World Journal of Dairy & Food Sciences 9 (2): 220-227, 2014
ISSN 1817-308X

DOI: 10.5829/idosi.wjdfs.2014.9.2.1139

Effect of Diet with Dried Plum, Calcium and Vitamin D on Osteoporosis in Ovariectomized Rats

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Abstract: The study aimed to assess the protective effect of diet supplemented with dried plum powder (DPP), calcium (Ca) and vitamin D (Vit.D) on osteoporosis in ovariectomized (OVX) rats and to elucidate the potential mechanisms. Forty two Sprague Dawley rats were randomized into 6 equal groups (n= 7). Group 1 was SHAM-operated (negative control), while the other 5 groups were OVX-operated. Three weeks post-ovariectomy, group 2 was kept positive control (OVX) and fed on basal diet. Groups 3, 4, 5 and 6 were fed on diets supplemented with DPP, Ca, Vit.D and DPP + Ca + Vit.D, respectively for 6 weeks. At the end of experiment, rats were weighted and blood samples were collected for estimating serum biomarkers of osteoporosis. Rats were then sacrificed and uteri were dissected out and weighed. Femur bones were dissected out for estimating bone markers of osteoporosis. The results showed that feeding diets supplemented with DPP + Ca + Vit.D reversed the increase in body weight gain and the decrease in uterine weight induced by ovariectomy. It also significantly increased serum calcium (Ca), bone-specific alkaline phosphatase (b-ALP) and osteocalcin (OC) but decreased serum interleukin-1beta (IL-1beta), interleukin-6 (IL-6) and pyridinoline (PD) levels in OVX-rats. There were significant increases in serum free thyroxin (T4) and calcitonin (CT) hormones and a decreased in parathormone (PTH). Femur bone mineral density (BMD) and contents of calcium and phosphorus in bone ash were also increased. These findings suggest that feeding of OVX rats on diets supplemented with dried plum, calcium and Vit. D together induces an anti-osteoporotic effect. This effect might be due to enhancement of bone formation, suppression of bone loss and regulation of some metabolic hormones which control calcium. Therefore, intake of dried plum and consumption of foods rich in calcium (as milk and dairy products) and Vit.D may be beneficial for the prevention of osteoporosis in postmenopausal women.

Key words: Plum · Calcium · Vitamin D · Osteoporosis · Biochemical analysis · Hormones · Bone mineral density

INTRODUCTION

Osteoporosis is the most common type of bone diseases which affects both men and women worldwide. It is characterized by a low bone mass and decreased bone mineral density, so it can lead to bone fragility and fractures [1]. Osteoporosis represents a serious health problem which prevails among elderly and young postmenopausal women. Moreover, menopause drastically increases the risk of osteoporosis [2]. Postmenopausal osteoporosis occurs due to imbalance between osteoblastic bone formation and osteoclastic bone resorption [3]. Estrogen deficiency is the most potent initiator of osteoclastic bone loss which is commonly associated with osteoporosis [4]. The preventive measures against osteoporosis include avoiding of smoking and excessive intake of alcohol and caffeine, adequate intake of calcium and vitamin D and practicing exercise [5]. Estrogen, calcium, vitamin D, calcitonin and several natural antioxidants can prevent postmenopausal osteoporosis [6]. Estrogen replacement therapy (ERT) has been established for the prevention of postmenopausal osteoporosis, but its long term may cause adverse effects and increase the risk of ovarian and uterine cancers [7]. Therefore, the search for natural antioxidants from fruits that can prevent osteoporosis is nowadays necessary.

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Dried plum (*Prunus domestica* L.) fruit is a rich source of nutritive and bioactive compounds. Plum contains large amounts of phenolic compounds (184 mg/100 g), mainly chlorogenic and neochlorogenic acids and high potassium content (745 mg/100 g). Moreover, dried plum is an important source of boron, which is postulated to play a role in the prevention of osteoporosis [8]. The phenolic and flavonoid compounds in dried plum are highly effective in modulating bone mass in estrogen-deficient rat model of osteoporosis [9]. Among the nutritional factors, dried plum is the most effective fruit in both preventing and reversing bone loss in postmenopausal women and this anti-osteoporotic effect is in part due to suppression of bone turnover rate [10]. Recently, it was reported that diet supplementation with dried plum fruit can prevent ovariectomy-induced bone loss in mice [11] and in rats [12]. Nutrition plays an important role in bone health and there is an increasing interest in dietary micronutrients which influence bone health. Adequate dietary intake of calcium and vitamin D improved bone turnover markers in postmenopausal women [13, 14] and in ovariectomized rats [15, 16]. Dietary supplementation with both calcium and vitamin D is more effective in reducing bone fracture risk than either each supplement alone because vitamin D helps intestinal absorption of calcium [17].

The present study was therefore performed to evaluate the effect of feeding diets supplemented with dried plum powder, calcium and vitamin D on serum and bone markers of osteoporosis in ovariectomized rats and to elucidate the potential mechanisms.

MATERIALS AND METHODS

Dietary Supplements: Dried plum powder was purchased from a local market of dried nuts and fruits, Giza, Egypt, in the form of dark dried powder packaged in plastic bags each containing 500 gram. It is added to basal diet at concentration of 10 %. (100 grams of powder added to 1 kg basal diet) according to Smith *et al*. [12]. Calcium carbonate was procured from El-Gomhoryia Company, Egypt in the form of fine white powder. Calcium carbonate is widely used as an inexpensive dietary calcium supplement. It was added to basal diet at 210 mg/kg diet according to Chen *et al*. [14]. Vitamin D (Cholecalciferol, vitamin D3) was obtained in the form of capsules and added to basal diet at 600 IU/kg diet according to Ghanizadeh *et al*. [16].

Rats: Forty two sexually mature female Sprague Dawley rats weighing 280-285 g b.wt and 10 to 12 weeks old were used in this study. The rats were purchased from Laboratory Animal Colony, Helwan, Egypt. The animals were housed under hygienic conditions at room temperature of 24°C, relative humidity of 50 % and 12 hr light/12 hr dark cycles. The rats were fed on either basal or experimental diets and water was provided as required. The experiment on rats was carried out according to the National regulations on animal welfare and Institutional Animal Ethical Committee (IAEC).

Basal and Experimental Diets: Basal diet is consisted of 20 % protein, 10 % sucrose, 5 % corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers and the remainder is corn starch up to 100 %. The dietary supply of protein, fat, carbohydrates, vitamins and minerals in basal was in accordance with the recommended dietary allowances for rats (American Institute of Nutrition, AIN93) according to Reeves *et al.* [18]. Four experimental diets were formulated as follows:

- Basal diet supplemented with 10 % dried plum powder (DPP).
- Basal diet supplemented with Ca (210 mg/kg diet).
- Basal diet supplemented with Vit. D (600 IU/ kg diet).
- Basal diet supplemented with DPP, Ca and Vit. D together.

Ovariectomy Procedure: The bilateral ovariectomy in rats was performed under ether anesthesia by making two dorsolateral incisions using sharp dissecting scissors. The skin and dorsal muscles were then cut and the peritoneal cavity was thus reached. The uterine horn was picked out and the fatty tissue around the ovary was removed. The connection between the Fallopian tube and the uterine horn was clamped by artery forceps and a cut was made under the clamped area to remove the ovary. Skin was closed bilaterally with one simple catgut suture. Tincture iodine solution (antiseptic) was applied locally on the skin at both sites of the operation. This technique was described by Lasota and Danowska-Klonowska, [19]. Similarly sham (SHAM) operation was performed where the ovaries were exposed out, but not removed.

Experiment Design: Forty two rats were randomized into to 6 equal groups of 7 animals each. Group 1 was sham-operated (SHAM) and fed on basal diet and the

other 5 groups were ovariectomized (OVX) and left for 3 weeks post-operation to ensure almost complete clearance of their bodies from sex hormone residues. Group 2 was kept OVX (positive control) and fed on basal diet. Groups 3, 4, 5 and 6 were fed on experimental diets supplemented with 10% DPP, Ca, Vit.D and DPP plus Ca plus Vit.D, respectively for 6 weeks. The initial and final body weights of rats were recorded and changes in body weight gains were calculated. Blood samples were collected for biochemical analyses. The rats were then euthanized by prolonged exposure to ether anesthetic and the uterus was dissected out and weighed. Femur bones were dissected out and prepared for bone analysis.

Biochemical Analyses: Blood samples were collected to separate the serum which kept frozen at -80°C till biochemical analyses. Serum calcium concentrations were colorimetrically determined using specific diagnostic reagent kits (BioMérieux, France) and measured using spectrophotometer [20]. Serum bone-specific alkaline phosphate was estimated by colorimetric assay using specific enzyme kits (Sigma-Aldrich Chemical Co. USA) [21]. Serum measurements of osteocalcin (OC), interleukin-1 beta (IL-1beta), interleukin-6 (IL-6), pyridinoline (PYD), calcitonin (CT) and parathyroid hormone (PTH) concentrations were performed using enzyme linked immunosorbent assay (ELISA) kits (San Jose, CA) as described by Norazlina *et al*. [22] and absorbance was read in at 540 nm according to manufacturer's instructions. Serum free thyroxin (T4) concentrations were measured using radioimmunoassay (RIA) method as described by Wang *et al.* [23].

Bone Analysis: Both femur bones were dissected out and the soft tissues were removed. Both femur epiphyses were removed and the length of each femur bone was measured using Vernier caliper. Femur bone volume and density (BMD) were calculated according to principle of Archimedes [24]. In brief, each femur was cut out at the mid diaphyses and bone marrow washed out. Each femur bone was placed in a vial filled with deionized water and the vial was placed in vacuum desiccator for 90 minutes. The femurs were removed from the vial, dried by blotted paper, weighed and placed again in other vial containing deionized water. The bone was reweighed and bone volume was measured. Femur bone density (BMD) was calculated using this formula:

BMD = Femur weight/femur volume

To obtain the ash, femur bones were dehydrated and defatted in acetone and anhydrous ether, dried for 6 hr in an oven at 700°C. The remaining ash was weighed, solubilized with 0.1Mol/L HCl, transferred into volumetric flask and completed to 100 ml with 0.1Mol/L HCl according to Yang *et al.* [25]. The final solution was used for estimation of calcium and phosphorus [26] in the ash using colorimetric methods with commercial kits (Biodiagnostics Company, Egypt).

Statistical Analysis: Data were presented as means ± standard errors (SE). The statistical analyses were performed using computerized statistical package of social sciences (SPSS, version 15) program with one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests according to Snedecor and Cochran [27].

RESULTS

The results of this study showed that OVX rats gained more body weight than sham (SHAM) negative control rats. The body weight gain was 17.34 % in OVX positive control group versus to 10.16 % in SHAM negative control group. The ovariectomy in rats caused a significant (P<0.05) decrease in the uterine weight when compared with SHAM control group. The mean value ± SE of the uterine weight was 1.25±0.01 g in OVX control rats versus to 2.10±0.02 g in SHAM control rats. Feeding of OVX rats on experimental diet supplemented with DPP, Ca and Vit. D together significantly antagonized the increase in body weight gain and the decrease in uterine weight when compared to the OVX positive control group as depicted in Table 1.

Bilateral ovariectomy in rats caused significant (P<0.05) decreases in serum levels of calcium (Ca), bone-specific alkaline phosphatase (b-ALP) and osteocalcin (OC) when compared with the SHAM negative control group. Feeding of OVX rats on experimental diet supplemented with DPP, Ca and Vit. D together significantly (P<0.05) increased serum levels of Ca, b-ALP and OC in OVX rats when compared to the OVX positive control group as recorded in Table 2.

Data in Table 3 showed that ovariectomy in rats significantly (P<0.05) increased serum levels of interleukin-1 beta (IL-1beta), interleukin-6 (IL-6) and

Table 1: Effect of diets supplemented with dried plum powder (DPP), calcium (Ca) and vitamin D (Vit. D) on body weight gain and uterine weight in ovariectomized (OVX) rats. (n=7 rats).

	Body weight (g)			
Groups	Initial weight	Final weight	Body weight gain (%)	Uterine weight (g)
Group 1: SHAM control	295.0±6.2[a]	325.0±3.6[d]	10.16	2.10±0.02[a]
Group 2: OVX control	294.0±4.7[a]	345.0±4.1[a]	17.34	1.25±0.01[d]
Group 3: 10% DPP	292.0±2.6[a]	337.0±3.2[c]	15.41	1.65±0.02[c]
Group 4: Ca	293.5±5.8[a]	338.0±2.7[c]	15.16	1.69±0.01[c]
Group 5: Vit. D	291.0±5.4[a]	336.0±2.2[c]	15.46	1.70±0.02[c]
Group 6: DPP + Ca + Vit. D	292.0±6.8[a]	340.0±2.4[b]	15.25	1.88±0.01[b]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test.

Table 2: Effect of diets supplemented with dried plum powder (DPP), calcium (Ca) and vitamin D (Vit. D) on serum calcium (Ca), bone specific alkaline phosphatase (b-ALP) and osteocalcin (OC) in ovariectomized (OVX) rats. (n=7 rats).

Groups	Ca (mg/dL)	b-ALP (U/L)	OC (µg/L)
Group 1: SHAM control	11.20±0.3[a]	125.0±2.7[a]	10.8±0.01[a]
Group 2: OVX control	9.10±0.2[d]	100.5±3.2[d]	8.3±0.03[d]
Group 3: 10% DPP	10.50±0.1[c]	115.5±3.4[c]	9.6±0.01[c]
Group 4: Ca	10.60±0.2[c]	115.6±4.1[c]	9.4±0.02[c]
Group 5: Vit. D	10.50±0.3[b]	114.6±3.5[c]	9.2±0.03[c]
Group 6: DPP + Ca + Vit. D	10.80±0.5[b]	119.6±4.2[b]	10.1±0.03[b]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P<0.05$ using one way ANOVA test.

Table 3: Effect of diets supplemented with dried plum powder (DPP), calcium (Ca) and vitamin D (Vit. D) on serum on serum levels of interleukin-1 beta (IL-1beta), interleukin-6 (IL-6) and pyridinoline (PD) in ovariectomized (OVX) rats. (n=7 rats).

Groups	IL-1beta (Pg/ml)	IL-6 (Pg/ml)	PD (nmol/L)
Group 1: SHAM control	33.15±2.2[d]	108.0±2.2[d]	2.77±0.14[d]
Group 2: OVX control	62.16±3.7[a]	205.0±4.8[a]	7.12±0.19[a]
Group 3: 10% DPP	57.44±2.2[b]	206.0±3.5[b]	4.62±0.14[b]
Group 4: Ca	55.55±3.4[b]	209.0±5.8[b]	4.82±0.21[b]
Group 5: Vit. D	56.95±2.5[b]	203.0±6.4[b]	4.55±0.16[b]
Group 6: DPP + Ca + Vit. D	39.13±2.2[c]	117.0±6.6[c]	2.52±0.15[c]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P<0.05$ using one way ANOVA test.

Table 4: Effect of diets supplemented with dried plum powder (DPP), calcium (Ca) and vitamin D (Vit. D) on serum levels of thyroxin (T4) and calcitonin (CT) and parathyroid hormone (PTH) in ovariectomized (OVX) rats. (n=7 rats).

Groups	T4 (ng/mL)	CT (ng/mL)	PTH (pg/mL)
Group 1: SHAM control	17.85±0.2[a]	16.0±0.72[a]	26.47±0.2[d]
Group 2: OVX control	11.23±0.7[d]	10.0±0.18[d]	38.22±0.7[a]
Group 3: 10% DPP	14.74±0.5[c]	12.3±0.13[c]	32.00±0.6[b]
Group 4: Ca	13.55±0.4[c]	12.2±0.18[c]	33.54±0.4[b]
Group 5: Vit. D	14.95±0.5[c]	12.6±0.24[c]	34.35±0.3[b]
Group 6: DPP + Ca + Vit. D	16.03±0.2[b]	14.8±0.16[b]	29.00±0.5[c]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P<0.05$ using one way ANOVA test.

Table 5: Effect of diets supplemented with dried plum powder (DPP), calcium (Ca) and vitamin D (Vit. D) on femur weight (Wt), length (L),volume (V) and bone mineral density (BMD) in ovariectomized (OVX) rats. (n=7 rats).

Groups	Femur Wt.(g)	Femur L (mm)	Femur V (cm^3)	BMD (g/cm^3)
Group 1: SHAM control	1.65±0.01[a]	47.01±3.75[a]	0.68±0.02[a]	2.43±0.06[a]
Group 2: OVX control	0.98±0.03[d]	46.09±3.71[a]	0.67±0.03[a]	1.31±0.02[d]
Group 3: 10% DPP	1.30±0.01[c]	45.10±3.25[a]	0.66±0.01[a]	1.96±0.03[c]
Group 4: Ca	1.33±0.03[c]	44.15±3.15[a]	0.67±0.02[a]	1.98±0.02[c]
Group 5: Vit. D	1.32±0.02[c]	46.10±3.05[a]	0.68±0.04[a]	1.94±0.03[c]
Group 6: DPP + Ca + Vit. D	1.45±0.06[b]	45.10±2.05[a]	0.69±0.03[a]	2.02±0.01[b]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P<0.05$ using one way ANOVA test.

pyridinoline (PYD) as compared to the SHAM control group. Experimental diet supplemented with DPP, Ca and Vit. D significantly ($P<0.05$) lowered the high serum IL-1beta, IL-6 and PYD (serum markers of bone loss) when compared to the positive OVX group.

Bilateral ovariectomy in rats induced significant ($P<0.05$) decreases in serum levels of free thyroxin (T4) and calcitonin (CT) and an increase in parathyroid hormone (PTH) as compared with the SHAM negative control group. Feeding OVX rats on experimental diet supplemented with DPP, Ca and Vit. D significantly ($P<0.05$) normalized serum levels of T4, CT and PTH when compared to OVX positive control rats as recorded in Table 4.

The results denoted that bilateral ovariectomy in rats induced significant ($P<0.05$) decreases in femur weight and bone mineral density (BMD) when compared to the SHAM control group. Feeding of OVX rats on experimental diets fortified with DPP, Ca and Vit. D significantly ($P<0.05$) increased femur weight and BMD when compared to the OVX control group as shown in Table 5.

Bilateral ovariectomy in rats produced significant ($P<0.05$) decreases in weight of femur ash and contents of calcium and phosphorus in the ash when compared to the SHAM control group. Experimental diets supplemented with DPP, Ca and Vit. D significantly ($P<0.05$) normalized the femur weight, ash weight and contents of calcium and phosphorus in the ash as depicted in Table 6.

Table 6: Effect of diets supplemented with dried plum powder (DPP), calcium (Ca) and vitamin D (Vit. D) on femur ash weight and contents of calcium (Ca) and phosphorus (P) in ash in ovariectomized (OVX) rats. (n=7 rats).

Groups	Ash Wt.(g)	Calcium (mg/g ash)	Phosphorus (mg/g ash)
Group 1: SHAM control	1.85±0.03[a]	11.5±0.02[a]	7.22±0.12[a]
Group 2: OVX control	0.90±0.02[d]	7.3±0.01[d]	4.21±0.13[d]
Group 3: 10% DPP	0.77±0.01[c]	9.0±0.03[c]	5.42±0.14[c]
Group 4: Ca	0.75±0.02[c]	8.9±0.01[c]	5.40±0.11[c]
Group 5: Vit. D	0.75±0.01[c]	9.1±0.02[c]	5.42±0.10[c]
Group 6: DPP + Ca + Vit. D	1.35±0.02[b]	10.6±0.02[b]	7.10±0.12[b]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P<0.05$ using one way ANOVA test.

DISCUSSION

The bilateral ovariectomy in rats caused dramatic decreases in the uterine weight, bone mineral content, density and biomechanical strength due to estrogen deficiency [28-31]. Estrogen is the most potent inhibitor of osteoclastic bone resorption (loss), so estrogen deficiency is a major risk factor in the pathogenesis of osteoporosis [32]. Ovariectomy causes also accumulation of reactive oxygen species (ROS) with subsequent oxidative stress and in turn it promotes the production of cytokines as interleukin 1 beta (IL1beta) and interleukin 6 (IL6) which causes osteoclast generation so increases bone loss [22]. Postmenopausal osteoporosis is commonly treated by estrogen replacement therapy and/or by some drugs such as Alendronate (one of Bisphosphonates series) which inhibits osteoclast-mediated bone resorption [30]. Accelerated bone loss that occurs in postmenopausal women has been linked to oxidative stress and increased generation of reactive oxygen radicals (ROS) which are chemically unstable atoms that cause damage to cell lipids, proteins. Oxidative stress occurs due to increased generation of ROS and decreased activity of antioxidant enzymes. Therefore, it has been suggested that the use of natural antioxidants especially from vegetables and fruits can prevent and reverse postmenopausal osteoporosis [33].

Calcium and vitamin D are two micronutrients essential for bone health [34]. Reduced intake of calcium is associated with a reduced bone mass and osteoporosis, whereas chronic and severe vitamin D deficiency leads to osteomalacia which is a metabolic bone disease characterized by a decreased mineralization of bone[35]. The most rational approach to reduce vitamin D deficiency is dietary supplementation. Cholecalciferol-D3, alfacalcidol, is a fat-soluble vitamin D which helps the body to absorb calcium that is necessary for bone building [22].

Results of the present study showed that diet supplemented with dried plum powder (DPP), Ca and Vit. D together prevented ovariectomy-induced the increase in body weight gain and the decrease in the uterine weight and turned these changes to nearly normal weights of SHAM-operated rats. The decrease in the uterine weight induced by ovariectomy could be attributed to estrogen deficiency in OVX rats. These findings were also reported by Srikanta *et al.* [30], who found that bilateral ovariectomy in rats significantly increased the body weight gain and decreased the uterine weight. Moreover, subcutaneous injection of estrogen to immature rats and mice was reported to increase the vascularity, growth and weight of the uterus [36]. Calcium, vitamin D and parathyroid hormone are critical regulators of bone remodeling [37]. Calcium is widely used as a marker for bone formation as it plays a vital role in bone mineralization [38, 39]. In the present study, the bilateral ovariectomy decreased serum calcium levels when compared to SHAM-operated rats. In a previous study, the decreased serum calcium levels were also reported to be due to estrogen deficiency in ovariectomized rats [39].

Serum calcium, bone-specific alkaline phosphatase (b-ALP) and osteocalcin (OC) are commonly used as biochemical markers of bone formation and building. The decrease in serum levels of calcium, b-ALP and OC induced by ovariectomy in rats as reported in this study was similar to the previous reports [29, 30, 40]. The previous authors concluded that decreases in serum calcium, b-ALP and OC are due to estrogen deficiency in OVX rats and mice. Experimental diet supplemented with DPP, Ca and Vit. D together significantly increased serum levels of these biochemical markers and this could be possibly due to an increased osteoblastic activity, consequently enhancing bone formation [30]. However, circulating osteocalcin hormone is a well known and commonly used marker for bone formation [41]. In this study, experimental diet supplemented with dried plum powder (DPP), Ca and Vit. D decreased serum levels of bone loss markers interleukin 1 beta (IL1beta) interleukin 6 (IL6) and pyridinoline (PD) in OVX rats. This effect could be possibly attributed to the powerful antioxidant effect of DPP due to its high content of phenolic compounds [8] and boron [9]. In addition, it has been reported that ovariectomy causes accumulation of reactive oxygen species (ROS) with subsequent oxidative stress and in turn increases the production of cytokines as interleukin 1 beta (IL1beta) and interleukin 6 (IL6) which causes generation of osteoclast and increases bone loss [22].

Regarding the metabolic hormones, the present results denoted that feeding OVX -rats on experimental diet supplemented with DPP, Ca and vitamin D together significantly elevated serum free thyroxin (T4) and calcitonin (CT) and decreased parathormone (PTH). These findings were partially in accordance with those reported by Norazlina *et al*. [22] and Dumic- Cule *et al*. [42]. The later authors reported that intermittent administration of thyroid-stimulating hormone (TSH) in a rat model with removed thyroid and parathyroid glands elevated free thyroxin (T4) and calcitonin (CT) serum levels, so inhibit calcium loss from bone into blood, stimulate calcium deposition into bone and improve bone health. On the contrary, parathormone (PTH) inhibits calcium deposition into bone and increases urinary exertion of calcium causing hypocalcaemia [23].

The results of this study showed that feeding OVX rats on diet supplemented with DPP, Ca and vitamin D significantly increased in femur bone mineral density (BMD) as well as calcium and phosphorus contents in bone ash. These findings were similar to the previous report by Chen *et al*. [14], who found that high -calcium plus vitamin D3 in diet plays a vital role in bone mineralization as it increases BMD and so can prevent osteoporosis. Adequate intake of calcium and vitamin D is essential for bone health [32]. On the contrary, Agata *et al.* [15] suggested that low calcium intake during period of rapid bone loss caused by estrogen deficiency in ovariectomized rats may be one possible cause for bone loss. The mechanisms of anti-osteoporotic activity of dried plum and micronutrients calcium, vitamin D could be due to enhancement of bone formation as dietary supplementation with dried plum prevented ovariectomy-induced bone loss in mice [11] and in rats [12]. Moreover, dried plum is an important source of boron, which is postulated to play a role in the prevention of osteoporosis [8]. High calcium plus vitamin D3 in diet was reported to play a vital role in bone mineralization and so prevent osteoporosis [14]. Moreover, the phenolic and flavonoid compounds in dried plum are highly effective in modulating bone mass in estrogen-deficient rat model of osteoporosis [9]. The second possible mechanism of anti-osteoporotic activity of dried plum, calcium and vitamin D could be due to prevention of bone loss in ovariectomized rats as evident in this study by low levels of serum biomarkers of bone resorption interleukin-1 beta (IL1beta); interleukin-6 (IL6) and pyridinoline (PD). The third possible mechanism could be attributed to the modulation of serum levels of metabolic hormone (T4, CT and PTH) which regulate calcium pathway by experimental diet containing DPP, Ca and Vit.D. The effect of dried plum, calcium and vitamin D on serum metabolic hormone which regulate calcium pathway is- for the first time- carried out in this work.

CONCLUSION

Diet supplementation with dried plum, calcium and vitamin D together induced anti-osteoporotic effect in ovariectomized rats. This effect may be due to enhancement of bone formation, suppression of bone loss and regulation of some metabolic hormones which control calcium pathway. The anti-osteoporotic activity of dried plum can be attributed to its high content of polyphenolic compounds and boron which improve bone health. The study recommends that intake of dried plum and consumption foods rich in calcium (as milk and dairy products) together with Vit.D as a dietary supplement may be effective, safe and cheap mean in preventing osteoporosis in postmenopausal women due to estrogen loss.

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