levels of calcium (Ca^{2+}), Phosphorous (P) and alkaline phosphatase (ALP), as well as, femur weight and thickness and femur contents of Ca^{2+} and P of male and female rats. Administration of L. sativum extract (500 mg/ kg) significantly protected against the reduction in the serum and bone levels of Ca^{2+} and P and femur physical parameters of PA-exposed male and female rats. Histopathological results supported protective effect of the extract. Our findings suggest a potential protective role of L. sativum against PA-induced osteoporosis in male and female rats. In addition, V. radiata did not produce any considerable protection against the deleterious effect of PA on the bone of male and female rats.

Key words: *Lepidium sativum; Vigna radiata; osteoporosis; rats*

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INTRODUCTION:

Glucocorticoids are widely used to treat inflammatory diseases [1]. However, the therapeutic use of glucocorticoids is always accompanied by substantial adverse outcomes. Osteoporosis induced by glucocorticoid is the most common form of secondary osteoporosis [2]. The disease involves both sexes and all races [3]. Presently, treatment of glucocorticoid-induced osteoporosis depends on drugs similar to those used in case of post-menopausal osteoporosis, such as PTH, calcium, calcitonin and vitamin D. These medications do not address the multi-factor driven glucocorticoid-induced osteoporosis. Currently, many natural products having an anti-osteoporotic activity were reported [4].

V. radiata L (Mung bean) family *Leguminoseae* is native to East and South Asia regions. It contains abundant amounts of flavonoids [5]. Earlier studies have demonstrated that *V. radiata* can act as an antioxidant [6], hepatoprotective [7] and antidiabetic agent due to its low glycemic index [8]. Leaves of the plant are used in cases of rheumatic pain, brain disorders, stomach, inflammatory disorders and jaundice [9].

L. sativum belongs to Cruciferae family and is known as Halim or Garden cress. In Saudi Arabia, *L. sativum* is called Hab el Rashaad and is a popular herbal plant grown in many regions such as AL-Qaseem and the Eastern Province [6]. The plant is used in traditional medicine in South Asia to treat asthma and is considered useful as diuretic, aphrodisiac, stomachic and gastrointestinal stimulant [10]. *L. sativum* is reported to exhibit antihypertensive, hypoglycemic and antioxidant properties [11]. Phytochemical analysis of the plant showed presence of coumarins, flavonoids, imidazole alkaloids, sulphur glycosides, sterols and triterpenes [12]. The plant seeds contain high percentages of protein and fat. The most abundant amino acids are glutamic acid, leucine and methionine, while the major fatty acid is linolenic acid [13].

Researchers are looking for better alternatives especially from natural sources. Therefore, the present study was an effort to explore the potential effect of *V. radiata* and *L. sativum* in experimental osteoporosis.

MATERIAL AND METHODS:

Plant Material and Extract Preparation

Vigna radiata (Mung bean) and *Lepidium sativum* (Garden cress- Hab elRashad) were purchased from a local market in Al-Kharj city. Samples were compared with voucher specimen deposited in the herbarium of the MAPPRC at the College of Pharmacy, King Saud University.

Powdered plant materials were extracted with ethanol by maceration at room temperature and combined extracts were evaporated under reduced pressure using rotary vacuum evaporator.

Experimental animals

Seven-week-old Sprague-Dawley male (200-210 g) and female (150-160 g) rats were obtained from Lab Animal Care Unit, College of Pharmacy, Prince Sattam bin Abdulaziz University, Saudi Arabia. Rats were maintained under standard situations of temperature (22±1 °C), relative humidity (55±5%), and 12 h/12 h light/dark cycle, and fed with a standard pellet diet with water *ad libitum.* The experiments and procedures used in this study were approved by the Ethical Committee of the College of Pharmacy, Prince Sattam Bin Abdulaziz University.

Acute toxicity test

Acute toxicity study of the ethanol extracts of *V. radiata* and *L. sativum* was carried in adult Sprague-Dawley rats according to OECD-423 guidelines [14]. Rats were divided into 3 groups (n = 6) and fasted overnight. Rats of the 1st and 2nd groups received *V. radiata* and *L. sativum* extracts, respectively at a dose of 5000 mg/kg (5 mL/kg) by the oral route. Rats of the 3rd group (control) treated with the vehicle (3% v/v Tween 80 in distilled water) and kept under the same conditions. Each animal was observed for symptoms of toxicity and/or mortalities during the first 30 min and periodically during 24 h, with special attention given during the first 4 h and daily there-after, for a total of 14 days.

Effect against bone loss induced by PA

After one week of acclimatization, 60 rats were divided into 2 main groups (30 males and 30 females). Each main group was randomly divided into five subgroups (n = 6) and treated as follows:
Group I (Normal control): Rats received the vehicle.
Group II (Osteoporotic control): Rats co-administered with PA at 5 mg/ kg and the vehicle.
Group III (Reference): Rats co-administered with PA at 5 mg/ kg and alendronate sodium at 3 mg/ kg. Alendronate sodium, an aminobisphosphonate, is a potent antiresorptive drug used in the treatment of osteoporosis.
Group IV: Rats co-administered with PA at 5 mg/ kg and *V. radiata* at 500 mg/ kg.
Group V: Rats co-administered with PA at 5 mg/ kg and *L. sativum* at 500 mg/ kg.
All treatments were given to rats once daily by oral route using gastric tube for 12 weeks. Body weights of all animals were measured each 3 weeks.

Serum bone markers

At the end of the experimental period, rats were anaesthetized with an intraperitoneal overdose of pentobarbital sodium and blood was collected from the retro-orbital region, centrifuged at 10,000 rpm for 10 min, and the collected sera were stored at -80°C for analysis of Ca^{2+} [15], P [16] and ALP [17].

Femur physical parameters

Soon after the collection of blood, the femurs were isolated and weighed using an electronic scale. The thickness of each femur was measured at their midshafts using a digital caliper. Then, the femurs were dried at 110 °C for 12 h, and weights of dried femurs were measured.

Bone mineral contents

The left femur of each rat was dried overnight at 100°C, then incinerated in a muffle furnace at 800°C for 6 h to obtain the ash. Bone ash was weighed then dissolved in 0.1 N HCl. Ca^{2+} and P contents in bone ash were determined using Furnace Atomic Absorption Spectrophotometer (FAAS) AAS-7000 Shimadzu, according to the method adopted by Teófilo *et al.* [18].

Histological examination of femurs

The right femurs were fixed in 10% formalin for 24 h, decalcified in 15% EDTA for 20 days, dehydrated in alcohol, then cleared in xylol and embedded in paraffin wax. Sections of 6 mm were prepared using rotary microtome and stained with haematoxylin–eosin, then observed under microscope [19].

Statistical analysis

Version 20 of SPSS, USA, was used for the statistical evaluation of the obtained data. Results were expressed as mean ± SE and as percentages. Statistical significance for data was determined using a one-way analysis of variance (ANOVA) with post-hoc test. The level of significance was accepted as P < 0.05.

RESULTS:

Acute oral toxicity:

Acute toxicity study in rats proved that *V. radiata* and *L. sativum* extracts were safe even at the dose of 5000 mg/kg. Both extracts did not provoke any visible signs of toxicity, stress or adverse behaviours. In addition, there was also no sign of diarrhea and none of the treated animals died in 14 days. Hence, the LD50 values of both extracts were thus found to be more than 5000 mg/kg body wt, p.o.

Effect against bone loss induced by PA

All data did not significantly differ between male and female rats.

Body weight

There was no significant difference between initial body weights of the male groups at the beginning of the study. Similarly, the five groups of female rats had a similar initial mean body weight. However, the body weights of osteoporotic control male and female groups were significantly decreased on week 6, 9, and 12 after PA administration, compared to their corresponding normal control groups (Tables 1-2). At the endpoint, the mean body weights of the osteoporotic control male and female rats were decreased by 22.41% and 25.18%, respectively compared to their corresponding normal control groups (Figures 1-2).

Administration of alendronate sodium + PA and *L. sativum* + PA significantly protected against the body weight loss in male and female rats in comparison to their corresponding osteoporotic control groups. No significant changes were observed in the body weights of male and female groups following *V. radiata* + PA intervention.

Table 1: Effects of *V. radiate* and *L. sativum* extracts on body weight (g) of male rats with PA-induced osteoporosis.

	Normal control	Osteoporotic control (PA)	Alendronate sodium + PA	*V. radiata* + PA	*L. sativum* + PA
0 w	208.07± 9.5	206.15± 10.6	205.45± 10.7	203.60± 12.7	210.27± 11.4
3 w	287.02± 10.2	249.85± 10.8#	273.36± 12.4	259.58± 14.5	269.43± 13.5
6 w	345.19± 11.7	286.77± 12.7#	342.20± 13.7*	302.52± 15.7	334.27± 13.7*
9 w	396.25± 14.9	312.81± 13.4#	384.62± 14.6*	344.38± 14.6	371.40± 14.6*
12 w	432.97± 15.1	335.92± 13.5#	410.17± 15.3*	380.22± 15.7	399.62± 12.5*

Values are expressed as Mean ± SE of six animals in each group.

 # indicate significance compared to normal control group at p< 0.05 (Dunnett's test).

*indicate significance compared to osteoporotic control group at p< 0.05 (Dunnett's test).

Table 2: Effects of *V. radiate* and *L. sativum* extracts on body weight (g) of female rats with PA-induced osteoporosis.

	Normal control	Osteoporotic control (PA)	Alendronate sodium + PA	*V. radiata* + PA	*L. sativum* + PA
0 w	158.64 ± 8.9	156.25 ± 9.4	155.61 ± 8.8	154.08 ± 10.1	157.62 ± 10.6
3 w	196.78 ± 9.2	170.28 ± 10.7	188.57 ± 9.4	180.44 ± 10.6	184.64 ± 10.1
6 w	245.42 ± 10.7	196.37 ± 9.5#	232.05 ± 9.6*	212.14 ± 9.7	227.50 ± 8.5*
9 w	282.80 ± 11.6	219.28 ± 11.4#	264.52 ± 10.7*	244.59 ± 10.4	255.62 ± 12.4*
12 w	316.57 ± 11.3	236.85 ± 11.7#	284.73 ± 11.3*	267.21 ± 10.3	281.73 ± 10.5*

Values are expressed as Mean ± SE of six animals in each group.
 # indicate significance compared to normal control group at p< 0.05 (Dunnett's test).
*indicate significance compared to osteoporotic control group at p< 0.05 (Dunnett's test).
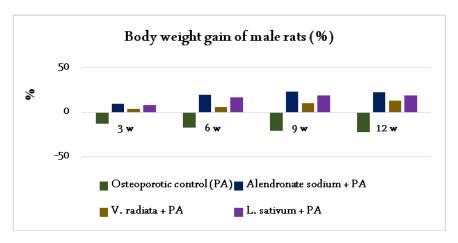

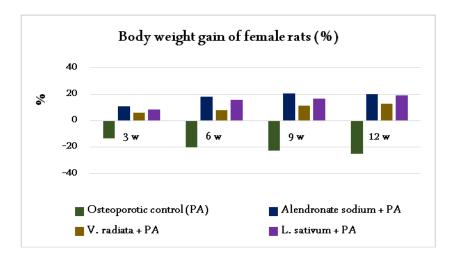**Fig 1: Effects of *V. radiate* and *L. sativum* extracts on the percentage of weight gain of male rats with PA-induced osteoporosis.**

Fig 2: Effects of *V. radiate* and *L. sativum* extracts on the percentage of weight gain of female rats with PA-induced osteoporosis.

Serum bone markers

The results of the present study show that serum Ca^{2+} and P levels were significantly decreased in osteoporotic control rats (males: 36.61% and 45.06%, respectively and females; 36.61% and 45.06%, respectively), compared to the corresponding normal control groups (Tables 3-4). The reduced serum levels of Ca^{2+} and P were significantly restored in rats supplemented with alendronate sodium + PA (males: 17.50% and 58.08%, respectively and females: 35.23% and 64.06%, respectively) and *L. sativum* + PA (males: 22.01% and 70.58%, respectively and females: 44.12% and 70.31%, respectively), as compared to the corresponding osteoporotic control rats. Serum ALP levels were significantly increased in the osteoporotic control male and female rats compared to the corresponding normal control groups. The increase in ALP level in the serum of osteoporotic male and female rats was significantly prevented following alendronate sodium and *L. sativum* supplementation (Tables 3-4). However, *V. radiata* extract could not reverse the decreased levels of Ca^{2+} and P levels or the increased activities of ALP in serum which were induced by PA administration.

Table 3: Effects of *V. radiate* and *L. sativum* extracts on serum biochemical parameters of male rats with PA-induced osteoporosis.

	Normal control	Osteoporotic control (PA)	Alendronate sodium + PA	*V. radiata* + PA	*L. sativum* + PA
Ca^{2+} (mg/dL)	5.15 ± 0.10	3.77 ± 0.17# (-26.79%)	4.43 ± 0.18* (17.50%)	4.18 ± 0.15 (10.87%)	4.60 ± 0.16* (22.01%)
P (mg/dL)	2.49 ± 0.05	1.36 ± 0.11# (-45.38%)	2.15 ± 0.12* (58.08%)	1.58 ± 0.16 (16.17%)	2.32 ± 0.15* (70.58%)
ALP (U/L)	172.5±8.74	294.7± 15.58# (70.84%)	192.7± 12.62* (-34.61%)	255.2 ± 16.81 (-13.40%)	192.7 ± 14.44* (-34.61%)

Values are expressed as Mean ± SE of six animals in each group.

 # indicate significance compared to normal control group at p< 0.05 (Dunnett's test).

*indicate significance compared to osteoporotic control group at p< 0.05 (Dunnett's test).

Values between brackets means % changes.

Table 4: Effects of *V. radiate* and *L. sativum* extracts on serum biochemical parameters of female rats with PA-induced osteoporosis.

	Normal control	Osteoporotic control (PA)	Alendronate sodium + PA	*V. radiata* + PA	*L. sativum* + PA
Ca^{2+} (mg/dL)	4.97 ± 0.18	3.15 ± 0.25# (-36.61%)	4.26 ± 0.23* (35.23%)	3.73 ± 0.22 (18.41%)	4.54 ± 0.21* (44.12%)
P (mg/dL)	2.33 ± 0.11	1.28 ± 0.11# (-45.06%)	2.10 ± 0.17* (64.06%)	1.42 ± 0.19 (10.93%)	2.18 ± 0.17* (70.31%)
ALP (U/L)	180.3±6.52	279.2± 17.80# (54.85%)	190.2± 15.50* (-31.87%)	236.8 ± 14.73 (-15.18%)	191.5 ± 15.17* (-31.41%)

Values are expressed as Mean ± SE of six animals in each group.

 # indicate significance compared to normal control group at p< 0.05 (Dunnett's test).

*indicate significance compared to osteoporotic control group at p< 0.05 (Dunnett's test).

Values between brackets means % changes.

Femur physical parameters

The femur weight and thickness were considerably decreased in the osteoporotic control male (35.43% and 16.53%, respectively) and female (27.08% and 18.58%, respectively) groups when compared with the corresponding normal control groups. After treatment of male and female rats with alendronate sodium and *L. sativum* extract, femur weight and thickness were restored, as compared with the corresponding osteoporotic control groups (Tables 5-6).

Bone mineral contents:

At the end of the experimental period, marked significant reduction in the femur ash weight was observed in the osteoporotic male (36.76%) and female (26.92%) control rats as compared with the corresponding normal control groups (Tables 5-6). Treatment with alendronate sodium and *L. sativum* extract significantly increased the femur ash weight in male (39.53% and 41.86%, respectively) and female (23.68% and 28.94%. respectively) rats compared with the corresponding osteoporotic

control groups. In addition, bone Ca^{2+} and P levels significantly decreased in osteoporotic control rats (males: 42.79% and 30.44%, respectively and females: 44.51% and 28.24%, respectively) as compared with the corresponding normal control groups. The changes in ash Ca^{2+} and P were significantly normalized in rats treated with alendronate sodium (male: 53.92% and 18.90%, respectively and females: 69.85% and 20.78%, respectively) or *L. sativum* extract (males: 50.11% and 16.57%, respectively and females: 65.37% and 20.88%, respectively), as compared to osteoporotic control rats (Tables 5-6).

Table 5: Effects of *V. radiate* and *L. sativum* extracts on bone parameters of male rats with PA-induced osteoporosis.

	Normal control	**Osteoporotic control (PA)**	**Alendronate sodium + PA**	***V. radiata* + PA**	***L. sativum* + PA**
Femur weight (g)	1.27± 0.02	0.82±0.06# (-35.43%)	1.08± 0.05* (31.70%)	0.90± 0.07 (9.75%)	1.10± 0.05* (34.14%)
Femur thickness (mm)	2.48 ±0.07	2.07±0.05# (-16.53%)	2.35 ±0.06* (13.52%)	2.15 ±0.10 (3.86%)	2.34 ±0.08* (13.04%)
Ash weight (g)	0.68 ± 0.03	0.43 ± 0.02# (-36.76%)	0.60 ± 0.04* (39.53%)	0.49 ± 0.05 (13.95%)	0.61± 0.03* (41.86%)
Ca^{2+} (mg/g ash)	485.26±11.36	277.58±16.30# (-42.79%)	427.26±14.28* (53.92%)	316.72±16.21 (14.10%)	416.68±15.27* (50.11%)
P (mg/g ash)	126.75 ±5.15	88.16±4.17# (-30.44%)	104.83 ±6.11* (18.90%)	100.45 ±5.17 (13.94%)	102.77 ±4.15* (16.57%)

Values are expressed as Mean ± SE of six animals in each group.
 # indicate significance compared to normal control group at p< 0.05 (Dunnett's test).
*indicate significance compared to osteoporotic control group at p< 0.05 (Dunnett's test).
Values between brackets means % changes.

Table 6: Effects of *V. radiate* and *L. sativum* extracts on bone parameters of female rats with PA-induced osteoporosis.

	Normal control	**Osteoporotic control (PA)**	**Alendronate sodium + PA**	***V. radiata* + PA**	***L. sativum* + PA**
Femur weight (g)	0.96± 0.04	0.70±0.06# (-27.08%)	0.88± 0.05* (25.71%)	0.78± 0.06 (11.42%)	0.90± 0.05* (28.57%)
Femur thickness (mm)	2.26 ±0.05	1.84±0.06# (-18.58%)	2.05 ±0.05* (11.41%)	2.00 ±0.06 (8.69%)	2.02 ±0.05* (9.78%)
Ash weight (g)	0.52 ± 0.02	0.38 ± 0.03# (-26.92%)	0.47 ± 0.02* (23.68%)	0.44 ± 0.03 (15.78%)	0.49± 0.02* (28.94%)
Ca^{2+} (mg/g ash)	408.25±14.47	226.50±28.72# (-44.51%)	384.72±27.66* (69.85%)	292.13±28.69 (28.97%)	374.58±29.60* (65.37%)
P (mg/g ash)	112.18 ±6.25	80.49±3.82# (-28.24%)	97.22 ±4.15* (20.78%)	87.12 ±5.24 (8.23%)	97.30 ±4.70* (20.88%)

Values are expressed as Mean ± SE of six animals in each group.
 # indicate significance compared to normal control group at p< 0.05 (Dunnett's test).
*indicate significance compared to osteoporotic control group at p< 0.05 (Dunnett's test).
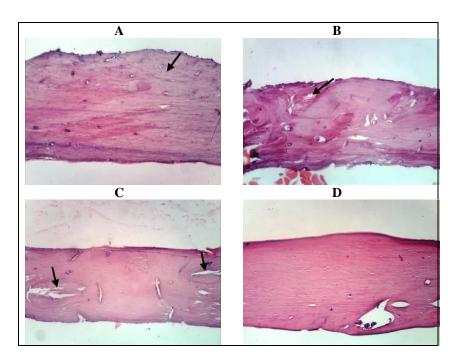*Values between brackets means % changes.*

Fig 3: Photomicrographs (x 100, H&E) of femur of a normal control rat revealed no histopathological alteration in the cortical bone with osteoblasts proliferation (A); femur of an osteoporotic control rat, showing thinning of the outer cortical bone with presence of cracks and fissures (B); femur of a rat treated with PA + *V. radiata* showing thinning of the outer cortical bone with presence of cracks and fissures (C) and femur of a rat treated with PA + *L. sativum* revealed marked improvement as compared to those of the osteoporotic rats (D).

Histological examination of femurs

Microscopically, the left femur of rat from normal control animals revealed no histopathological alteration in the cortical bone with osteoblasts proliferation as well as normal bony trabeculae (Figure 3-A). Bone sections in the osteoporotic rats revealed thinning of the outer cortical bone with presence of cracks and fissures in addition to deteriorated architecture of trabecular bone (Figure 3-B). Like the osteoporotic group, bone sections from rats treated with PA + *V. radiata* showed thinning of the outer cortical bone with presence of cracks and fissures (Figure 3-C). Femur sections from the PA + *L. sativum* extract rats revealed marked improvement as compared to those of the osteoporotic rats. The cortical bone thickness was very similar to the normal control group. The bony trabeculae partially regained near normal structure and appeared more continuous with less widened bone marrow spaces (Figure 3-D).

DISCUSSION:

Osteoporosis is a metabolic bone disorder characterized by loss of bone mass and the mechanical impairment of the bone tissue in addition to an increase in the bone weakness and tendency to fracture [20].

Synthetic glucocorticoids play an important role in the normal regulation of bone remodeling. They are used to treat inflammatory and immune disorders. A common side effect of prolonged glucocorticoid therapy is glucocorticoid-induced osteoporosis which is the most common cause of secondary osteoporosis [21]. This adverse effect is due to the direct inhibitory effect of glucocorticoids against the osteoblasts [22], a reduction in renal tubular calcium reabsorption [23] or a reduction in the amount of calcium absorbed by the intestine [24].

In the present study, PA was used as a model for the evaluation of potential osteoporosis treatments [25, 26]. After 12 weeks of PA administration, the mean body weights of the osteoporotic control male and female groups were significantly lower than that of the corresponding normal controls. The body weight changes during the study as a result of PA administration support the observations of the other investigators [27,28]. Since PA reduced the body weights of male and female rats by 22.41% and 25.18%, respectively, it was possible that the reduced body weights might have contributed to the PA-induced bone loss. The administration of ethanolic

extract of *L. sativum* significantly normalized the PA-induced decreases in body weight of male and female rats.

In our study, administration of PA resulted in significant reduction in Ca^{2+} and P levels in the serum of osteoporotic control male and female rats compared with their corresponding normal groups. These results are in agreement with Banji et al [29], who reported that PA-exposed rats had impaired Ca^{2+} and P balance. The decline in the serum levels of Ca^{2+} and P could be due to enhanced renal excretion and alteration in their transport across the brush border membrane [29]. In this present study, *L. sativum* extract is effective in protection against PA-induced hypocalcemia in male and female rats. In this connection, *L. sativum* extract is a good source of linolenic acid, which has been shown to inhibit bone reabsorption and markers of bone turnover and decrease the elimination of Ca^{2+} [30].

Serum ALP is a biochemical marker of bone turnover, good indicator of internal bone activity and is used to monitor metabolic bone diseases. The increased bone turnover and fracture risk was manifested by the ALP activity [31]. Serum ALP is increased in osteoporosis and other bone metabolic disorders [32]. Similarly, the present finding showed that 12 weeks of PA medication, increased the level of ALP in serum of male and female rats compared to their normal control groups. The marked increased activity of serum ALP in osteoporotic control male and female rats indicates increased bone turnover due to the induction of osteoporosis. The positive role of medical herbs was achieved by the observed improvement of bone metabolic marker; ALP [33]. In this study, the ethanolic extract of *L. sativum* significantly improved serum ALP level after 12-week administration, suggesting that the plant can activate the osteoblast differentiation and bone formation; meanwhile, it can inhibit osteoclast function and bone resorption.

Bone has a persistent and dynamic turnover with sequential cycles of resorption and renewal of bone packets as a result of the coupled action of bone-forming cells, osteoblasts and bone-resorbing cells, osteoclasts. The processes of formation and resorption of bone are coupled in both time and space and the amount of bone resorbed and formed is at the equilibrium. Glucocorticoid treatment is suggested to influence bone physiology and calcium homeostasis [28]. Our finding demonstrated that 12 weeks of treatment with PA significantly decreased the femur weight and thickness in male and female rats. These results are in agreement with Banji et al [29] and Lin et al [28] who recorded that the levels of bone Ca^{2+} and P were decreased in the PA-treated rats. This may be because PA enhances urinary excretion of Ca^{2+} and P and reduces their intestinal absorption [29]. The reduced weight and thickness of femurs of male and female animals exposed to PA could be attributed to apoptosis of osteoblasts and osteocytes [4]. In this connection, accelerated osteoblast apoptosis has been manifested in patients with osteoporosis induced by glucocorticoid [34]. This bone loss was accompanied by a marked increase in bone remodeling, as manifested by the enhanced biochemical bone turnover marker. In addition, mice implanted with glucocorticoids also have a higher number of apoptotic osteoblasts that inhibit bone formation [35]. *In vitro* studies have also revealed that glucocorticoids can induce the apoptosis of osteoblasts [36]. These results suggested that increased osteoblast apoptosis is responsible for glucocorticoid-induced bone loss.

In the present study, oral administration of *L. sativum* extract at a dosage of 500 mg/kg significantly decreased the bone loss in the femurs of PA-treated male and female rats. In this connection, phytosterols and phytoestrogens are some important compounds present in higher quantities in *L. sativum* plant. Sirotkin and Harrath [37] reported that phytoestrogens can be useful for the prevention and treatment of many diseases including osteoporosis. Krishnaraju et al [38] added that seeds of *L. sativum* are useful in the treatment of fracture.

CONCLUSION:

In conclusion, the present data indicated that *L. sativum* protected male and female rats against osteoporosis associated with glucocorticoid use, as represented by the elevation of bone minerals (Ca^{2+}, P). Thus, *L. sativum* might represent a natural therapy to help in preventing bone loss associate with PA osteoporosis.

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