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Research article

Preventive effect of dried plum extract against dexamethasone-induced osteoporosis in male rats through inhibiting cathepsin-K activity, lipogenesis and trabecular bone loss

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Key words: Osteoporosis; Dexamethasone, Dried plum, Cathepsin-K, Collagen type-1.

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Abstract

Objectives: Bone protective effect of dried plum extract (DPE) was investigated in dexamethasone (DEX) treated male rats, as an animal model of osteoporosis. **Material and methods:** Rats received intramuscular injection of DEX (7 mg/kg b.wt.) once a week for 4 weeks, whereas DPE (150 mg/kg b.wt.) was given orally for the same duration. **Results:** DEX-treated rats exhibited significant decline in the body weight accompanied by marked reduction in serum and bone minerals (Ca, P), bone mineral density (BMD) and serum total protein (TP) with elevation in serum creatinine (CR) level. Serum parathyroid hormone (PTH), osteocalcin (OC) and hydroxyproline (HYP) were increased, whereas calcitonin (CT), insulin like growth factor-I (IGF-I) and prostaglandin E_2 (PGE_2) were decreased, along with notable reduction in bone collagen type-1 (Col-1). Marked elevation in serum and bone lipids (TL, TG, TC), alkaline and acid phosphatases (ALP, ACP), as well as bone cathepsin-K (Cath-K) and oxidative stress markers [hydrogen peroxide (H_2O_2), malondialdhyde (MDA)] were recorded with decreased antioxidant components [reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT)] in bone of DEX treated rats. Bone histopathological alterations were also observed with DEX treatment, as reflected by thinning of trabecular bone and loss of connection with noticeable porosity, indicating bone fragility. Consumption of DPE showed high ability to protect against DEX-induced biochemical and histological alterations in bone tissue, particularly through normalizing BMD and improving trabecular bone thickness and structure. **Conclusion:** Dried plum could be considered as a potential dietary approach for preventing bone loss and structural deterioration caused by DEX treatment.

Introduction

Dexamethasone (DEX) is a type of glucocorticoids (GCs) extensively used as a treatment of allergic disorders, ulcerative colitis, arthritis, pulmonary disorders and organ transplantation, owing to its potent anti-inflammatory and immunomodulatory effects [1]. Although the therapeutic effectiveness of this drug, its frequent use inevitably produces variable health problems. Among which, is a severe form of secondary osteoporosis affecting 30-50% of patients with GCs therapy [2]. The pathological mechanisms of GCs-induced osteoporosis include diminished bone formation by decreasing differentiation and maturation of osteoblasts and increasing life span of osteoclasts [3]. Osteocytes are also affected, with decreased cell function and increased apoptosis resulting in impairment of their ability to detect and repair bone microdamage [4]. The combination of increased bone resorption and attenuated bone formation may thus explain early and fast loss of bone mineral density (BMD) and bone strength/quality in patients undergoing GCs therapy [5].

Currently, use of medicinal plants has emerged as one of the most common and preferred modalities of complementary and alternative medicine. Evidence provided that certain vegetables and fruits are essential for maintaining bone mass and preventing osteoporosis [6]. Plum (*Prunus domestica* L.) is a type of drupe fruits belongs to genus Prunus (family Rosaceae). It is a delicious and nutritious fruit eaten either fresh or dried, however dried plum has attracted attention as concentrated source of phytonutrients than the fresh fruit [7]. Dried plum is extremely low in fat, rich in macronutrients including both soluble and insoluble fibers, oligosaccharides and simple sugars, as well as micronutrients like vitamins and minerals. Dried plum is also a rich source of bioactive compounds, such as flavonoids, flavones and flavanols which have found to possess numerous pharmacological effects, including antioxidant activity, anticancer, antimutagenic and anti-inflammatory properties [8]. Nevertheless, the relatively high content of phenolic compounds such as chlorogenic acid, neochlorogenic acid and cryptochlorogenic acid may be implicated in number of health effects, including

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anti-diabetic action, cardiovascular benefits, improved immune function, memory capability, muscular degeneration and gastrointestinal system [9]. Consumption of dried plum can limit food intake and promote satiety, concurrent with suppressed plasma glucose levels [10]. Other studies have documented that dried plum is known for its laxative and metabolic stimulant effects [11].

Besides, dried plum has considered as one of the most extensively studied botanicals for its role in bone health [12]. Animal studies suggested that dried plum and/or its extracts enhance bone formation and inhibit bone resorption through its actions on cell signaling pathways that influence osteoblast and osteoclast differentiation [13]. These studies are consistent with clinical trials showing that dried plum helps to increase BMD, owing to presence of polyphenolic compounds that may inhibit osteoclastogensis and prevent bone loss [14]. Regarding the beneficial effects of dried plum on bone, the present study was undertaken to evaluate the possible anti-osteoporotic effect of dried plum extract (DPE) in DEX-treated male rats, as a model of GCs-induced osteoporosis.

Material and methods

Animals care and diet

Ten- week old male Wistar rats weighing about 160g were used in this study. Rats were housed in stainless-steel cages at well controlled environmental conditions (25± 2°C and 12- h light/ dark cycle). Rats received normal laboratory diet (60% ground corn meal, 15% ground beans, 10% bran, 10% fat, 3% casein, 1% minerals, 1% vitamins) obtained from Meladco Feed Co.(Aubor City, Cairo, Egypt) and provided water *ad libitum*. All experiments were performed in accordance with the guidelines of Animal Care and Use Committee of Mansoura University and were conformed to the National Institute of Laboratory Animal Resources, National Research Council "NRC" [15].

Induction of osteoporosis

For induction of osteoporosis, animals were injected intramuscularly with DEX sodium phosphate purchased from Amriya Pharmaceutical Industries, (Alexandria-Egypt) at dose (7 mg/ kg b.wt.) once a week up to four weeks [16].

Preparation of dried plum extract

One kg of dried plum (*Prunus domestica* L.) was obtained from a local market at Mansoura city. The plant was authenticated by professors of Taxonamy, Botany Department, Mansoura University. Dried plums were peeled and extracted using 80% ethanol at room temperature for one week. The extract was filtered and obtained filtrate was evaporated to dryness in a rotatory vacuum evaporator to yield 40g of dried plum extract (DPE) [17].

Animal grouping

Following one week of acclimation, rats were randomly allocated into five groups of six rats/each. The 1st group served as normal control, the 2nd group received saline solution (0.9%) as vehicle, while the 3rd group was given DPE orally at dose (150 mg/kg b.wt) [17]. The 4th group was injected intramuscularly with DEX at dose (7 mg/kg b.wt.) once weekly and the 5th group received DPE plus DEX as described in the above groups. All experiments were continued for four weeks and animals were weighed once per week during the course of experiment to determine body weight changes.

At the end of experimental period, rats were fasted for 12h, anesthetized and scanned to assess femur bone mineral density (BMD), using GE Lunar DXA bone densitometer provided by GE Healthcare (Chicago, IL, USA). BMD was represented in g/cm^2 and all samples were measured 3 times and the mean values were calculated [18]. Next, animals were sacrificed and blood samples were collected, centrifuged and sera were separated for further analysis. Rats were then dissected and both right and left femurs from each rat were removed and washed using chilled saline solution. Left femurs were weighed, homogenized in ice cold saline solution and the collected supernatants were kept at -20℃ for later biochemical analysis, while right femurs were fixed in 10% neutral formalin for histological examination.

Biochemical analysis

Serum and bone levels of minerals [calcium (Ca), phosphorus (P)], alkaline phosphatase (ALP), acid phosphatase (ACP), lipid fractions [total lipid (TL), triglyceride (TG), total cholesterol (TC)], besides serum total protein (TP), creatinine (CR), bone oxidative stress markers [hydrogen peroxide (H_2O_2), malondialdehyde (MDA)] and antioxidants [reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT)] were assessed using commercial kits supplied by Bio-diagnostic Co. (Dokki, Giza, Egypt). Other biochemical parameters including, parathyroid hormone (PTH), collagen type-1 (Col-1), cathepsin-K (Cath-K) and prostaglandin E_2 (PGE_2) were estimated using enzyme-linked immunosorbent assay (ELISA) kits provided by CUSABIO (Baltimore, MD, USA). Insulin like growth factor-I (IGF-I) and osteocalcin (OC) were estimated using ELISA kits provided by Cloud-Clone Crop (Katy, TX, USA) and Kamiya Biomedical Co. (Seattle, WA, USA), respectively. Levels of hydroxyproline (HYP) and calcitonin (CT) were determined using ELISA kits obtained from LSBio (Seattle, WA, USA).

Histopathological examination

Fixed femur bones were decalcified in 10% EDTA for a minimum of 72h and then processed for paraffin embedding. Longitudinal 5µm sections were obtained, deparaffinized and stained with hematoxylin and eosin (H&E) dye for histopathological examination [19].

Statistical analysis

Results were represented as mean ± standard error (SE) and differences were considered significant at $P < 0.05$. GraphPad Prism software version 5.0 (San Diego, CA, USA) was used and all statistical analyses were performed using one-way ANOVA. Coefficient of variation test has been applied to check the variability of data in relation to the mean.

Results

Body weight

Results showed marked body weight reduction in DEX-treated rats as compared to normal control. Administration of DPE to DEX group showed marked increase in the body weight compared to animals receiving DEX only. However, no alterations were seen when DPE was given to untreated normal rats (Figure 1).

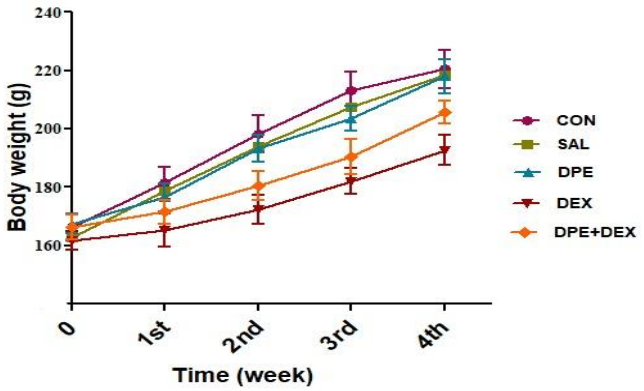


Figure 1. Body weight changes in different animal groups. Data are expressed as means ±SE.CON: control, SAL: saline, DPE: dried plum extract, DEX: dexamethasone.

Serum total protein and creatinine

DEX-treated rats showed significant decrease in serum TP with marked elevation in serum CR level as compared to intact control group. Indeed, a reverse pattern was exhibited when DEX group was supplemented by DPE, showing significant amelioration in tested parameters compared to DEX group. Administration of DPE to normal rats showed no significant alterations in serum TP and CR levels compared to control animals (Table 1).

BMD, serum and bone minerals

Marked reduction in serum and bone minerals (Ca, P), as well as BMD was observed in DEX treated rats. Indeed, these changes appeared to be significantly improved following consumption of DPE, where significant elevation in BMD and mineral contents were recorded. No significant changes were observed in these parameters in animals received DPE alone compared to healthy control rats (Figure 2).

Serum biomarkers of bone formation and resorption

DEX-treated rats showed significant reduction in serum CT, IGF-I and PGE2 with increased levels of PTH, OC and HYP compared to control group. Nevertheless, marked improvement was observed in all these biomarkers when DEX group was supplemented with DPE, however, no significant alterations were noticed in normal rats that received DPE only (Table 2).

Serum and bone alkaline and acid phosphatases

Serum and bone ALP and ACP activities were found to be increased in DEX-treated rats as compared to control animals. Administration of DPE to DEX-treated rats produced significant reduction in tested enzymes compared to DEX group. Indeed, no marked changes were detected when DPE was given alone to normal rats (Table 3).

Bone collagen type-1 and cathepsin-K

Significant decline in bone Col-1 with marked elevation in Cath-K activity were demonstrated in DEX-treated rats as compared to control group. DPE administration seemed to be effective in reducing observed alterations in both Col-1 and Cath-K compared to DEX group. Meanwhile, no significant changes were recorded when DPE was administered to normal untreated rats (Figure 3).

Table 1. Serum total protein and creatinine in different animal groups.

	CON	SAL	DPE	DEX	DPE+DEX
TP (g/dl)	6.03 ±0.27	6.13 ±0.20	6.12 ±0.15	4.11 ±0.03[a]	5.26 ±0.12[a,b]
CR (mg/dl)	1.32 ±0.82	1.28 ±0.11	1.29 ±0.03	2.54 ±0.08[a]	1.70 ±0.05[a,b]

Data are expressed as means ±SE. CON: control, SAL: saline, DPE: dried plum extract, DEX: dexamethasone. **a:** significantly (P<0.05) different from control. **b:** significantly (P<0.05) different from DEX group.

Table 2. Serum biomarkers of bone formation and resorption in different animal groups.

	CON	SAL	DPE	DEX	DPE+DEX
CT (pg/ml)	4.74 ±0.16	4.48 ±0.13	4.45 ±0.09	3.00 ±0.03[a]	3.57 ±0.14[a,b]
IGF-I (ng/ml)	1.12 ±0.05	1.13 ±0.06	1.24 ±0.04	0.63 ±0.04[a]	0.89 ±0.24[a,b]
PGE_2 (ng/ml)	26.97 ±0.65	26.49 ±0.47	28.37 ±0.38	20.83 ±0.26[a]	23.89 ±0.25[a,b]
PTH (pg/ml)	23.27 ±0.40	22.80 ±0.53	21.98 ±0.29	29.38 ±0.49[a]	25.88 ±0.28[a,b]
OC (ng/ml)	14.01 ±0.30	14.12 ±0.37	13.14 ±0.38	18.90 ±0.33[a]	16.03 ±0.21[a,b]
HYP (mmol/l)	0.225±0.02	0.219±0.03	0.222±0.03	0.283±0.020[a]	0.238±0.02[a,b]

Data are expressed as means±SE. CON: control, SAL: saline, DPE: dried plum extract, DEX: dexamethasone. **a**: significantly (P<0.05) different from control. **b**: significantly (P<0.05) different from DEX group.

Table 3. Serum and bone alkaline and acid phosphatases in different animal groups.

		CON	SAL	DPE	DEX	DPE+DEX
Serum	**ALP (U/L)**	97.22±2.01	97.12±1.32	97.26±3.03	156.90±6.70 [a]	117.4±2.92[a,b]
	ACP (U/L)	28.21±0.28	27.96±0.37	27.74±0.31	42.23±0.56 [a]	39.89±0.36[b]
Bone	**ALP (U/g)**	31.12±0.09	31.46±2.13	29.55±0.97	40.58±0.74[a]	34.05±1.36[b]
	ACP (U/g)	37.53±0.92	37.17±0.96	37.38±0.76	62.18±0.60[a]	48.04±0.36[a,b]

Data are expressed as means ±SE.CON: control, SAL: saline, DPE: dried plum extract, DEX: dexamethasone. **a**: significantly (P<0.05) different from control. **b**: significantly (P<0.05) different from DEX group.

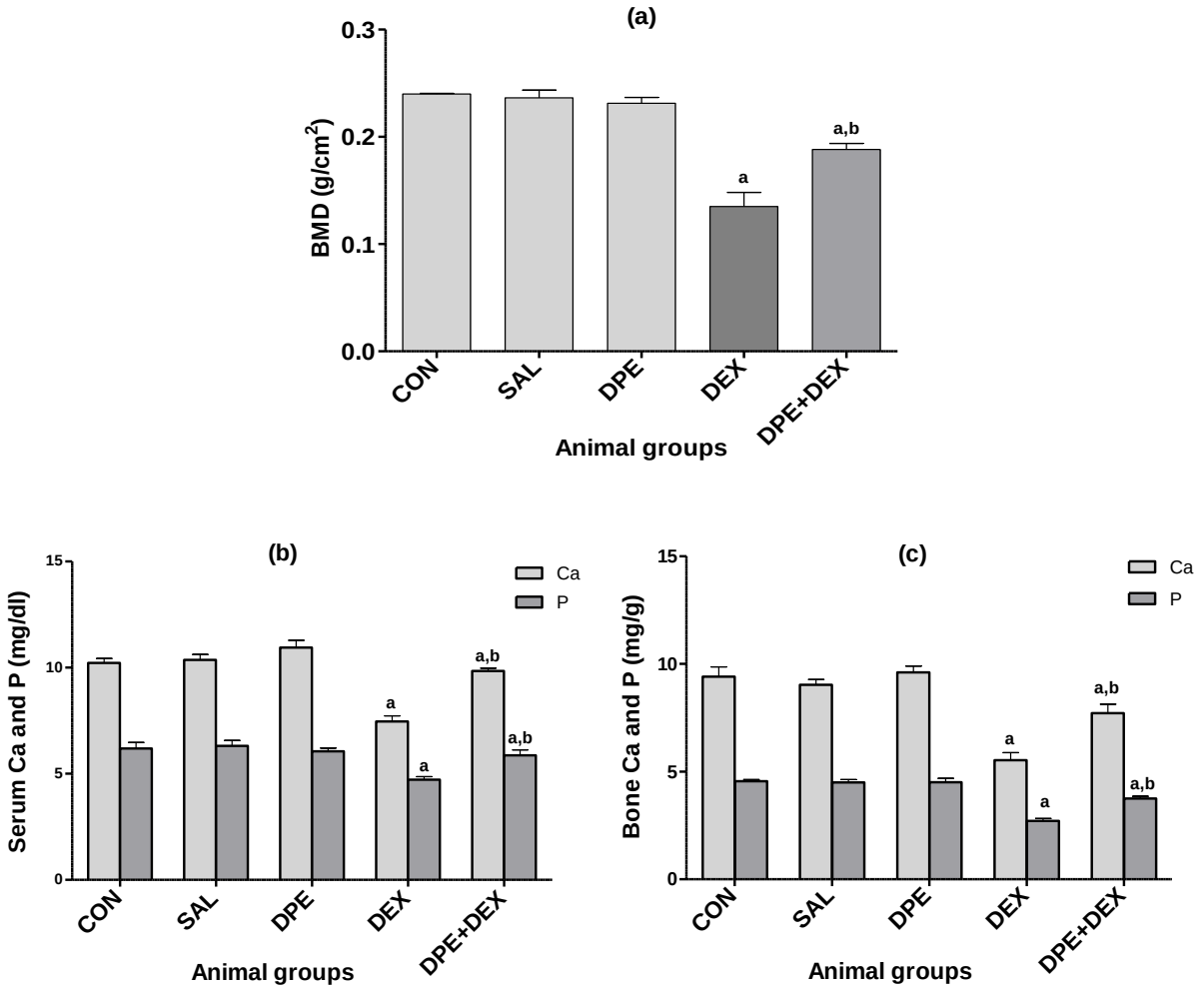


Figure 2. BMD (a), serum (b) and bone (c) minerals (Ca, P) in different animal groups. Data are expressed as means ±SE. CON: control, SAL: saline, DPE: dried plum extract, DEX: dexamethasone. a: significantly (P<0.05) different from control. b: significantly (P<0.05) different from DEX group.

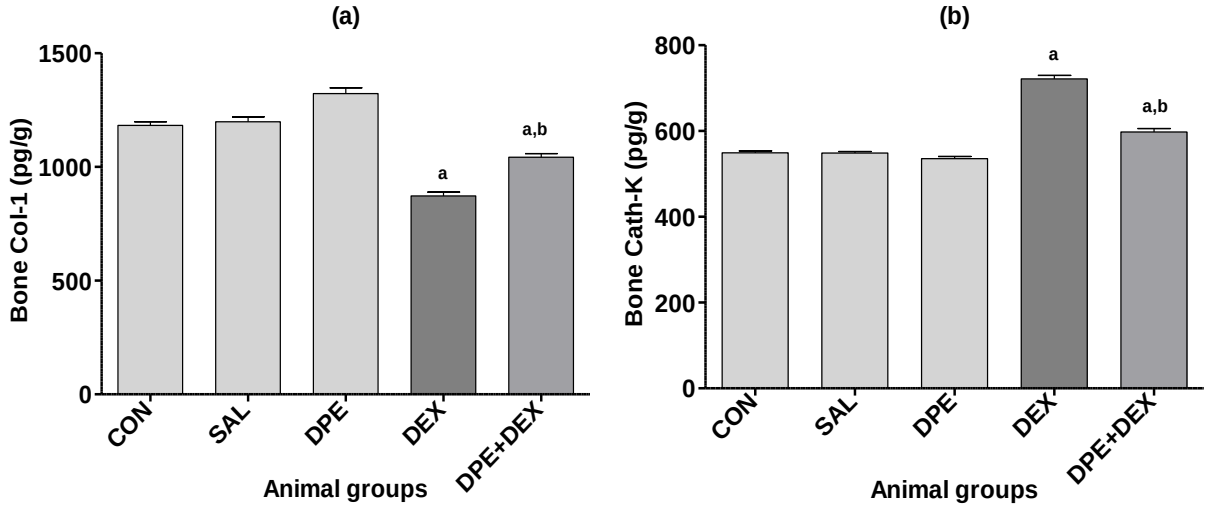


Figure 3. Bone collagen type-1 (a) and cathepsin-K (b) in different animal groups. Data are expressed as means ±SE. CON: control, SAL: saline, DPE: dried plum extract, DEX: dexamethasone. a: significantly (*P*<0.05) different from control. b: significantly (P<0.05) different from DEX group.

Serum and bone lipid fractions

DEX-treated rats revealed significant elevation in both serum and bone lipids (TL, TG, TC) when compared with control rat group. Interestingly, this elevation was markedly abolished following administration of DPE to DEX group, however, no alterations were noticed when DPE was given to normal untreated rats (Figure 4).

Bone oxidative stress and antioxidant biomarkers

Changes in bone oxidative stress and antioxidant markers are summarized in Figure 5. Results showed significant elevation in MDA and H_2O_2 levels, accompanied with notable decline in GSH content, SOD and CAT activities in DEX treated rats when compared with control ones. On contrast, the above alterations were found to be markedly ameliorated when rats were administered DEX, concurrent with DPE comparing with animals receiving DEX alone. Indeed, no significant changes were noticed upon administration of DPE to normal rats.

Histopathological examination

Sections of femur trabecular (cancellous) bones in control (A), vehicle (B) and DPE (C) groups showed normal structure with no alterations in the trabeculae and bone marrow tissue. DEX group (D) revealed marked degenerative changes characterized by thinning of trabecular bone and disconnection with increased porosity identified by excessive appearance of resorption or osteoporotic cavities, indicating bone fragility. In DPE+DEX group (E), trabecular bone showed near normal thickness and structure with slight irregularities and lowered porosity (Figure 6).

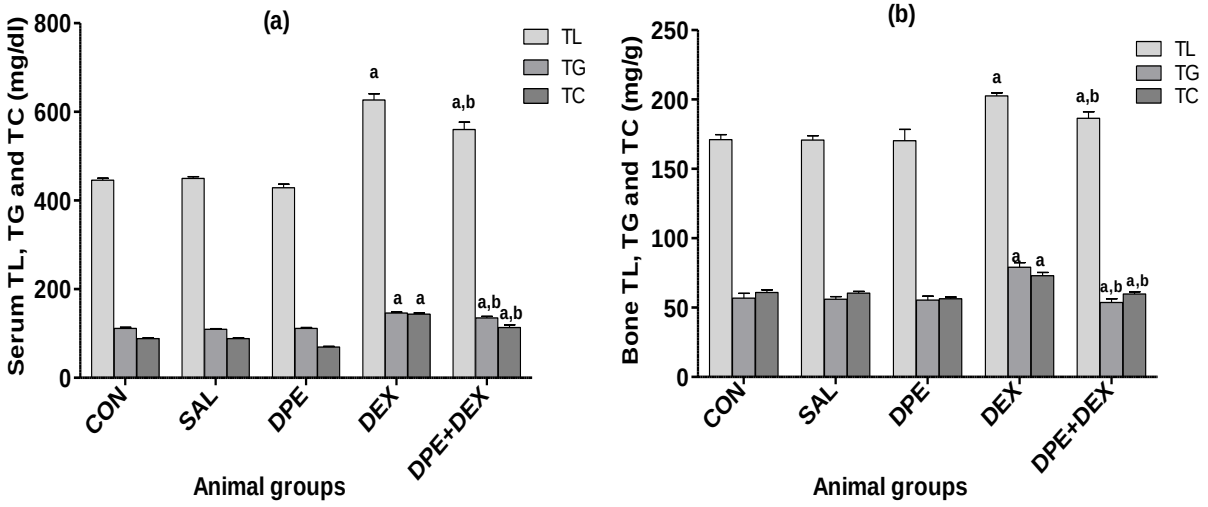


Figure 4. Serum (a) and bone (b) lipids (TL, TG, TC) in different animal groups. Data are expressed as means ±SE. CON: control, SAL: saline, DPE: dried plum extract, DEX: dexamethasone. a: significantly (*P*<0.05) different from control. b: significantly (*P*<0.05) different from DEX group.

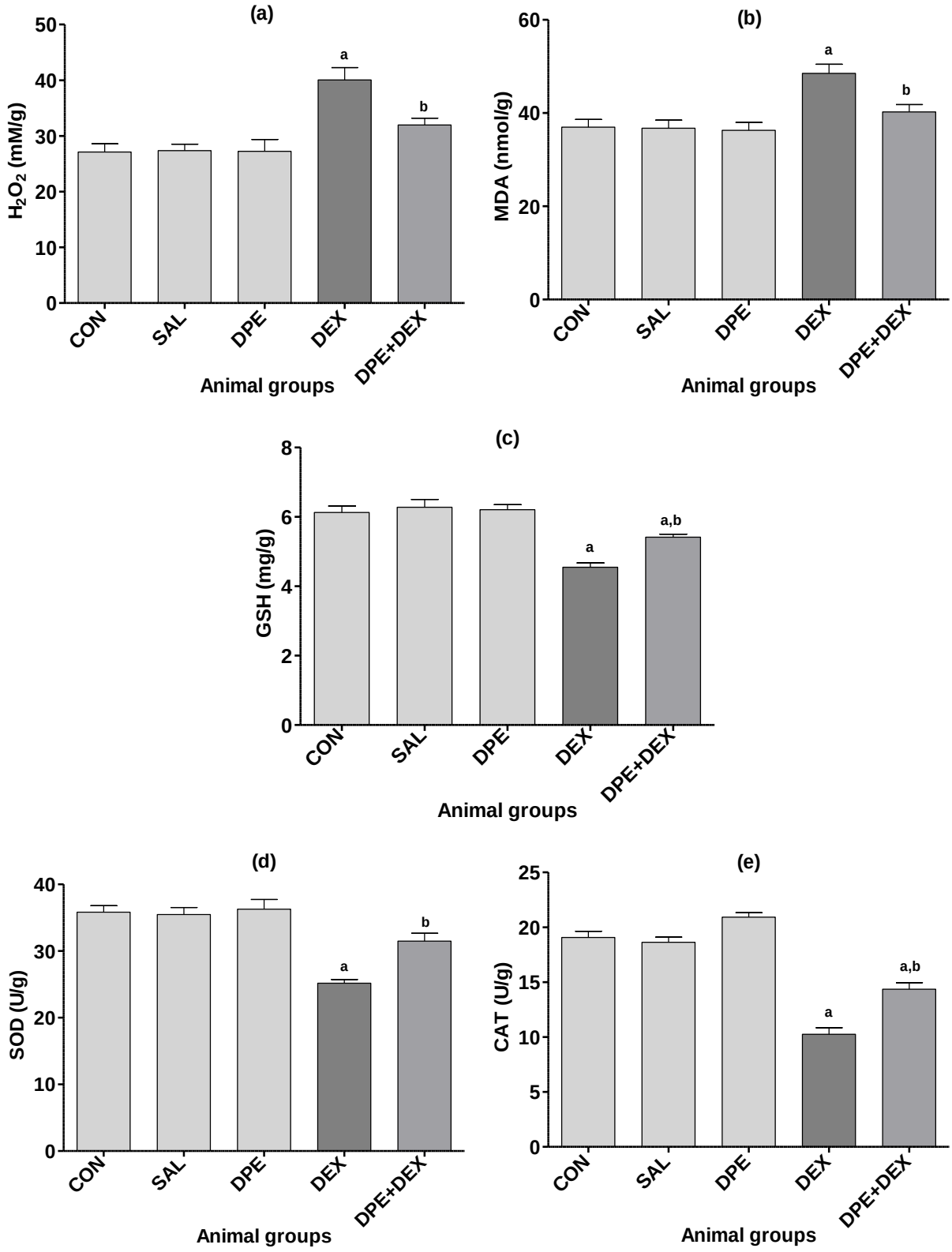


Figure 5. Bone oxidative stress [H_2O_2(a), MDA(b)] and antioxidant [GSH(c), SOD(d), CAT(e)] biomarkers in different animal groups. Data are expressed as means±SE. CON: control, SAL: saline, DPE: dried plum extract, DEX: dexamethasone. a: significantly (P<0.05) different from control. b: significantly (P<0.05) different from DEX group.

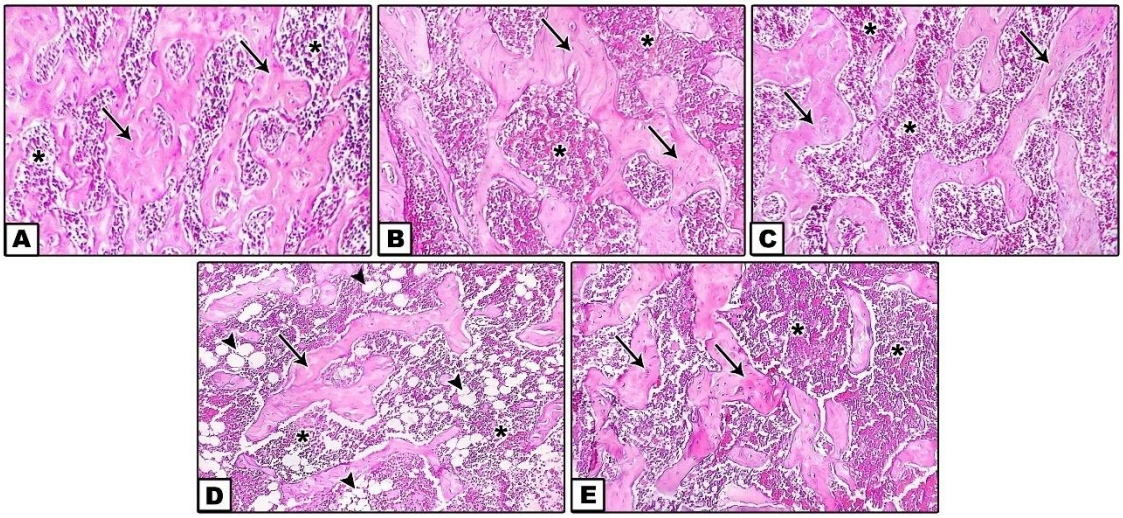


Figure 6. Photomicrograph of femur sections in control (A), vehicle (B) and DPE (C) groups showing normal trabeculae (arrow) and bone marrow structure (star). DEX-treated rats (D) showed marked degenerative changes presented as thinning of trabecular bone and loss of connection (arrow) with increased porosity and appearance of osteoporotic cavities (arrow head). However, administration of DPE+DEX showed notable trabecular thickness with lowered porosity (H&E ×400).

Discussion

Dexamethasone (DEX) is a synthetic GC widely used as an effective therapeutic option in the treatment of several inflammatory diseases [1]. However, administration of DEX at moderate to high doses often leads to multiple unwanted effects ranging from mild to severe complications [2]. Most attention has directed toward body weight loss as one of the basic indices reflecting adverse health consequences of the drug [20]. In the present study, DEX treated rats clearly demonstrated decreased body weight, along with lowered serum total protein and increased serum creatinine level compared to control rats. These effects suggested a role of DEX in suppression of protein synthesis and stimulation of its degradation which may lead to muscle atrophy and weight loss [21]. Interestingly, dried plum administration realized an effective role in preventing body weight loss and changes in protein metabolism in DEX-treated rats, most likely through its ability to promote healthy metabolism and maintain normal body weight [12].

More evidence suggested deleterious effects of DEX on bone formation and integrity which can end in osteoporosis and bone loss [5]. Signs of osteoporosis were similarly observed in the present study as manifested by reduced serum and bone minerals (Ca, P) coupled with decline of BMD in DEX-treated rats. This could be a consequence of alterations in Ca and P renal excretion [22] which may derive bone tissue toward enhanced resorptive activity. In this concern, several studies have been carried out to verify benefits of natural vegetables and fruits for preventing bone loss [9]. Herein, dried plum administration to DEX-treated rats has shown to increase BMD and decrease minerals loss in both serum and bone, indicating bone protecting effect of dried plum mostly through its high minerals content [23]. Plum is a good source of potassium that maintains bone health due to its ability to reduce blood acidity, thereby reduces bone resorption [8]. Plum also has higher amount of boron than most fruits which seems to stimulate bone growth and play an important role in preserving BMD, bone microarchitecture, and bone strength [24]. Besides, plum is specially rich in magnesium which acts as a cofactor of glycosyltransferase enzyme required for synthesis of proteoglycans being important for bone formation and mineralization [6].

Elevated level of PTH is a major contributing factor for development of osteoporosis. GCs-induced negative calcium balance generally leads to secondary hyperparathyroidism [25]. However, a direct stimulatory action of GCs on PTH secretion seems also to be involved [26]. As consequence, PTH stimulates bone resorption indirectly through increasing expression of RANKL/RANK pathway, which ultimately represents activating impact on osteoclasts and bone resorption [27]. GCs additionally lead to reduced serum CT level which is a potent bone forming hormone acts directly to suppress bone resorption *via* binding to high affinity receptors on osteoclasts [28]. Thus, suggesting a relation between GCs bone effect and disruption of both CT and PTH secretory mechanisms. The present study showed similar hormonal alterations following DEX treatment which seemed to be successfully attenuated upon DPE administration. This may be related to dried plum high content of polyphenol compounds which are known for their bone health effects probably through modulating elevated PTH levels and enhancing the effect of CT on calcium metabolism [29].

Alkaline phosphatase (ALP) is a biochemical indicator for osteoid formation and mineralization [30]. On the

other hand, ACP is a lysosomal enzyme secreted by osteoclasts for facilitating degradation of bone matrix [30]. Researches proved that elevated levels of alkaline and acid phosphatases are contributing factors in the pathogenesis of several bone diseases [31]. Elevated ALP could contribute to higher bone turnover rate characterized by an increase in bone formation and resorption, but bone resorption is predominating. However, increased ACP activity detects pathological bone resorption when osteoclasts were stimulated at an increased rate [32]. Therefore, raised activities of ALP and ACP, as notably seen herein and in other investigations [33] may be due to imbalance between osteoclastic and osteoblastic activities leading to gradual shifting of normal bone status to osteoporosis. Of concern, DPE administration significantly attenuated levels of ALP and ACP near to normal control value, suggesting decreased bone turnover rate with consequent anti-osteoporotic effects. Particularly, the osteoprotective properties of DPE may be in part mediated by its polyphenols and their effects on osteoclast precursors and osteoblast-mediated signaling for osteoclastogenesis [6].

Collagen type-1 (Col-1) is the primary bone matrix protein synthesized by osteoblasts and is important for maintaining mechanical properties of bone [34]. GCs have shown to suppress collagen synthesis and promote its degradation with increased accumulation of serum HYP, the breakdown product of Col-1 [35]. In the present study, elevated levels of serum HYP may thus reflect potentiating action of DEX on collagen breakdown, as evident by increased activity of Cath-K, the main collagenolytic enzyme of osteoclasts that facilitates degeneration of bone matrix. This finding and other data by Ma *et al*. [36] suggested that Cath-K is likely to play a key role in bone damage by DEX treatment. In this respect, DPE administration to DEX-treated rats was useful in preventing matrix degradation, as reflected herein by increased bone Col-1 and decreased serum HYP, together with marked inhibition of Cath-K activity in bone tissue. In support, dried plum has shown to increase Col-1cross-linking by up regulating transcription of runt-related factor2 (RUNX2) and ostrix (OSX), the growth factors required for osteoblastogenesis [37]. This effect may be related to the presence of querectin among other flavonoid-polyphenols available in DPE. Querectin has important role in differentiation of bone mesenchymal stem cells (BMSCs), the ideal seed cells of bone tissue reportedly to give several cell types including osteoblasts which promote osteogenesis through RUNX2/OSX-mediated pathway [38]. Beyond this, DPE is also rich in copper which is necessary co-factor of lysyl oxidase enzyme concerned with cross-linking of the extracellular matrix proteins, collagen and elastin which is clearly important for maintaining bone integrity. Thus, indicating bone anabolic effect of dried plum.

Insulin like growth factor-I (IGF-I) is a critical mediator in bone growth and is believed to stimulate bone matrix synthesis [39]. IGF-I is produced primarily by hepatocytes, although it can be also synthesized by osteoblasts and other cell types. Of note, GCs have suggested to decrease synthesis of IGF-I by osteoblasts through blocking transcription of IGF-I messenger RNA [40]. GCs treatment also reduce biological activity of IGF-I mostly through increasing binding proteins that may trap IGF-I [41]. This action is ultimately related to increased risk of osteoporosis and bone fracture occurring with GCs. Even though, GCs may also increase serum level of OC that is the major non-collagenous bone matrix protein produced by osteoblasts and is concerned with synthesis of hydroxyapatite and bone mineralization [42]. Increased serum OC may be a result of bone breakdown, allowing release of OC into the blood. Similar effects were achieved by the present study where marked reduction in serum IGF-I, coupled with notable elevation in serum OC levels were observed following DEX treatment. Nevertheless, administration of DPE has shown to attain positive effects regarding DEX-induced changes in both IGF-I and OC mostly related to plum vital supply of micronutrients and phenolic compounds. Similar studies reported that polyphenols enriched dried plum has the ability to increase production of IGF-I by osteoblasts *via* increasing IGF-I mRNA, which in turn may enhance bone formation [43]. More evidence is that dried plum is a rich source of vitamin K which is a cofactor needed for δ- carboxylation of OC, thereby promotes bone mineralization and preserves normal bone architecture. Taken together, DPE can be considered as a natural medication with specific bone protective properties.

Hyperlipidemia has established as one of the prime factors influencing bone mass. In the present study, DEX treatment has shown to increase accumulation of lipids (TL, TG and TC) in serum and bone tissue. Thus, indicating hyperlipidemic effect of DEX [44] that may be related to the ability of GCs to stimulate differentiation of BMSCs to adipocytes rather than osteoblasts, enhancing activity of adipogenesis. Of concern, prostaglandins (PGs), particularly PGE_2 are lipid mediators sensed as relevant factors for inhibiting lipogenesis [45]. Herein, DEX treatment tended to reduce PGE_2 primarily by suppressing its regulatory enzyme cyclooxegenase-2 (COX-2) which is thought to impair osteogenesis due to increased accumulation of marrow adipose tissue. In this respect, a number of studies have indicated an association between hyperlipidemia and lowered BMD [46]. Other studies described that hypercholesterolemia may be linked to an increase in osteoclastogenesis and bone resorption [47]. Nevertheless, uptake of DPE notably attenuated DEX-induced hyperlipidemia in this study which may be mediated by excessive amounts of soluble fibers in this fruit. Soluble fibers such as pectin has

excellent binding and jell forming properties which may help in reducing absorption of lipids with enhanced bile acid excretion, causing lowered total cholesterol levels [48]. Soluble fibers may also be fermented by gut microbiota giving short chain fatty acids (SCFA) which seemed important for increasing lipid oxidation and energy expenditure [49]. Thereby, preventing outcomes of bone osteoporosis with DEX treatment.

Increased reactive oxygen species (ROS) and induction of oxidative stress have been suggested as a potent risk factor for development of osteoporosis [51]. Oxidative stress represents an imbalance between production of ROS and the ability of antioxidant defense system to detoxify these reactive intermediates which eventually accelerates bone damage [50]. ROS act directly via inhibiting osteoblasts differentiation and increasing survival of osteoclasts, leading to diminished osteogenesis and bone loss [4]. ROS can also increase peroxidation of membrane lipids, in which MDA is the end product known as indicator for bone disorders [52]. Of note, DEX-associated bone loss is directly mediated via oxidative stress dependent mechanism. One possible cause is that existing hyperlipidemia with DEX is a strong promoter for ROS generation [26]. However, DEX can directly augment oxidative stress *via* boosting ROS generation or suppressing the activities of antioxidant system [9]. In support, the present study revealed marked elevation in lipid peroxidation product MDA and H_2O_2 levels, with suppression of endogenous antioxidants; GSH, SOD and CAT in bone of DEX treated rats. Of importance is that increased ROS like H_2O_2, with impaired bone defense mechanisms are considered as regulatory factors within activation of osteoclastic bone resorption and destruction [4]. These events may be closely related to the presently observed bone structural alterations evidenced as reduction in thickness of femur trabecular bone and loss of connection with noticeable porosity, as previously described by McNerny *et al.* [53]. Herein, administration of DPE successfully attenuated progression of bone lipid peroxidation and lowering of antioxidant defenses which in turn protect against trabecular bone loss, suggesting potential antioxidant capacity of DPE. This effect seemed to be mediated through its main polyphenolic compounds, chlorogenic acid and its isomers (neocholorogenic acid and cryptocholorogenic acid) which are known to have high free radicals scavenging activity [8]. However, this effect may also be related to the ability of polyphenols to activate endogenous antioxidant pathways and to oppose lipid peroxidation [9]. Thus, consumption of DPE could be useful in reducing oxidative bone damage caused by DEX.

Conclusion

The present study provided evidence for the preventive effect of dried plum against DEX-induced osteoporosis in male rats. This effect is ultimately mediated through suppressing hormonal alterations, lipogenesis and oxidative stress, which in all may aid in maintaining BMD and improving trabecular bone structure. Therefore, dried plum can be recommended as a promising and efficacious antiosteoporotic medication in patients undergoing DEX treatment.

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