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Original Article

Effects of the Hydroalcoholic Extract of the *Psidium guajava* Fruit on Osteoporosis Prevention in Ovariectomized Rats

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Abstract

Background: Several plants have been shown to possess antioxidant and estrogenic properties that can be useful in postmenopausal bone-loss prevention. The present study aimed to investigate the anti-osteoporotic effects of the hydroalcoholic extract of the *Psidium guajava* (*PG*) fruit in ovariectomized (OVX) rats.

Methods: Sixty female Sprague–Dawley rats were randomly divided into 6 groups: a control positive group, a sham-operated group, an OVX group given normal saline (OVX-only group), and 3 treatment groups comprising 2 OVX groups treated orally with 500 and 1000 mg/kg/d of the hydroalcoholic extract of the *PG* fruit respectively and an OVX group treated with an injection of 0.15 mg/kg of estradiol. The study was conducted over a 12-week period. Samples from the animals' blood, femoral bones, and uteri were collected for stereological and biochemical analyses. The data were analyzed using SPSS, version 19. A P value equal to or less than 0.05 was considered statistically significant.

Results: The results revealed a significant decrease in the levels of calcium, total antioxidant capacity, and phosphorus as well as uterus weight, femoral ash density, femoral volume and weight, and numbers of osteocytes and osteoblasts. Moreover, there was an increase in the levels of alkaline phosphatase and urine deoxypyridinoline together with a rise in the number of osteoclasts in the OVX-only group compared to the control and treatment groups ($P \leq 0.05$). The hydroalcoholic extract of the *PG* fruit increased femoral weight and volume, femoral ash density, numbers of osteocytes and osteoblasts, and trabecular volume of the bones in comparison with the OVX-only group in a dose-dependent manner. No significant difference was observed between the groups in the levels of malondialdehyde and interleukin-6.

Conclusion: The hydroalcoholic extract of the *PG* fruit prevented OVX-induced bone loss in the rats, with no proliferative effect on atrophic uteri; it should, therefore, be considered for treatment purposes.

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What's Known

- For all the studies evaluating osteoporosis treatment, its prevention by herbal medicine remains debatable.

What's New

- *Psidium Guajava* because of its range of phytochemicals and minerals can be considered for the treatment of osteoporosis.

Introduction

Osteoporosis is one of the most prevalent chronic diseases,[1] and it is growing especially in the elderly.[2] In various studies,

height, weight change, nutrition, lifestyle, length of menopause, puberty, steroids, calcium intake, and vitamin D are considered risk factors for osteoporosis.[3] Estrogen deficiency, as a major reason for osteoporosis in both sexes, causes oxidative stress and increases reactive oxygen species.[4] Estrogen supplement can inhibit oxidative stress.[5] Osteoporotic women have low antioxidant levels due to estrogen deficiency, which has a negative effect on bone mass.[6] Duggan et al.[7] indicated inflammation as a related factor to bone turnover in patients with osteoporosis. *Psidium guajava* (*PG*) is a member of the *Myrtaceae* family, whose fruit has a variety of phytochemicals, especially lycopene, vitamin C, vitamin A, iron, zinc, calcium, manganese, magnesium, saponins, tannins, and other flavonoids such as quercetin. The *PG* fruit and leaves have high antioxidant, antimicrobial, liver protective, antiplaque, anticancer, analgesic, anti-inflammatory, immune-system-regulating and antiarthritic properties.[8,9] This family is phytoestrogenic[10] and is used for the treatment of osteoporosis[11] in hormone replacement therapy. Additionally, because of its unique nutritional constituents, this family can be effective in bone-loss prevention.

In light of the abovementioned information, we conducted the present study to assess the efficacy of the hydroalcoholic extract of the *PG* fruit in the prevention of osteoporosis.

Materials and Methods

Extract Preparation

Fresh fruits of *PG* (Genus: *Psidium*, Species: *guajava*; herbarium number 771) were purchased from Chabahar (Iran). The fruits were washed, sliced, and dried in shade for 2 days. The dried fruits were then powdered; the powder was kept in 70% ethanol for 72 hours and shaken several times a day (300 g/1070 mL). Thereafter, the powder was filtered to obtain a semi-solid extract. The concentrated extract was kept in a desiccator for 12 hours (high vacuum) at 45 °C, and the semi-solid extract was kept in a refrigerator at 4 °C. The extract (4 g) was dissolved in 20 mL of distilled water and after surgery, the hydroalcoholic extract was gavaged to the rats every day according to the following formula:[12]

volume of the extract (*ml*)=[*weight of the rat* (*kg*)×*dose rate* (*mg/kg*)]/*stock concentration* (*mg/ml*)

Antioxidant Assays

The antioxidant potential of the *PG* extract was determined by using the Brand–Williams 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.[13] Quercetin was used as the reference antioxidant with the half maximal inhibitory concentration (IC_{50}) of 9.1±0.42. The results of the DPPH assay were expressed as IC_{50} values by the Curve Expert software (for Windows, version 1.34). The reactions were carried out in 96-well microplates, and the *PG* extract was tested at different concentrations. Ethanol was regarded as a blank for the microplate reader. The test sample solutions (270 µL), containing different concentrations of the plant extract in ethanol, were mixed with an ethanolic solution of DPPH (100 µM). This mixture was incubated at room temperature for 30 minutes, and finally absorbance was measured at 517 nm with a spectrophotometer.[14] Each experiment was repeated 3 to 5 times. All the data were presented as means±standard deviations (SDs).

Determination of the Total Phenolic Content

The Folin–Ciocalteu colorimetric method, described by Waterhouse (2002), was applied to determine the total phenolic content of the plant extract. To that end, 40 µL of the 1 mg/mL extract was mixed with 3.16 mL of distilled water and 200 µL of Folin–Ciocalteu reagent.[6,15] After 10 minutes, 600 µL of a 0.25% sodium carbonate solution was added to this mixture. The absorbance of the resulting solution was measured at 765 nm against the blank after 2 hours of incubation at room temperature by using a UV-visible spectrophotometer. Quantitative measurements were done based on a calibration curve of gallic acid as the standard phenolic compound in ethanol. The concentration of the total phenolic was expressed as milligrams of gallic acid equivalents per gram of dry extract.

Animals

Sixty adult female Sprague-Dawley rats, 6 months old and weighing 200±20 g, were purchased from the Laboratory Animal Breeding Center, Shiraz University of Medical Sciences. The animals were kept in a room at a temperature of 23±2 °C and humidity of 55±5% at a 12-hour light/dark cycle; they were fed standard pellet and water *ad libitum*. After 1 week, the rats were randomly divided into 6 groups (10 rats in each group): a control positive group, a sham-operated group, an ovariectomized (OVX) group given normal saline (OVX-only group), and 3 treatment groups comprising 2 OVX groups treated with 500 and 1000 mg/kg/d of the hydroalcoholic extract of the *PG* fruit respectively and an OVX group treated with 0.15 mg/kg of estradiol. The *PG* extract was gavaged daily and estradiol was injected weekly. The intervention

was started a day after surgery and continued for 12 weeks. All the experiments were in accordance with the recommendations of the European Council Directive (86/609/EEC) of November 24, 1986, regarding the protection of animals used for experimental purposes (http://data.europa.eu/eli/dir/1986/609/oj). The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences (No. 94-01-84-10444).

Ovariectomy

Ovariectomy was done bilaterally, and sham surgery was carried out through the manipulation of the ovaries without removal to induce surgical stress. The animals were subjected to surgery after anesthesia with 10% ketamine (100 mg/kg, Alfasan, Netherlands) and 2% xylazine (10 mg/kg, Alfasan, Netherlands).

Collection of Specimens and Biochemical Tests

Deoxypyridinoline (Dpd) was measured during the last 24 hours of intervention by placing the rats in metabolic cages separately to collect 24-hour urine samples. Subsequently, the samples were centrifuged (2500 rpm in 15 min at 4 °C) and Dpd was measured using an ELISA kit. After 12 weeks, the rats having been anesthetized with 10% ketamine (100 mg/kg) and 2% xylazine (10 mg/kg) underwent cardiocentesis for blood collection. The blood samples were centrifuged to separate the serum (3500 rpm for 10 min). The serum samples were used to evaluate biochemical markers, comprising calcium, phosphorus, alkaline phosphatase (ALP), urine Dpd, malondialdehyde (MDA), interleukin-6 (IL-6), and total antioxidant capacity (TAC), with ELISA kits. The levels of calcium, phosphorus, and ALP were also quantified by calorimetric methods using kits supplied by Biosystems S.A., Spain.

Euthanasia was done rapidly with diethyl ether. The rats' right and left femurs and uteruses were harvested and fixed in 10% formalin after all the connective tissues were removed. The weight of the uteruses and bones was measured using electronic laboratory balances with 0.001 mg accuracy (Secura 213-1S, England). To investigate ash density after weighing was investigated by incinerating the bones at 700 °C for 7 hours. Ash density was calculated as ash weight per bone volume (g/cm^2).[16]

Stereological Methods

Estimation of Bone Volume

As the histomorphology of the bone may be modified in different preparation conditions of samples, well-grounded stereological examinations provide quantitative morphological data on the most important characteristics.

The right femurs were removed and decalcified in 10% acid formic for 1 week. The primary volume "V primary" was measured via the immersion method.[12] During tissue fixation, processing, and staining, tissue shrinkage was measured. Shrinkage estimation required isotropic uniform random slabs and the orientator method.[13] In this method, the right femur was placed on a circle (A) and a number was randomly chosen between 0 and 9. Thereafter, the bone was sectioned into 2 parts along into the chosen number. The first portion was placed on another circle (B) and a random number was chosen again and cut into a parallel portion. The second portion was vertically placed on another circle (C), and a new random number was selected again and cut into a direction parallel to the number.

In this method, 10 to 12 slabs were sampled from each bone. A spherical piece, about 2 mm in diameter, was punched out of a random slab using a trocar. Thereafter, the diameter and area of the circular pieces were calculated. The sampled slabs and the circular pieces of each animal's bone were embedded in 1 paraffin block. Then, 5- and 25-µm sections were obtained. After the tissue sections were stained with hematoxylin and eosin, the area of the circular pieces were measured again to estimate the global degree of bone tissue shrinkage.[17]

Before and after fixation/processing/embedding, the area of the circular piece was estimated according to the following formula:[18]

$$V\left(shrinkage\right) = 1 - \frac{Area\,after}{Area\,before}^{1.5}$$

where "area after" and "area before" were the areas of each circular piece of the bones. The final volume of the bone was estimated using the following formula:

$$V_{(final)} = (1\text{-}Dsh) \times V_{(primary)}$$ [6,19,20]

Estimation of the Volume Density of the Bones' Trabeculae

Sections of 5 µm were used to estimate the volume and volume density of the trabeculae and bones. The volume density of the trabeculae "Vv (trabeculae)" refers to the ratio of the volume of the bone ossification that is filled by trabeculae, and it was estimated by the following point-counting method:

$$V_v\left(trabeculae\,/\,bone\right) = \frac{P(Trabeculae)}{P\left(reference\right)}$$

where "P (trabeculae)" was the grid points number falling on the trabeculae and "P

(reference)" was the grid points number falling on the reference bone tissues. The following formula was used to estimate the femur bone volume:[6]

$V\ (bone) = V\ (final) \times V_v(bone)$

The Estimation of Numerical Density and the Number of Bone Cells

Sections of 25 µm were used to estimate the number of bone cells and numerical density. Numerical density "NV (cells/bone)" refers to the number of cells per unit volume of the trabeculae and was evaluated by using the disector method. For this purpose, a microscope (Nikon E-200, Japan) was linked to a monitor and a counting grid was laid on the bone.[20] The upper and lower guard zones of the bone were set to be 5 µm using a microcator (MT 12, HEIDENHAIN, Germany). The thickness "t" of the final section on the bone was measured using the microcator (20 µm). The disector height of the section (10 µm) was considered the disector height. The numerical density of the bone cells was estimated using the following formula:[20]

$$NV(cells / bone) = \frac{\sum_{i=1}^{n} Q}{(\sum P \times h \times a / f)} \times \frac{t}{BA}$$

where "ΣQ" was the number of the bone cells counted, "h" was the optical disector height, "a/f" was the area of the counting grid, "ΣP" was the number of the counted grid, "BA" or block advance was the sum of the microtome to cut the block of paraffin, and "t" was the mean of the final cut thickness.

The total number of the bone cells was estimated by applying the following formula[19]:

$N\ (bone\ cells) = N_V\ (cells/bone)\ V\ (final)$

Statistical Analysis

The data were analyzed using a computerized statistical program (SPSS, version 19.0). The analysis of the pathological findings was done using the Mann–Whitney *U*-test. The data of the wound area were analyzed using one-way ANOVA. A P value equal to or smaller than 0.05 was considered statistically significant.

Results

Biochemical Markers

The levels of serum calcium, phosphorus, ALP, urine Dpd, TAC, IL-6, and MDA in the experimental groups are shown in table 1.

A significant decrease in the concentration of serum calcium in the OVX-only group was observed in comparison with the control (P=0.04) and treatment (P≤0.05) groups. The level of serum calcium in the treatment groups with estradiol and 1000 mg/kg/d of the *PG* extract (P=0.04) was significantly increased in comparison with the OVX-only group (P≤0.05).

The level of serum phosphorus was increased significantly in all the treatment groups compared with the OVX-only group (P≤0.05). The level of serum phosphorus in the group treated with 1000 mg/kg/d of the *PG* extract was significantly higher than that in the estradiol- treated group (P=0.04).

The levels of both serum ALP (P≤0.05) and urine Dpd (P≤0.05) were significantly increased in the OVX-only group compared with all the other groups, but their concentrations were not significantly different between the treatment group and the control group or the sham- operated group (P≤0.05).

The level of serum TAC was significantly increased in all the treatment groups compared with the OVX-only group (P≤0.05). There were no significant differences in the levels of serum MDA and IL-6 between the groups (P≤0.05).

Uterus Weight

The results showed a significant reduction in the uterus weight in the OVX-only group (P=0.001) and the groups treated with the *PG*

Table 1: Measurement of serum biochemical parameters in the experimental and control groups

Group	Ca (mg/dL)	P (mg/dL)	ALP (mg/dL)	Urine Dpd (% of basal ratio)	MDA (nmol/mL)	TAC (nmol/mL)	IL-6 (nmol/mL)
Control	10.96±0.14	6.40±0.20	392.0±55.5	50.08±6.06	2.42±0.06	0.209±0.015	144.4±43.9
Sham-operated	10.98±0.08	5.91±0.28	371.0±63.5	50.28±5.42	2.38±0.20	0.226±0.014	179.8±56.2
OVX	10.01±0.35†	4.17±0.39†	642.7±48.4 †	84.26±9.62†	2.91±0.13	0.149±0.014†	213.2±46.2
OVX+PG 500 mg/kg	10.41±0.45#	6.29±0.34*	454.0±111.1	50.49±6.17*	2.52±0.24	0.240±0.012*	197.9±47.6
OVX+PG 1000 mg/kg	11.02±0.23*	6.56±0.48*#	442.0±114.1	51.62±4.34*	2.69±0.23	0.221±0.009*	204.2±32.2
OVX+estrogen	11.53±0.12*	5.39±0.28*	556.1±52.7	61.37±6.00*	2.50±0.15	0.203±0.008*	147.6±52.3

OVX, Ovariectomized; *PG, Psidium guajava*; Ca, Calcium; P, Phosphorus; ALP, Alkaline phosphatase; Urine Dpd, Urine deoxypyridinoline; MDA, Malondialdehyde; TAC, Total antioxidant capacity; IL-6, Interleukin-6; †P<0.05, OVX vs. control or sham-operated; *P<0.05, OVX vs. estrogen or *PG* (500 or 1000 mg/kg) extract groups, #P<0.05, estrogen vs. OVX+*PG* (500 or 1000 mg/kg)

extract at both doses (P<0.001 and P=0.01). Further, there was a significant increase (P≤0.05) in the uterus weight of the estradiol group compared with the treatment and control groups (P=0.02) (figure 1).

Left Femoral Ash Density

There was a significant decrease in the left femoral ash density in the OVX-only group compared with the control (P=0.02) and treatment (P≤0.05) groups. In all the treatment groups, the left femoral ash density was significantly increased compared to the OVX-only group (P=0.04, P=0.01, and P=0.03) (figure 2).

Weight of the Right Femur

In the treatment groups with estradiol and 1000 mg/kg/d of the *PG* extract, there was a significant increase compared to the OVX-only group (P<0.001) in the average weight and volume of the right femur; the difference was not statistically significant between the OVX group treated with 500 mg/kg/d of the *PG* extract and the OVX-only group (P=0.12).

Stereological Study

The weight of the femur; volume of the femur; total volume of the bones' trabeculae; and total number of the osteocytes, osteoblasts, and osteoclasts are presented in table 2. Additionally, the qualitative microscopic evaluation of the femur is shown in figure 3.

Our results indicated a significant decrease in the right femoral trabecular volume in the OVX-only group compared with the control (P<0.001) and treatment (P≤0.05) groups.

A significant reduction was observed in the number of right femoral osteocytes in the OVX-only group compared to the control (P=0.01) and treatment (P≤0.05) groups. In the estradiol group and the group treated with 1000 mg/kg/d of the *PG* extract, there was a significant rise in the number of right femoral osteocytes compared to the OVX-only group (P=0.01 and P=0.01).

The number of osteoblasts in the right femur was significantly lower in the OVX-only group than in the control (P<0.001) and treatment (P<0.05) groups. The number of the osteoclasts in the right femur was higher in the OVX rats than in the sham-operated (P<0.001) and control (P=0.04) groups. The number of the osteoclasts was significantly decreased in all the treatment groups compared to the OVX-only group (P<0.05).

Antioxidant Activity and Total Phenolic Content

The total polyphenol content was 252.67±2.71 milligrams of gallic acid equivalents per gram of dry extract. Free radical scavenging activity was defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration to 50% in 30 minutes (IC_{50}). The DPPH radical scavenging activity, expressed as the IC_{50} of the *PG* extract, was 161.66±4.479 mg/mL.

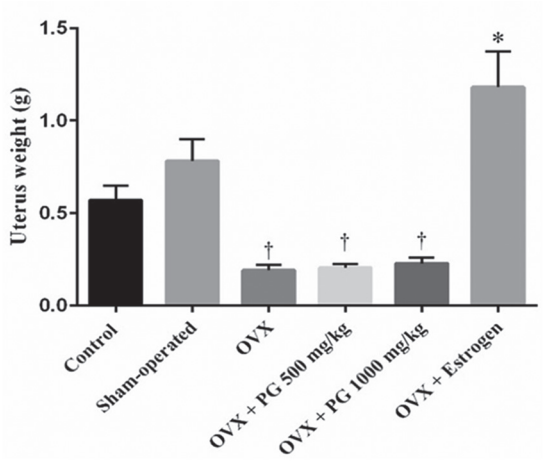

Figure 1: Uterus weights of all the groups after 12 weeks. †P<0.05, OVX vs. control or sham-operated group; *P<0.05, OVX vs. OVX+estrogen or *PG* extract (500 or 1000 mg/kg) groups; OVX: Ovariectomized; *PG*: *Psidium guajava*.

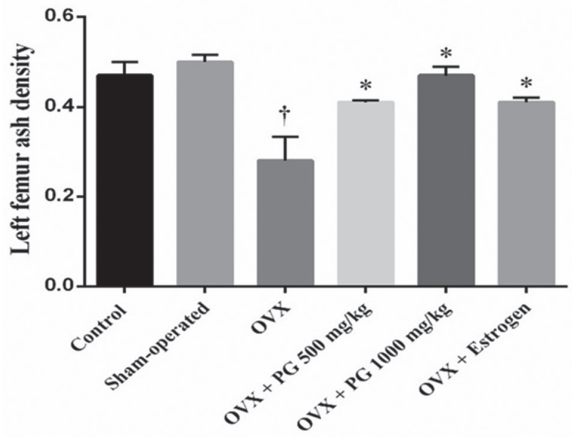

Figure 2: Left femoral ash density of all the groups after 12 weeks. †P<0.05, control or sham-operated group vs. OVX or OVX+*PG* extract (500 or 1000 mg/kg) *P<0.05, OVX+ estrogen vs. *PG* extract (500 or 1000 mg/kg) or control or sham-operated group; OVX: Ovariectomized; *PG*: *Psidium guajava*.

Histopathology of the Liver

There was no evidence of liver toxicity with 1000 mg/kg/d of the *PG* extract during the present study (figure 4).

Discussion

According to our study, the hydroalcoholic extract of the *PG* fruit prevented OVX-induced bone loss in rats with no proliferative effect on atrophic uteri. We evaluated the effects of

Table 2: Measurements of weight, volume and stereological parameters of the femur on the experimental and control groups in male rats

Groups	Weight (mg)	Volume (mm^3)		Number		
	Femur	Femur	Trabeculae	Osteocytes ($\times10^6$)	Osteoblasts ($\times10^6$)	Osteoclasts ($\times10^4$)
Control	510.0±23.0	478.3±22.8‡	144.1±12.6‡	51.79±15.38	7.90±0.86	24.28±4.8
sham-operated	504.6±24.3	468.6±27.3	146.3±7.3‡	50.44±7.26	8.70±1.39	19.80±3.9
OVX	385.1±20.5†	359.5±22.4†	90.27±7.5†	21.52±1.95†	3.70±0.75†	39.51±3.8†
OVX+PG500mg/kg	435.3±18.3 #	402.6±18.6	106.7±6.0#	33.00±2.50	7.70±1.05*	22.66±2.16*
OVX+PG1000mg/kg	485.1±13.2*	459.3±13.5*	132.38±15.4*	48.23±6.19*	8.20±1.55 *	21.93±4.35*
OVX+estrogen	499.0±23.6*	460.5±22.5*	137.9±8.2*	47.09±10.47*	7.30±1.38 *	24.69±6.47*

PG: *Psidium Guajava*. †p<0.05, OVX vs. control or sham-operated groups. *P<0.05, OVX vs. OVX+estrogen or PG extract groups, #P<0.05, Estrogen vs. OVX+PG (500 or 1000mg/kg), ‡P<0.05, OVX+PG 500 mg/kg vs. control or sham

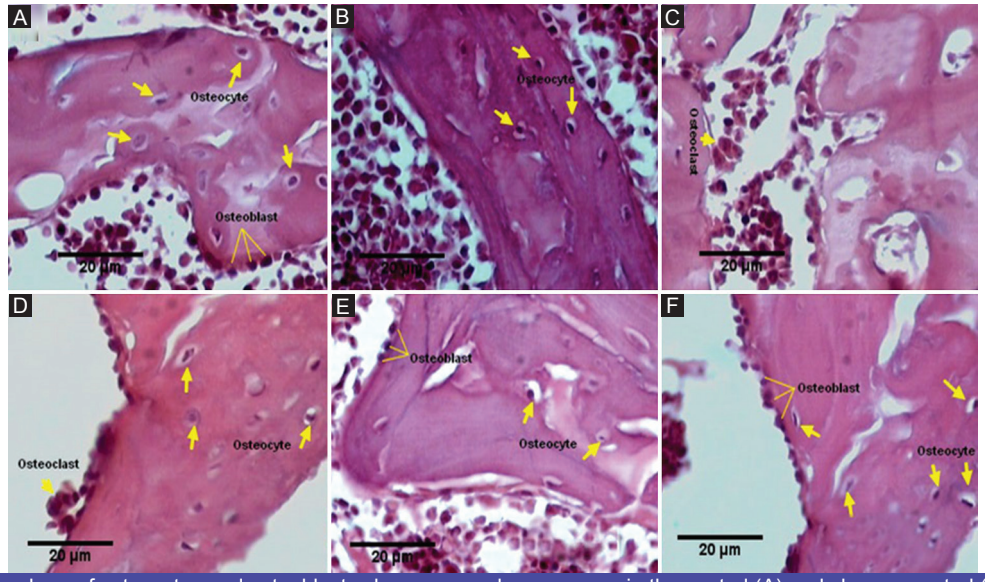

Figure 3: Numbers of osteocytes and osteoblasts show a normal appearance in the control (A) and sham-operated (B) rats. The trabecular atrophic changes and the increased number of the osteoclasts can be seen clearly in the OVX rats (C). Treatment of the OVX animals with *Psidium guajava* (D, E) and estrogen (F) showed protective changes in the bone (stained with hematoxylin and eosin, ×400). OVX: Ovariectomized.

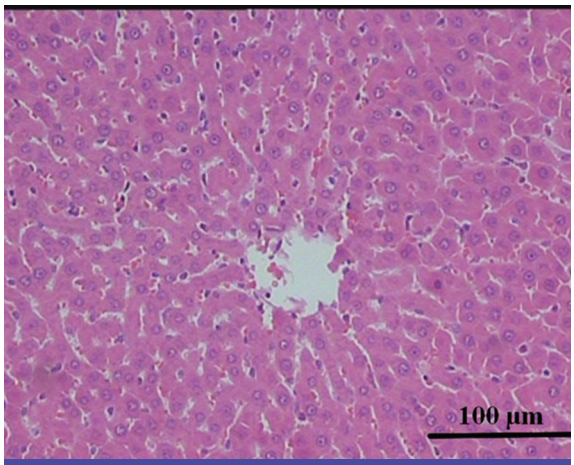

Figure 4: Histopathology of the liver with at a dose of 1000 mg/kg/d after 12 weeks in a male rat (stained with hematoxylin and eosin, ×100).

the hydroalcoholic extract of the *PG* fruit, in comparison with estradiol as a major treatment for osteoporosis, on the metabolism and femoral structure of OVX rats.

Menopause reduces calcium intestinal absorption despite its urinary excretion.[6] Bone loss is accompanied by increased bone remodeling, as evidenced by increased biochemical bone turnover markers such as serum ALP.[21] Dpd is a biomarker of bone reabsorption. Consistent with our findings, Iimura et al.[22] demonstrated that lycopene significantly reduced the urine Dpd concentration, which was predictable because *PG* is a rich source of lycopene. The authors also concluded that oxidative stress was a risk factor of bone loss and that it was indirectly correlated with bone mineral density.

Oxidant/antioxidant imbalance can activate osteoclasts and progress bone loss.[23] In the present study, the extract of the *PG* fruit increased serum TAC significantly unlike

the OVX-only group. It is suggested that the aqueous extract of the *PG* fruit peel supplementation has the ability to reduce oxidative stress (MDA) in the pancreas of diabetic rats and may play a role in reducing the development of diabetic complications.[24] Similar to our results, Lei et al.[25] (2009) reported that osteoclasts were increased while osteoblasts were decreased among their OVX rats with very thin trabecular bones and explained that estrogen loss in OVX rats inhibited the differentiation of mesenchymal stem cells into osteoblasts.

Chiming in with our results, Høegh-Andersen et al.[26] reported estrogen deficiency following ovariectomy and also a reduction in uterus weight due to estrogen withdrawal in rats, capable of changing bone metabolism. Estrogen is an anabolic hormone with proliferative effects on the uterus.[27] Lack of estrogen causes uterine atrophy as we found in our OVX-only group. Previous studies have indicated that the biomechanical competence of bone depends on bone mass (ash density) and the continuity of bone lattice changes with an increase in age.[28]

The ethanol extract of the *PG* fruit because of its antioxidant and phytoestrogen compounds[10] stimulates bone formation, reduces the involvement of free radicals in bone resorption, enhances the serum levels of calcium and phosphorous, and prevents hydrogen peroxide production, thus inhibiting ovariectomy-induced bone loss.[29]

Changes in the bone remodeling cycle occur with aging, menopause, and various pathologic situations and render the skeleton more fragile.[30] Research during the last decade has revealed that estrogen regulates bone homeostasis through unexpected regulatory effects on the immune system and oxidative stress and exerts a direct impact on bone cells.[31] The stereological surveys in the current study demonstrated an increased number of osteoblasts and a decreased number of osteoclasts in the rats' right femur. Furthermore, in contrast to the OVX-only group, the estradiol and *PG* extract-treated groups showed the supportive effects on bone. Dabbaghmanesh et al.[32] (2017) used the hydroalcoholic extract of the *Elaeagnus angustifolia* fruit in OVX rats in order to prevent osteoporosis and reported that this extract significantly increased the number of osteoblasts and decreased the number of osteoclasts. These effects were analogous to those of the hydroalcoholic extract of the *PG* fruit due to their similar constituents such as phytoestrogens and flavonoids. Noorafshan et al.[6] (2015) reported a significant increase in the number of osteoclasts and a reduction in the number of osteoblasts and osteocytes in OVX rats. The authors also demonstrated that their OVX rats had a lower total volume of bone trabeculae than their control group. Nonetheless, a reduction in the level of ALP and in the number of osteoclasts as well as a rise in the number of osteocytes and osteoblasts was previously reported in OVX rats treated with the hydroalcoholic extract of black olives due to its components such as estrogen,[6] which can inhibit the production of proinflammatory cytokines and improve bone remodeling. These findings are concordant with the results of the current study. We found that these changes were ameliorated in the *PG*-treated groups. This effect might result from the antioxidant and phytoestrogenic properties of *PG*. In point of fact, bone mass is synchronized by the stability between osteoclastic bone resorption and osteoblastic bone formation.[33] Derakhshanian et al.[34] (2013) showed that quercetin was able to prevent osteoporosis by the stimulation of bone formation. Polyphenolic compounds play a bone-protective role by both reducing osteoclastic resorption and increasing osteoblastic activity.[35,36] This action can be also related to the antioxidant capacity of polyphenols.[37] The present study showed a significant increase in the TAC. Guaijava polyphenolic flavonoid, which is also present in *PG*,[9] and quercetin can inhibit bone loss without effects on the uterus in OVX rats.[38] The increase and decrease in the numbers of osteoblasts and osteoclasts, respectively, have made the rat a valuable model in osteoporosis studies.[39]

For all the merits of the present study, the limitations in our work should be addressed by future investigations focusing further on the detection of major components and performance of thorough liver function tests.

Conclusion

The hydroalcoholic extract of the *PG* fruit conferred a significant osteoprotective activity in the OVX rats in the present study. The extract, therefore, seems to be a potential candidate for the development of new approaches to osteoporosis treatment.

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