ORIGINAL ARTICLE

Eur. J. Anat. 17 (2): 102-114 (2013)

Histological and morphometric effects of $CdCl_2$ and ginger on osteoporosis induced by bilateral ovariectomy in adult albino rats

Mohamed S. Mustafa[1], Omayma M. Mahmoud*[,2] and Hoda H. Hussein[2]

[1]*Orthopedic Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*
[2]*Anatomy Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*

SUMMARY

Cadmium is a widespread environmental pollutant. Low-level environmental cadmium exposure induced osteoporosis especially in postmenopausal women. Ginger is a strong antioxidant that may play an important role in bone formation. The purpose of the present study was to assess the effects of cadmium chloride and ginger on osteoporosis induced by bilateral ovariectomy in adult albino rats. Seventy-two adult albino rat females were used in the present study. They were divided into non-operated groups and operated groups. Cadmium chloride was received at a dose of 3.5 mg/kg daily by subcutaneous injection for 8 weeks, and ginger was fed on a diet containing 5% ginger for 8 weeks. Rats were sacrificed; femurs were dissected out, fixed and decalcified. Serial transverse and longitudinal sections from the diaphysis and metaphysis of femurs were stained by haematoxylin and eosin (H&E) and Masson trichrome stainings and examined using light microscopy. Femurs of Cd-treated, ovariectomized non-treated, and ovariectomized +Cd-treated groups showed histological and morphometric osteoporotic changes that were marked and exaggerated in the ovariectomized +Cd-treated group. Whereas Cd+ginger, ovariectomized +Cd+ginger and ovariectomized+ginger treated groups showed less bone resorption, more bone formation, and improvement in bone structure and morphometric parameters compared to other groups. Cadmium chloride exposure is a risk factor for osteoporosis, especially in postmenopausal women. Ginger effectively ameliorated cadmium and ovariectomy -induced osteoporosis in rats, and is a promising candidate for the prevention and treatment of postmenopausal osteoporosis.

Key words: Cadmium chloride – Ginger – Osteoporosis – Bilateral ovariectomy

INTRODUCTION

Cadmium (Cd) is a widespread environmental pollutant, which can be absorbed into biological systems through direct uptake and by accumulation in food chains. It has an extremely long half-life (20-30 years), and acts as a cumulative poison causing disorders in the respiratory, renal, skeletal, and vascular systems (Lin et al., 1995; Warren et al., 2000). Cadmium is widely used in paints, electrolysis industry (Prozialeck et al., 2006), fertilizers and battery manufacturing (ATSDR, 1989). Human acute and chronic Cd exposures occur through food, air, water, industrial products, and smoking and occupational exposure (Duruibe et al., 2007). It has been demon-

Corresponding author: Omayma Mahfouz Mahmoud. Department of Anatomy, Faculty of Medicine, Suez Canal University, 41111 Ismailia, Egypt Tel: 01272759000.
E-mail: omima34@hotmail.com

Submitted: 13 April, 2012. *Accepted:* 25 January, 2013.

strated that Cd stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals (Waisberg et al., 2003). Low-level environmental cadmium exposure induced osteoporosis and leads to a higher risk of fractures, especially in postmenopausal women (Staessen et al., 1999). Women are at greater risk of developing cadmium toxicity than men (Choudhury et al., 2001). Animal and *in vitro* studies suggest that cadmium might have direct toxic effects on bone (Wang et al., 1994; Regunathanet al., 2003). Ginger had been used as a spice for over a thousand years (Bartley and Jacobs, 2000). Its roots contain polyphenol compounds (6-gingerol and shogaols), which have a high antioxidant activity (Stoilova et al., 2007). The strong antioxidant action of ginger was one of the major possible mechanisms for the protective actions against toxicity and lethality of radiation (Jagetia et al., 2003; Haksar et al., 2006). Ginger contains Mg, Ca, and Po, which play important roles in bone formation (Srivastava and Mustafa, 1992). Osteoporosis is a skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhance bone fragility and a consequent increase in fracture risk (Turner, 2002). Hormonal factors strongly determine the rate of bone resorption. Lack of estrogen as a result of menopause increased bone resorption, as well as decrease the deposition of new bone that normally takes place in weight-bearing bones (Raisz, 2005). The ovariectomized rat was considered as a reliable animal model of postmenopausal osteoporosis which is the commonest type of osteoporosis (Frost and Jee, 1992). Free radical may play a role in postmenopausal osteoporosis pathogenesis by increasing bone resorption through the activation of oxidative stress responsive nuclear factor NF-kB, which has been proved to be associated with osteoclastogenesis (Basu et al., 2001). So the aim of the present study was to assess the effects of cadmium chloride and ginger on osteoporosis induced by bilateral ovariectomy in adult albino rats.

MATERIALS AND METHODS

Ginger powder was obtained from a natural herb market in Ismailia City, Egypt. It was mixed with normal rat pellets at 5: 95 wt. /wt. ratios. Cadmium chloride was obtained from the chemical store of the Faculty of Medicine at Suez Canal University, and dissolved in distilled water.

Animals

Seventy-two adult albino rat females weighing 200-250 g. were purchased from the animal house of the Faculty of Veterinary at Zagazeg University, and were used throughout this study. They were housed in wire mesh cages at room temperature under daylight in good hygienic conditions, and they received water and rodent pellets *ad libitum*. Rats were randomly divided into two main groups: I. Non-operated group and II. Operated group. Each group was subdivided into subgroups (8 rats in each) as follows:

I. Non-operated group: (A) Control group received 0.5 ml saline subcutaneously. (B) Cadmium-treated group (Cd-treated) received cadmium chloride (3.5 mg/kg body weight daily by subcutaneous injection for 8 weeks) and fed on rodent pellets. (C) Cadmium- and ginger-treated group (Cd+g treated) received the Cd (3.5 mg/kg body weight daily by subcutaneous injection) (Kara et al., 2005) and fed on a diet containing 5% ginger for 8 weeks. (D) Ginger-treated group (G treated) fed on a diet containing 5% ginger for 8 weeks.

II. Operated group: (1) Sham-operated control group (S-O control): fed on rodent pellets and received 0.5 ml saline subcutaneously after sham operation. (2) Ovariectomized non-treated group (OVX): fed on rodent pellets after bilateral ovariectomy. (3) Ovariectomized cadmium-treated, group (OVX+Cd treated) received the Cd (3.5 mg/kg body weight daily by subcutaneous injection for 8 weeks) and fed on rodent pellets after bilateral ovariectomy. (4) Ovariectomized cadmium- and ginger-treated group (OVX+Cd+G treated) received Cd (3.5 mg/kg body weight daily by subcutaneous injection) and fed on a diet containing 5% ginger for 8 weeks after bilateral ovariectomy. (5) Ovariectomized ginger-treated group (OVX+G treated) fed on a diet containing 5% ginger for 8 weeks after bilateral ovariectomy.

Operation: Bilateral ovariectomy and sham operations (in group II) were performed under ether anesthesia. The fur of the abdomen was shaved and the skin was sterilized using 70% alcohol and Providence iodine solution. A midline longitudinal incision was made inferior to the costal margin, the uterine tubes were ligated and the periovarian fatty tissue was identified and exteriorized. The ovaries were dissected and excised by cutting above the clamped area, while the uterine horn, other blood vessels were ligated and intra peritoneal injection of crystalline penicillin (3ml/kg) was done to avoid postoperative infection. Muscles and skin were stitched separately. Sterilization and bandages were performed. Sham operation was performed in a group (1) using the same procedure, without removing the ovaries.

Experimental procedures

All experiments were carried out in accordance with the guidelines of the Institutional Animals

Ethics Committee. Rats were observed for water intake and physical signs of toxicity following treatment. Rats of all groups were weighed daily, and were observed for signs of postoperative infection. They were sacrificed at the end of the 8 weeks of experiment by ether overdose.

Histological examination of bone

Rat femurs were dissected out, fixed in 10% neutral buffered formaldehyde for 2 days and decalcified in EDTA solution during 4 weeks (Hermizi et al., 2007). The decalcified specimens were dehydrated and processed to form paraffin blocks. Serial transverse and longitudinal sections (5 µm thick) from the diaphysis and metaphysis of femurs were prepared and stained by haematoxylin and eosin (H&E) and Masson trichrome stainings (Bancroft, 1994). All sections were examined using light microscope.

Morphometric analysis

The morphometric parameters were measured in transverse and longitudinal sections according to the report by the American Society for Bone and Mineral Research Committee (Parfitt et al., 1987). Cortical bone thickness or width (µm) were determined at five levels (0.5mm apart), starting 0.2 mm below the lowest point of the growth plate (Surve et al., 2001) and number of osteoclasts /10 HPF. All parameters were measured in 10 fields/ slide from 5 slides for each rat by the image analyzer (Leica Q 500 MC program) in the Histology Department, Faculty of Medicine, El Azhar University.

Statistical analysis

The chi-square and One-way analysis of variance (ANOVA) tests were used to evaluate statistically the results obtained from all groups. Differences among groups were considered significant when P value < 0.05.

RESULTS

Body weight changes

Rats of Cd-treated and OVX+Cd-treated groups showed significant loss of body weight when

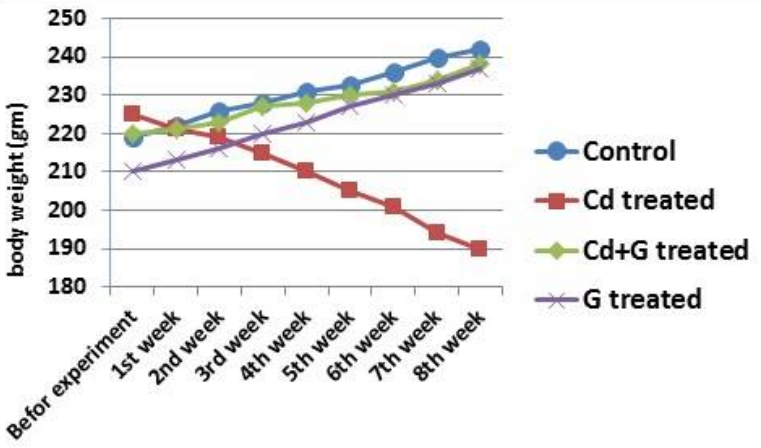

Fig. 1. Effects of cadmium chloride and ginger on body weight of adult albino rats in non-operated subgroups.

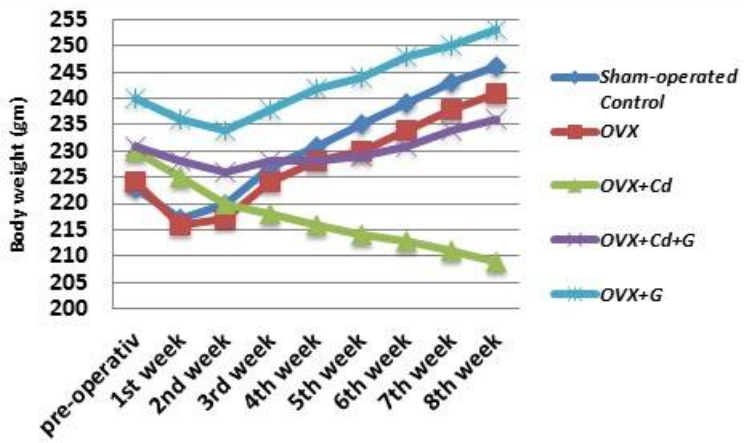

Fig. 2. Effects of cadmium chloride and ginger on body weight of adult albino rats in operated subgroups.

compared with other studied groups. All rats of operating group (II) showed weight loss during the 1st post-operative week. Sham-operated control group showed weight gain starting from the second week until the end of the 8th week of the experiment, whereas the OVX-non-treated, OVX+Cd+G-treated and OVX+G-treated groups showed weight loss during the 2nd week, followed by weight gain until the end of the experiment (Figs. 1 and 2).

Histology

Control and Sham-operated control groups (Figs. 3-5): Examination of femur metaphysis sections in control and S-O control groups showed an outer periosteum, shell of cortical (compact) bone and inner cancellous bone. The inner cancellous bone consisted of a network of bony trabeculae separated by interconnecting spaces containing bone marrow. The bone marrow was formed of hematopoietic tissue and scattered adipocytes. The bone trabeculae consisted of irregular bone lamellae and osteocytes within their lacunae in between bone lamellae. The endosteal surface of trabeculae was lined by osteoprogenitor cells, osteoblasts and osteoclasts in Howship's lacunae. Femur diaphysis sections revealed an outer periosteum, shell of cortical bone, endosteum and bone marrow. The periosteum was composed of a thick outer fibrous layer and inner osteogenic layer. The endosteal surface of the cortical bone appeared smooth and lined with osteoprogenitor cells and osteoblasts. The cortical bone was formed of compact bone consisting of outer, inner, and interstitial bone lamellae containing osteocytes inside their lacunae with densely stained oval nuclei. The cortical bone showed sub-

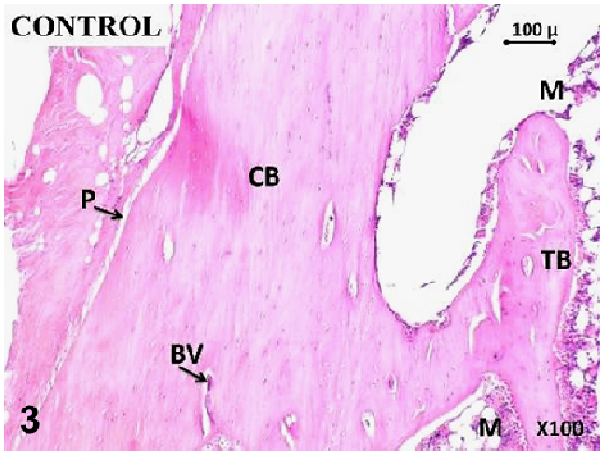

Fig. 3. Longitudinal section of the femur metaphysis of the control group showed normal bone architecture: compact bone (CB), trabecular bone (TB), periosteum (P), marrow cavities (M) and blood vessel (BV). H&E staining. x 100.

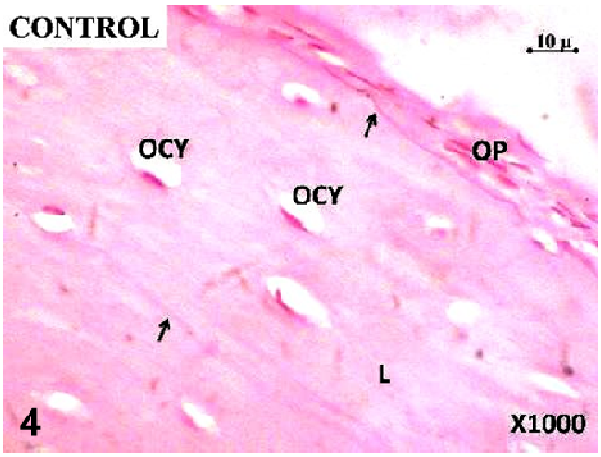

Fig. 4. Cross section of femur diaphysis compact bone of the control group showed osteocytes inside their lacunae (OCY), osteoproginator cells (OP) and distinct bone deposition lines (arrows). H&E staining. x 1000.

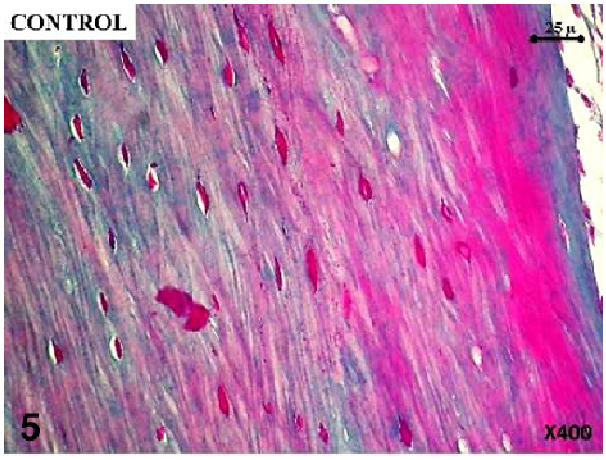

Fig. 5. Longitudinal section of femur diaphysis compact bone of the control group showed normal collagen fiber staining of bone lamellae. Masson's trichrome stain. x 400.

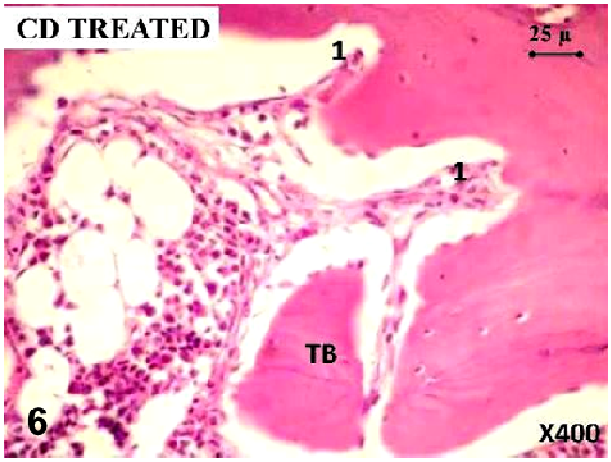

Fig. 6. Longitudinal section of femur metaphysis cancellous bone of Cd-treated group showed widely separated bone trabeculae (TB) and areas of bone erosion (1). H&E staining. x 400.

periosteal bone deposition appearing as a distinct dark line demarcating the border between newly added bone matrix and the older bone. Masson's trichrome stained metaphysis and diaphysis sections showed collagen fibers stained homogenously with light green.

Cadmium-treated group (Figs. 6-8): Femur metaphysis sections in Cd-treated rats revealed loss of normal architectures of trabecular bone, surface erosion and irregularities, widening of bone marrow spaces and increased inter-trabecular distances. Trabecular bone showed bone resorption, cavitation with the presence of osteoclasts inside bone cavities. The number, size, and density of trabeculae decreased with the appearance of widely separated bony spicules compared to control and sham-operated control groups. Femur diaphysis, compact bone sections revealed resorption, cavitation erosion and irregularities in the endosteal and periosteal surfaces of the cortical bone, few distinct lines of sub-periosteal bone deposition. Also decreased collagen fibers staining intensity (Masson's trichrome stained) was observed compared to control and sham-operated control groups.

Cadmium- and ginger-treated group (Figs. 9-11): Femur metaphysis sections in Cd+G-treated group revealed normal trabecular bone architectures and density. Few sites of bone resorption observed in the trabecular bone surrounded by areas of bone deposition by the presence of osteoclasts and osteoblasts on the site of bone cavities. Femur diaphysis sections revealed less resorption and cavitation of compact bone, with the

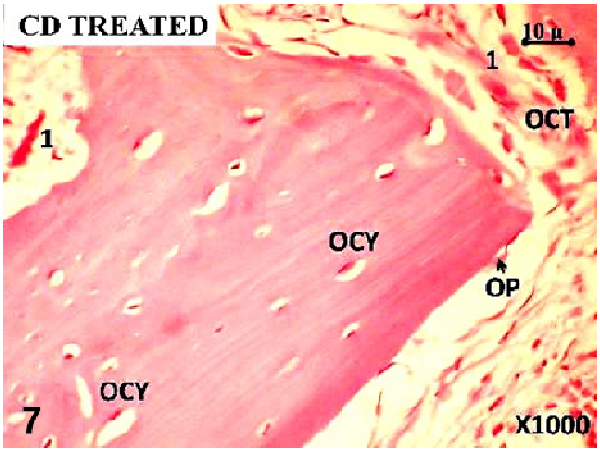

Fig. 7. Longitudinal section of femur diaphysis compact bone of Cd-treated group showed bone resorption, osteoclast cells (OCT) located in an area of bone resorption (1), osteoproginator cell (OP) and osteocyte inside its lacuna (OCY). H&E staining. x 1000.

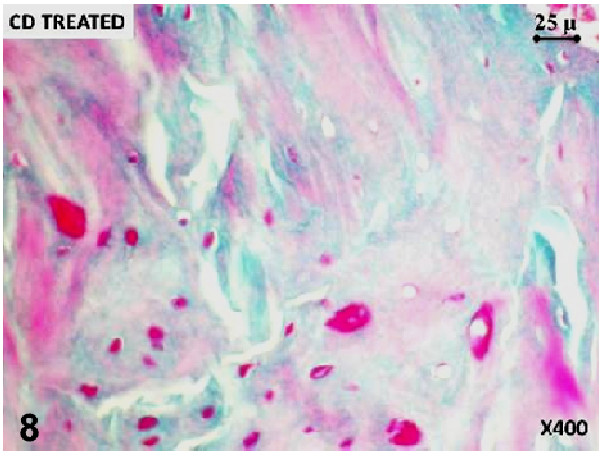

Fig. 8. Cross section of femur diaphysis compact bone of Cd-treated group showed areas of bone resorption and decreased collagen fiber staining of bone lamellae. Masson's trichrome staining. x 400.

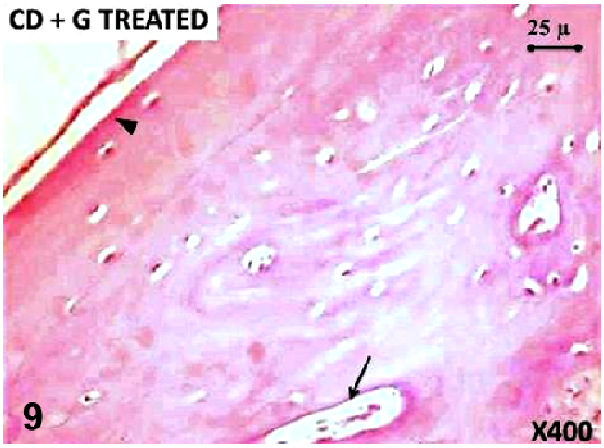

Fig. 9. Cross section of femur diaphysis compact bone of Cd +G-treated group showed smooth periosteal surface (arrowhead) and small area of bone resorption (arrow) surrounded with dark lines of bone deposition. H&E staining. x 400.

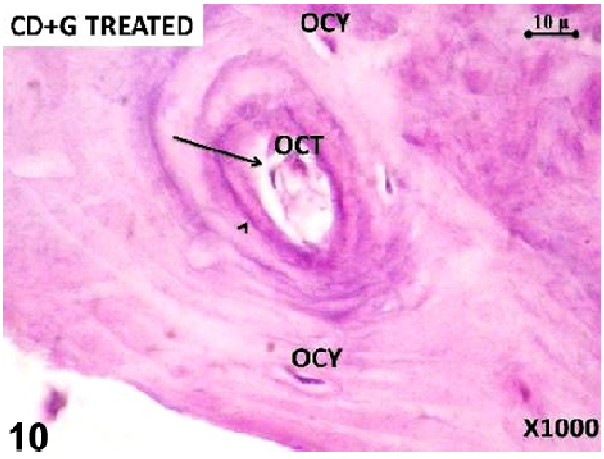

Fig. 10. Cross section of femur diaphysis compact bone of Cd +G-treated group showed osteoclast cell (OCT) inside a small area of bone resorption (arrow) surrounded with dark lines of bone deposition and osteocyte cells inside their lacunae (OCY). H&E staining. x 1000.

presence of distinct lines of sub-periosteal bone deposition compared to Cd-treated group. The periosteal and endosteal surfaces appeared smooth with few sites of erosion, which lined with osteoprogenitor cells and osteoblasts with few numbers of osteoclasts. Masson's trichrome stained showed normal collagen fibers staining intensity with the appearance of decreased staining intensity in sites near resorption areas compared to control, sham-operated control and other treated groups.

Ginger-treated group (Figs. 12-14): Femur diaphysis and metaphysis sections of ginger-treated group resembled those of the control and sham-operated control groups.

Ovariectomized non-treated group (Figs. 15-17): Femur metaphysis and diaphysis sections in OVX -non-treated rats revealed loss of normal trabecular architecture, increased inter-trabecular distances, surface erosion and irregularities, widening of bone marrow spaces, trabecular and compact bone resorption and cavitation with the presence of osteoclasts inside the sites of bone cavities, decreased bone density and collagen fiber staining intensity compared to control and sham-operated control groups.

Ovariectomized cadmium-chloride-treated group (Figs. 18-21): metaphysis and diaphysis sections in OVX+Cd-treated rats revealed marked trabecular, compact bone resorption, cavitation with the presence of osteoclasts inside bone cavities, loss of normal architectures of trabecular bone, marked widening of bone marrow spaces, marked increase in inter-trabecular distances, erosion and irregularities in the endosteal, periosteal surfaces and decreased sub-periosteal bone deposition compared to control, sham-operated,

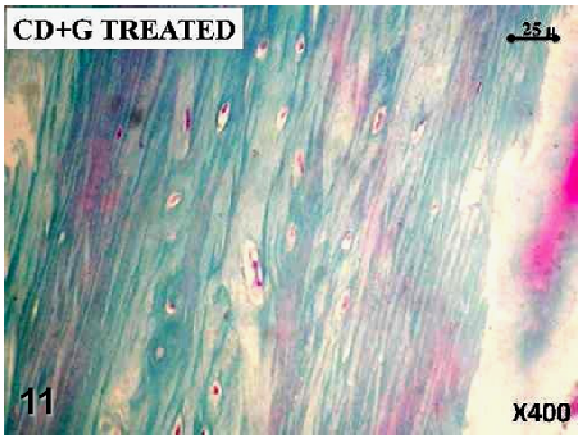

Fig. 11. Longitudinal section of femur diaphysis compact bone of Cd+G-treated group showed decreased collagen fiber staining of bone lamellae. Masson's trichrome staining. x 400.

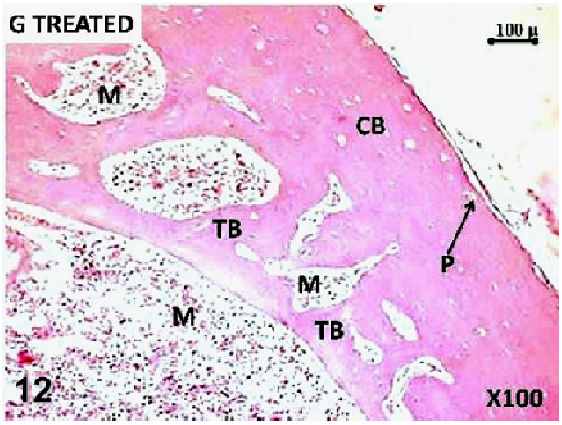

Fig. 12. Cross section of femur metaphysis of G-treated group showed compact bone (CB), normal architecture of trabecular bone (TB), marrow cavities (M) and smooth periosteal surface (P). H&E staining. x 100.

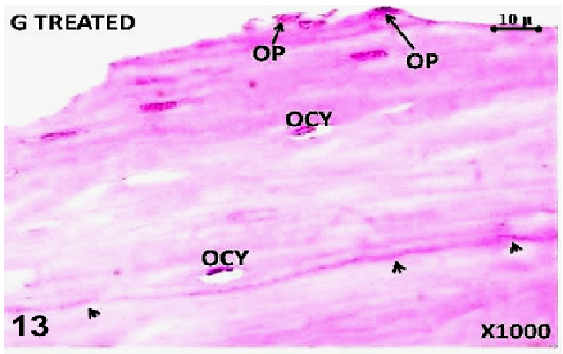

Fig. 13. Cross section of femur diaphysis compact bone of G-treated group showed osteoproginator cells on the periosteal surface (OP), dark lines of bone deposition (short arrows) and scattered osteocytes(OCY). H&E staining. x 1000.

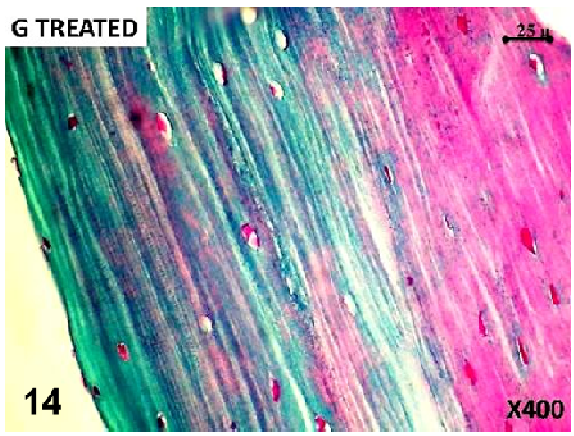

Fig. 14. Longitudinal section of femur diaphysis compact bone of G-treated group showed normal collagen fiber staining of bone lamellae. Masson's trichrome staining. x 400.

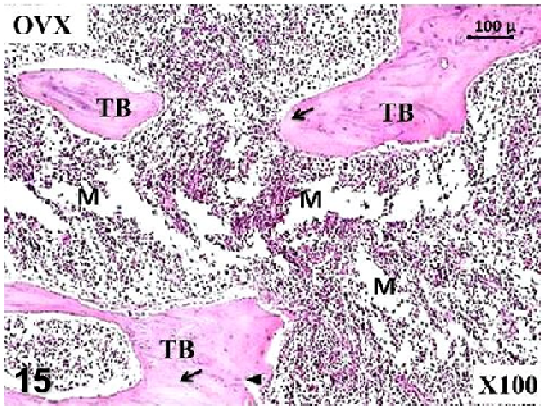

Fig. 15. Longitudinal section of femur metaphysis cancellous bone of ovariectomized non-treated group showed widely separated bone trabeculae (Tb), areas of trabecular erosion (arrow head), faintly stained areas of bone matrix (arrows) and wide marrow spaces (M).H&E staining. x 100.

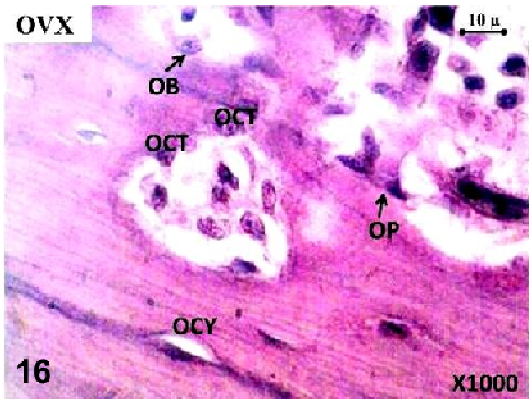

Fig. 16.- Cross section of femur diaphysis compact bone of ovariectomized non-treated group showed osteocyte (OCY), osteoproginator cell (OP), osteoblast (OB), osteoclast cell (OCT) located in an area of bone resorption. H&E staining. x 1000.

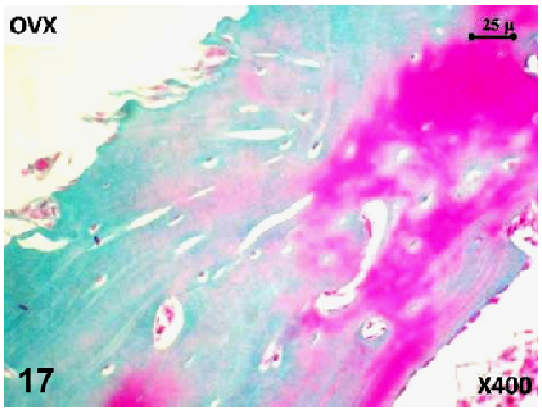

Fig. 17. Longitudinal section of femur diaphysis compact bone of ovariectomized non-treated group showed bone resorption and decreased collagen fiber staining of bone lamellae. Masson's trichrome staining. x 400.

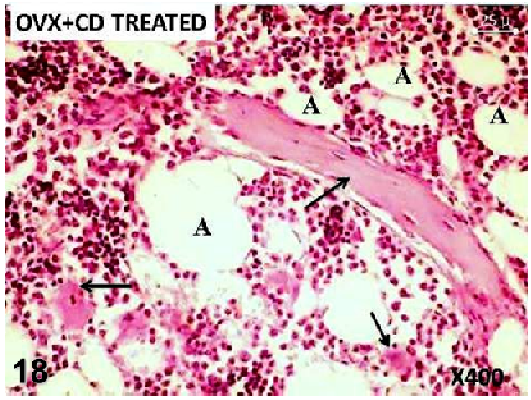

Fig. 18. Longitudinal section of femur metaphysis cancellous bone of ovariectomized Cd-treated group showed faintly stained bone trabeculae (arrows) that appeared as widely separated bony ossicles in widened bone marrow spaces and increased marrow adipocytes (A). H&E staining. x 400.

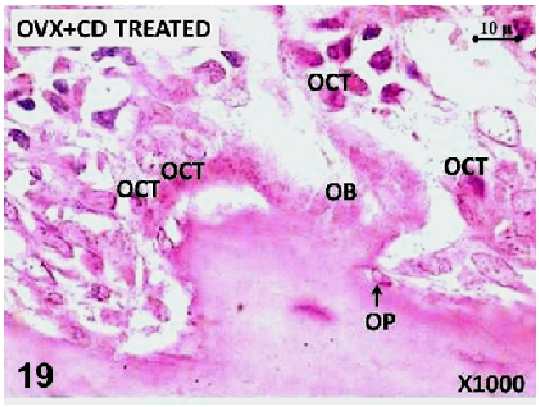

Fig. 19. Longitudinal section of femur metaphysis trabecular bone of ovariectomized Cd-treated group showed osteoblast (OB), osteoproginator (OP) and osteoclast cells (OCT) housed in the resorption area. H&E staining. x 1000.

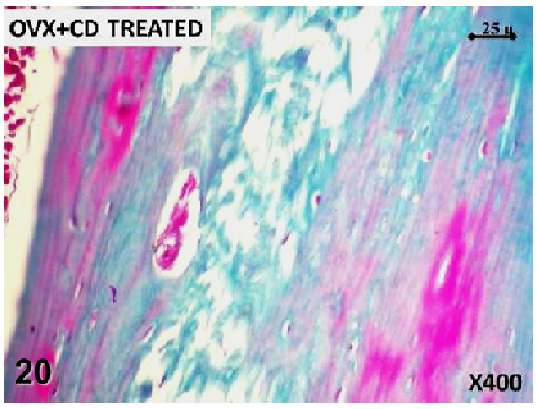

Fig. 20. Longitudinal section of femur diaphysis compact bone of ovariectomized Cd-treated group showed decreased collagen fiber staining of bone lamellae and areas of bone resorption. Masson's trichrome staining. x 400.

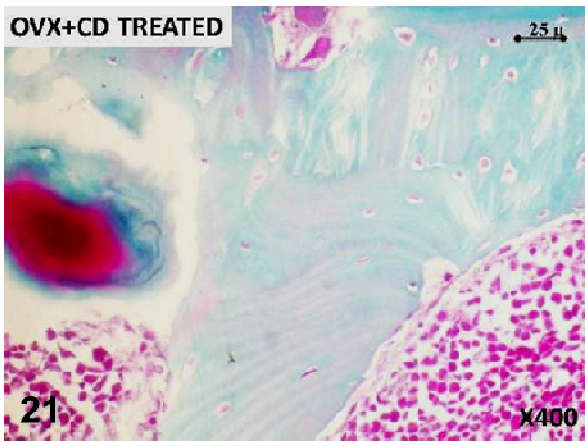

Fig. 21. Longitudinal section of femur metaphysis cancellous bone of ovariectomized Cd-treated group showed areas of bone resorption and decreased collagen fiber staining of bone lamellae. Masson's trichrome staining. x 400.

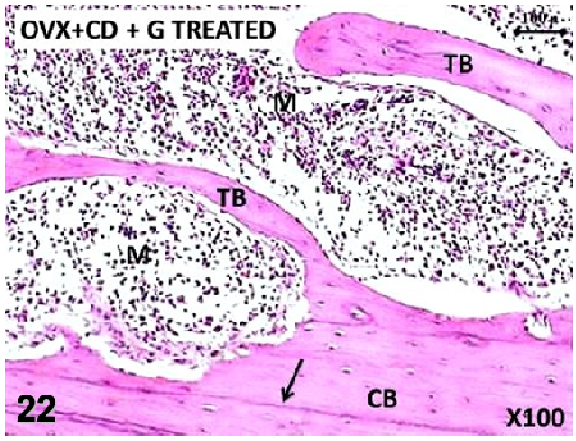

Fig. 22. Longitudinal section on femur metaphysis of ovariectomized Cd +G-treated group showed compact bone (CB), trabecular bone (TB), marrow cavities (M) and the dark line of bone deposition (arrow). H&E staining. x 100.

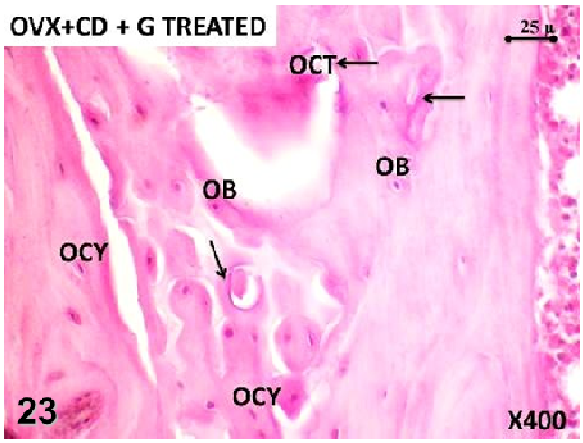

Fig. 23. Longitudinal section of femur metaphysis compact bone of ovariectomized Cd +G-treated group showed an area of bone resorption with osteoclast cell inside (OCT) and osteoblast cells around (OB), scattered osteocytes (OCY) and dark lines of bone deposition (arrows).H&E staining. x 400.

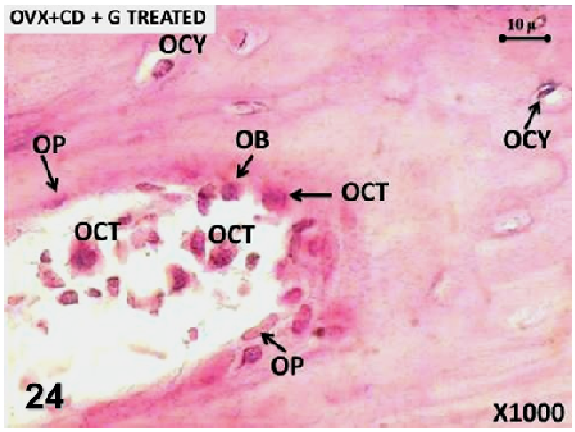

Fig. 24. Cross section of femur diaphysis compact bone of ovariectomized Cd +G-treated group showed an area of bone resorption with osteoclast cell inside (OCT), osteoproginators (OP) and osteoblast (OB) and scattered osteocytes (OCY). H&E staining. x 1000.

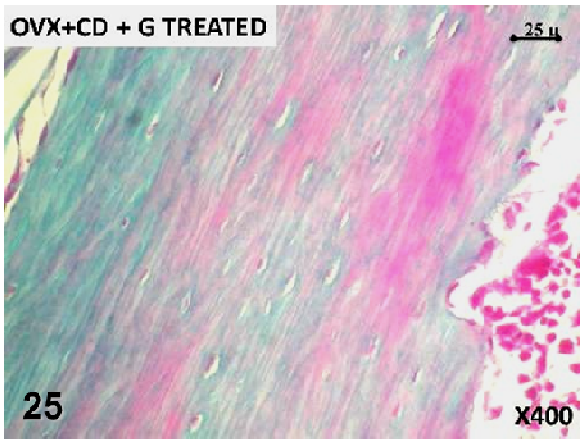

Fig. 25. Cross section of femur diaphysis compact bone of ovariectomized Cd + G-treated group showed an area normally stained and areas of decreased collagen fiber of bone lamellae and endosteal surface erosion. Masson's trichrome staining. x 400.

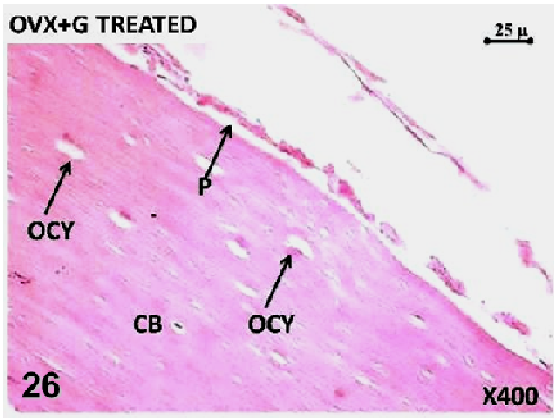

Fig. 26. Cross section of femur diaphysis compact bone of ovariectomized G-treated group showed smooth periosteal surface (P), normal compact bone matrix (CB) and scattered osteocytes (OCY). H&E staining. x 400.

Cd-treated, Cd+g-treated, and OVX-non-treated groups. There was a decreased number, size and density of trabeculae, with the appearance of islands of bony spicules inside wide bone marrow spaces. Masson's trichrome-stained showed decreased collagen fibers staining intensity with alteration in staining pattern in some areas compared to other groups.

Ovariectomized cadmium-chloride- + ginger-treated group (Figs. 22-25): Femur metaphysis sections in OVX+Cd +G-treated group revealed normal trabecular bone architectures and density. Few sites of bone resorption were observed in the trabecular bone surrounded by areas of bone deposition by the presence of osteoclasts and osteoblasts on the site of bone cavities. Femur diaphysis sections revealed less resorption and cavitation of compact bone, with the presence of distinct lines of sub-periosteal bone deposition compared to Cd-treated and OVX+Cd-groups. The periosteal and endosteal surfaces appeared smooth with few sites of erosion, lined with osteoprogenitor cells and osteoblasts with the presence of fewer numbers of osteoclasts. Masson's trichrome-stained showed decreased collagen fibers staining intensity compared to control, sham-operated control, OVX-non-treated, Cd-treated and ginger-treated.

Ovariectomized ginger-treated group (Figs. 26-29): Femur diaphysis and metaphysis sections of the OVX+G-treated group were near those of the control group.

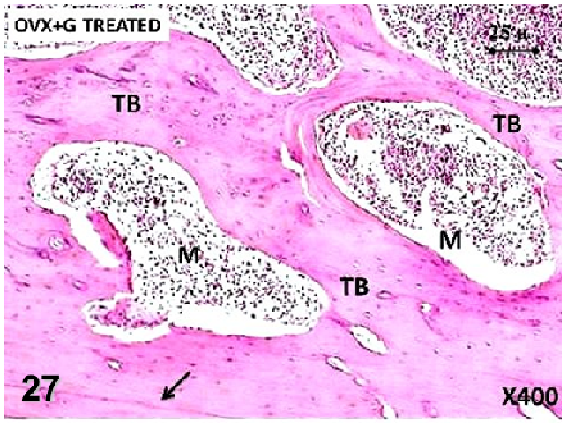

Fig. 27. Longitudinal section of femur metaphysis cancellous bone of ovariectomized G-treated group showed normal architecture of trabecular bone (TB), marrow cavities (M) and dark lines of bone deposition (arrow). H&E staining. x 400.

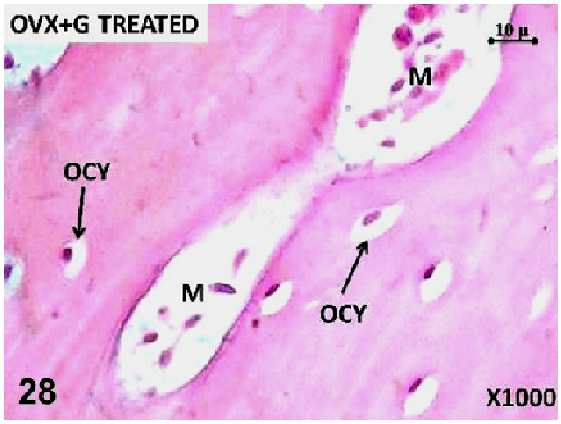

Fig. 28.- Cross section of femur metaphysis cancellous bone of ovariectomized G-treated group showed marrow spaces (M) between bone trabeculae and scattered osteocytes (OCY).H&E staining. x 1000.

Morphometry

Table 1 showed that compact bone thickness (CBT) was significantly decreased and the number of osteoclast cells was significantly increased in Cd-, Cd+g-, OVX-non-treated, OVX+Cd-,

Table 1. Compact bone thickness (CBT) and number of osteoclast cells (Oct No.) in femoral bones of the different studied groups.

Group	CBT (µm) Mean±SD	Oct No./10HPF Mean±SD
Control	658 ± 31.6	2±0.2
Sham-operated control	650 ± 47.02	3±0.43
Cd-treated	302± 29.69 [a, b]	14±0.33 [a, b]
Cd+G-treated	444.4 ± 26.3 [a, b, c]	8±0.5 [a, b, c]
G-treated	630 ± 12.41	3±0.39
OVX-nontreated	365.56 ± 26.0 [a, b]	13±0.72 [a, b]
OVX+Cd-treated	170± 41.5 [a, b, c, d, f]	17±0.2 [a, b, c, d, f]
OVX+Cd+G-treated	287.5 ± 20.2 [a, b, d, e, f]	13±0.6 [a, b, e, f]
OVX+G-treated	476± 26.9 [a, b, d]	7±0.36 [a, b, d]

ANOVA test: a) P<0.001 as compared to control and sham-operated control groups; b) P<0.001 as compared to G-treated group; c) P<0.001 as compared to Cd-treated group; d) P<0.001 as compared to OVX-non-treated group; e) P<0.001 as compared to OVX+Cd treated-group; f) P<0.001 as compared to OVX+G-treated group.

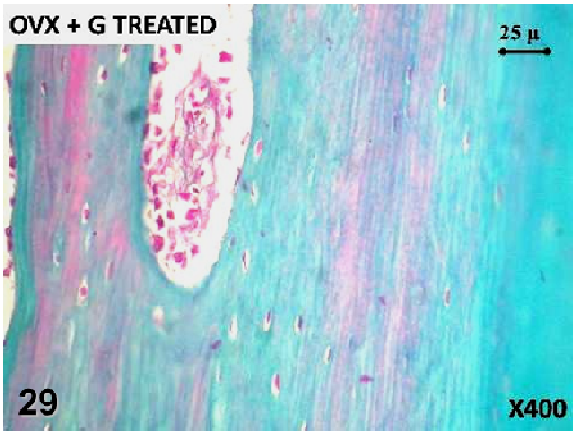

Fig. 29. Longitudinal section of femur diaphysis compact bone of ovariectomized G-treated group showed an area of bone resorption and normal collagen fiber staining of bone lamellae. Masson's trichrome staining. x 400.

OVX+Cd+G- and OVX+G- treated groups compared to the control, sham-operated control and ginger-treated groups. OVX+Cd treated group showed the lowest CBT value followed by OVX+Cd+G-treated group. Number of osteocytes was markedly increased in OVX+Cd-treated group compared to other groups.

DISCUSSION

The present study revealed a significant decrease in body weight in Cd-treated and OVX+Cd -treated groups compared to other studied groups. These agreed with another study which reported that cadmium chloride produced a significant decrease in body weight in rats of Cd-treated group compared with the other groups (Gaurav et al., 2010), whereas another study reported that a single intra-peritoneal administration of Cadmium chloride had no significant effect on the body weight (Martiniakova et al., 2011).

Cadmium is a risk factor for the development of osteoporosis even at low doses by altering mineral metabolism before the occurrence of kidney lesions (Noëlet al., 2004). A single intraperitoneal administration of cadmium chloride induced significant bone osteoporotic changes (Martiniakova et al., 2011). Changes in bone osteopenia, osteoporosis, and osteomalacia, with increased bone fragility and pathological fractures in humans and experimental animals, were noticed as a result of exposure to Cd (Bhattacharyya, 2009; Brzoska et al., 2010). This agreed with the results of Cd-treated group in this study, which revealed compact and trabecular bone resorption, surface erosion, decreased bone deposition, loss of normal trabecular architecture, wide bone marrow spaces, increased inter-trabecular distances, decreased collagen fiber staining intensity, decreased compact bone thickness (CBT), and increased the number of osteoclast cells compared to control. These findings are considered osteoporotic changes and micro-architectural deterioration of bone structure.

Decreasing concentration of both Ca and Zn in the femur of rats after a single injection of cadmium chloride was reported (Bonner et al., 1981). Ovariectomized rats showed a reduction in cortical bone thickness and intra-cortical resorption cavities (Weber et al., 2004). Ovariectomy in rats resulted in an impaired calcium balance and significant hypocalcaemia. Calcium metabolism plays a significant role in bone turnover in postmenopausal osteoporosis, as deficiency of calcium leads to impair bone deposition (Mattix et al., 2003). Estrogen deficiency caused acceleration of bone remodeling where osteoclastic bone resorption outpaced the anabolic activity of osteoblasts (Goss et al., 2004; Grassi et al., 2006; Orlice et al., 2007). The distal femur metaphysis of OVX-non-treated rats revealed significant decrease in the mean trabecular bone volume, causing widening of the bone marrow spaces and increased inter-trabecular distances. Moreover, loss of normal architecture thinned some trabeculae, which appeared as islands of widely separated specules (Kostandy, 2011). There was evidence that there is a biochemical link between increased oxidative stress and reduced bone density in postmenopausal women (Maggio et al., 2003). Reactive oxygen species (ROS) were considered to be responsible for the aging process and osteoporosis (Isomura et al., 2004). Loss of estrogens also accelerates the effects of aging on bone by decreasing defense mechanisms against oxidative stress. Both aging and loss of sex steroids has adverse effects on skeletal homeostasis (Almeida et al., 2007). This agreed with and explained the results of the OVX -non-treated group in this study, which showed osteoporotic changes in femur diaphysis and metaphysis, decreased CBT and increased the number of osteoclast cells in overctomised groups compared to control and sham-operated control.

Bone strength, bio-mechanic parameters and bone histology were more affected after Cadmium exposures in osteoporotic than normal rats (Comelekoglu et al., 2007). This agreed with the results of this study, which revealed thatovariectomized Cd treated group showed marked osteoporotic changes in compact and trabecular bones with decreased bone formation and CBT and increased osteoclasts compared to control, sham-operated control and OVX-non-treated groups.

Bone strength depends on bone mineral density (BMD) and bone quality (Ferretti et al., 2001). Stiffness, strain, stress, and toughness were also important biomechanical parameters for bone strength. The mineral component confers strength and stiffness to the tissues. Stress, strain and toughness contribute to bone collagen integrity (Burr, 2002). Cd influences collagen content and its solubility in the femoral bone of 3-week-old female rats (Galicka et al., 2004). Bone stress, strain and toughness were reduced in the OVX+Cd group. This may be related to deformation of collagen integrity (Orlic et al., 2007). This was in agreement with present study which revealed that collagen fiber staining was decreased in Cd- and OVX+Cd- groups. However, this reduction was more in the OVX+Cd-group than in the Cd-group.

Cadmium + ginger-treated, group in the present study revealed less compact and trabecular bone resorption few sites of erosion, with the presence of fewer numbers of osteoclasts and more bone

formation compared to Cd-treated, OVX-non-treated and OVX+ Cd-treated groups. CBT was increased and the number of osteoclast cells was, decrease compared to Cd treated, OVX-non -treated and OVX+ Cd-treated groups. OVX+Cd+G-treated group, revealed less osteoporotic changes with more bone formation than OVX+Cd-treated, group. So ginger administrations caused improvements in bone microarchitectures and structure, and decreased the osteoporotic changes in femur diaphysis and metaphysis caused by cadmium chloride and bilateral ovariectomy in our study.

Long-term exposure to cadmium leads to an increase in lipid peroxidation and inhibition of superoxide dismutase (SOD) activity, showing oxidative damage in different body tissues (El-Missiry and Shalaby, 2000). The increase in lipid peroxidation may be attributed to alterations in the antioxidant defense system (AOS). This defense system includes the enzymes, glutathione peroxidase (GPx) and SOD, which normally protect against free radical toxicity (Rana and Verma, 1996). It was known that chelators and antioxidants protect against potentially damaging effects of reactive oxygen and lipid peroxides (Gupta et al., 2005).

Ginger has been listed in "Generally Recognized as Safe" (GRAS) document of the US FDA (Langner et al., 1998). Ginger was known as strong antioxidant having a strong antioxidant defense mechanism against toxicity and lethality of radiation (Jagetia et al., 2003; Haksar et al., 2006). Its antioxidant activity might be due to polyphenol compounds (6-gingerol and shogaols) from its roots (Stoilva et al., 2007). Ginger also contains acid resins, vitamin C compounds, gingerol, vitamins B3 and B6, volatile oils, and biotrace which play important roles in bone formation (Srivastava and Mustafa, 1992). It was reported that ginger had both prophylactic and therapeutic Cd de-toxicities effects, and ginger therapy was more effective as more Cd intake was avoided (Egwurugwu et al., 2007). So one mechanism that may explain the improvement in bone structure in the Cd+g-treated group in this study was the antioxidant effect of ginger, as well as its bone protection against oxidative damage induced by cadmium.

Many authors suggested that Cd caused osteoporosis through alteration of several essential metal metabolism involved in the formation of bone matrix and mineralization, and acted as cofactor for specific bone enzymes besides its effect on calcium metabolism. The most strikingly decreased metals were iron, zinc, copper, and magnesium, which contributed to collagen maturation (Brzoska and Monuszko 2001; Medeiros et al., 2002).

Concerning the ginger-treated group in the present study, the histological and morphometric examinations of compact and trabecular bones showed a normal bone architecture and structure, resembling those of the control and sham-operated control group. Also OVX+G-treated group showed improvement in the collagen fiber staining intensity compared to OVX-non-treated group. Estrogen exerts beneficial actions through suppression of reactive oxygen species (ROS). ROS stimulate osteoclasts, thus, estrogen might prevent bone loss by enhancing oxidant defenses in bone. Estrogen deficiency after ovariectomy caused bone loss by lowering thiol antioxidants in osteoclasts, thus promoting osteoclastic bone resorption (Lean et al., 2003). Recently, it was observed that other antioxidants such as a-lipoic acid (ALA) improved the imbalance between osteoclastic bone resorption and osteoplastic bone formation and ameliorated the osteoporotic changes that accompanied ovariectomy (Kostandy, 2011). As far as the results of the present study are concerned, cadmium chloride is toxic to bone and causes osteoporotic changes, which were more marked and exaggerated in bilateral ovariectomized rats. Ginger ameliorated the osteoporotic effect of bilateral ovariectomy and cadmium chloride through its composition and strong antioxidant properties.

So we concluded that Cadmium chloride exposure is a risk factor of osteoporosis especially in postmenopausal women. Ginger represents a promising therapeutic option for the prevention and treatment of postmenopausal osteoporosis.

REFERENCES

ALMEIDA M, HAN L, MARTIN MILLAN M, PLOTKIN LI, STEWART SA, ROBERSON PK (2007) Skeletal involution by age-associated oxidative stress and its acceleration by loss of sex steroids. *J Biol Chem*, 282: 27285-27297.

ATSDR {Agency for Toxic Substances and Disease Registry} (1989) Toxicological profile for cadmium. *ATSDR/US Public Health Service*, ATSDR/TP-88/08.

BANCROFT JD (1994) Bone staining In: Manual of histological techniques and their diagnostic application. *Bancroft JD*, 4: 400-412.

BARTLEY J, JACOBS A (2000) Effects of drying on flavor compounds in Australian-grown ginger (*Zingiber officinale*). *J Sci Food Agric*, 80: 209-215.

BASU S, MICHAELSSON K, OLOFSSON H, JOHANSSON S, MELHAUS H (2001) Association between oxidative stress and bone mineral density. *Biochem Biophys Res Commun,* 288: 275-277.

BHATTACHARYYA MH (2009) Cadmium osteotoxicity in experimental animals: mechanisms and relationship to human exposures. *Toxicol Appl Pharmacol*, 238: 258-265.

BONNER FW, KING LJ, PARKE DV (1981) The acute and subacute effects of cadmium on calcium homeostasis and bone trace metals in the rat. *J Inorg Biochem*, 14: 107-114.

BRZOSKA MM, MONUSZKO-JAKONIUK J (2001) Interactions between cadmium and zinc in the organism. *Food Chem Toxicol*, 39: 967-980.

BRZÓSKA MM, MAJEWSKA K, KUPRASZEWICZ E (2010) Effects of low, moderate and relatively high chronic exposure to cadmium on long bones susceptibility to fractures in male rats. *Environ Toxicol Pharmacol*, 29: 235-245.

BURR DB (2002) The contribution of the organic matrix to bone material properties. *Bone*, 31: 8-11.

CHOUDHURY H, HARVEY T, THAYER WC, LOCKWOOD TF, STITELER WM, GOODRUM PE (2001) Urinary cadmium elevation as a biomarker of exposure for evaluating a cadmium dietary exposure biokinetic model. *J Toxicol Environ Health Part A*, 63: 321-350.

COMELEKOGLU U, YALIN S, BAGIS S, OGENLER O, OZLEN SAHIN N, YILDIZ A, COSKUN B, HATUNGIL R, TURAC A (2007) Low-exposure cadmium is more toxic on osteoporotic rat femoral bone: Mechanical biochemical and histopathological evaluation. *Ecotoxico Enviro Safety*, 66: 267-271.

DURUIBE JO, OGWUEGBU MOC, EGWURUGWU JN (2007) Heavy metal pollution and human biotoxic effects. *Int J Phys Sci*, 2: 112-118.

EGWURUGWU JN, UFEARO CS, ABANOBI OC, NWOKOCHA CR, DURUIBE JO, ADELEYE GS, EBUNLOMO AO, ODETOLA AO, ONWUFUJI O (2007) Effects of ginger (Zingiber officinale) on cadmium toxicity. *African Jof Biotech*, 6: 2078-2082.

EL-MISSIRY MA, SHALABY F (2000) Role of β-carotene in ameliorating the cadmium induced oxidative stress in rat brain and testis. *J Biochem MolToxicol*, 14: 238-243.

FERRETTI JL, COINTY GR, CAPOZZA RF, CAPIGLIONI R, CHIAPPE MA (2001) Analysis of biomechanical effect on bone and on the bone muscle interactions in small animal models. *J Musculoskel Neuron Interact*, 1: 263-274.

FROST HM, JEE WS (1992) On the rat model of human osteopenias and osteoporoses. *Bone Miner*, 18: 227-236.

GALICKA A, BRZOSKA MM, SREDZINSKA K, GINDZIENSKI A (2004) Effect of cadmium on collagen content and solubility in rat bone. *Acta Biochim Pol,* 51: 825-829.

GAURAV D, PREET S, DUA KK (2010) Chronic cadmium toxicity in rats: treatment with combined administration of vitamins, amino acids, antioxidants and essential metals. *J Food Drug Anal,* 18: 464-470.

GOSS PE, QI S, CHEUNG AM, HU H, MENDES M, PRITZKER KP (2004) Effects of the steroidal aromatase inhibitor exemestane and the nonsteroidal aromatase inhibitor letrozole on bone and lipid metabolism in ovariectomized rats. *Clin Cancer Res,* 10: 5717-5723.

GRASSI F, FAN X, RAHNERT J, WEITZMANN MN, PACIFICI R, NANES MS, RUBIN J (2006) Bone re/modeling is more dynamic in the endothelial nitric oxide synthase in mouse. *Endocrinology*, 147: 4392-4399.

GUPTA R, KANNAN GM, SHARMA M, FLORA SJA (2005) Therapeutic effects of *Moringa oleifera* on arsenic-induced toxicity in rats. *Environ Toxicol Pharmacol*, 20: 456-464.

HAKSAR A, SHARMA A, CHAWLA R, KUMAR R, ARORA R, SINGH S, PRASAD J, GUPTA M, TRIPATHI RP, ARORA MP, ISLAM F, SHARMA RK (2006) Zingiber officinale exhibits behavioral radioprotection against radiation. *Pharmacol Biochem Behav*, 84: 179-188.

HERMIZI H, FAIZAH O, IMA NIRWANA S, AHMAD NAZRUN S, LUKE DA, NORAZLINA M (2007) Nicotine impaired bone histomorphometric parameters and bone remodeling biomarkers in Sprague-Dawley male rats. *Ann Microsc*, 7: 10-24.

ISOMURA H, FUJIE K, SHIBATA K, INOUE N, IIZUKA T, TAKEBE G (2004) Bone metabolism and oxidative stress in post menopausal rats with iron overload. *Toxicol,* 197: 93-100.

JAGETIA GC, BALIGA MS, VENKATESH P, ULLOOR JN (2003) Influence of ginger rhizome (Zingiber officinale Rosc.) on survival, glutathione and lipid peroxidation in mice after whole-body exposure to gamma radiation. *Radiat Res*, 160: 584-592.

KARA H, KARATAŞ F, CANATAN H, SERVI K (2005) Effects of exogenous metallothionein on acute cadmium toxicity in rats. *Biol Trace Elem Res*, 104: 223-232.

KOSTANDY NK (2011) Histological and morphometric studies on the effect of alpha-lipoic acid on postovariectomy osteoporosis induced in adult female albino rats. *Egyptian J Histology*, 34: 139-155.

LANGNER E, GREIFENBERG S, GRUENWALD J (1998) Ginger: history and use. *Adv Ther,* 15: 25-30.

LEAN JM, DAVIES JT, FULLER K, JAGGER CJ, KIRSTEIN B, PARTINGTON GA (2003) A crucial role for thiol antioxidants in estrogen-deficiency bone loss. *J Clin Invest*, 112: 915-923.

LIN CJ, WU KH, YEW FH, LEE TC (1995) Differential cytotoxicity of cadmium to rat embryonic fibroblasts and human skin fibroblasts. *Toxicol Appl Pharmacol,* 133: 20-26.

MAGGIO D, BARABANI M, PIERANDREI M, POLIDORI MC, CATANI M, MECOCCI P (2003) Marked decrease in plasma antioxidants in aged osteoporotic women: results of a cross-sectional study. *J Clin Endocrinol Metab,* 88: 1523-1527.

MARTINIAKOVÁ M, CHOVANCOVÁ H, OMELKA R, GROSSKOPF B, TOMAN R (2011) Effects of a single intraperitoneal administration of cadmium on femoral bone structure in male rats. *Acta Vet Scand,* 53: 1-5.

MATTIX KHJ, GRODSTEIN F, STAMPFER MJ, CURHAN GC (2003) Menopause and postmenopausal hormone use and risk of incident kidney stones. *J Am Soc Nephrol*, 14: 1272-1277.

MEDEIROS DM, PLATTNER A, JENNINGS D, STOECKER B (2002) Bone morphology, strength and density are compromised in iron-deficient rats and exacerbated by calcium restriction. *J Nutr,* 32: 3135-3141.

NOËL L, GUÉRIN T, KOLF-CLAUW M (2004) Subchronic dietary exposure of rats to cadmium alters the metabolism of metals essential to bone health. *Food Chem Toxicol*, 42: 1203-1210.

ORLIC I, BOROVECKI F, SIMIC P, VUKICEVIC S (2007) Gene expression profiling in bone tissue of osteoporotic mice. *Arh Hig Rada Toksikol*, 58: 3-11.

PARFITT AM, DREZNER MK, GLORIEUX FH, KANIS JA, MALLUCHE H, MEUNIER PJ (1987) Bone histomorphometry: standardization of nomenclature, symbols and units. Report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res*, 2: 595-610.

PROZIALECK WC, EDWARDS JR, WOODS JM (2006) The vascular endothelium as a target of cadmium toxicity. *Life Sci,* 79: 1493-1506.

RAISZ L (2005) Pathogenesis of osteoporosis: concepts, conflicts, and prospects. *J Clin Invest,* 115: 3318-3325.

RANA S VS, VERMA S (1996) Protective effects of GSH, vitamin E and selenium on lipid peroxidation in cadmium-fed rats. *Biol Trace Elem Res,* 51: 161-168.

REGUNATHAN A, GLESNE DA, WILSON AK, SONG J, NICOLAE D, FLORES T (2003) Microarray analysis of changes in bone cell gene expression early after cadmium gavage in mice. *Toxicol Appl Pharmacol,* 191: 272-293.

SRIVASTAVA KC, MUSTAFA T (1992) Ginger (Zingiber officinale) in rheumatic and muscoskeletal disorders. *Med Hypoth*, 4: 342-348.

STAESSEN JA, ROELS HA, EMELIANOV D, KUZNETSOVA T, THIJS L, VANGRONSVELD J (1999) Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. *Lancet*, 353: 1140-1144.

STOILOVA I, KRASTANOV A, STOYANOVA A, DENEV P, GARGOVA S (2007) Antioxidant activity of a ginger extract (Zingiber officinale). *Food Chem,* 102: 764-770.

SURVE VV, ANDERSSON N, LEHTO AXTELIUS D, HAKANSON R (2001) Comparison of osteopenia after gastrectomy, ovariectomy and prednisolone treatment in the young female rat. *Acta Orthop Scand*, 72: 525-532.

TURNER CH (2002) Biomechanics of bone: determinants of skeletal fragility and bone quality. *Osteoporos Int,* 13: 97-104.

WAISBERG M, JOSEPH P, HALE B, BEYERSMANN D (2003) Molecular and cellular mechanisms of cadmium carcinogenesis: a review. *Toxicol,* 192: 95-117.

WANG C, BROWN S, BHATTACHARYYA MH (1994) Effect of cadmium on bone calcium and 45Ca in mouse dams on a calcium-deficient diet: evidence of Itai-Itai-like syndrome. *Toxicol Appl Pharmacol,* 127: 320-330.

WARREN S, PATEL S, KAPRON CM (2000) The effect of vitamin E exposure on cadmium toxicity in mouse embryo cells *in vitro*. *Toxicol,* 142: 119-126.

WEBER K, KASCHIG C, ERBEN RG (2004) 1a-Hydroxyvitamin D2 and 1a-hydroxyvitamin D 3 have anabolic effects on cortical bone, but induce intracortical remodeling at toxic doses in ovariectomized rats. *Bone,* 35: 704-710.