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Therapeutic Potential of Opuntia ficus Indica Extract Against Cadmium-Induced Osteoporosis and DNA Bone Damage in Male Rats

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Abstract

The purpose of the present study was to assess the protective effects of *'Opuntia ficus indica'* (family Cactaceae) against osteoporosis induced by cadmium chloride in female Wistar rats. Experiments were carried out on 36 male Wistar rats (6-8 weeks old) divided into four groups of nine each: a control group, a group treated with cadmium (3,5 mg/kg /day) by subcutaneous injection, a group treated with *Opuntia ficus indica* extract (100 mg/Kg/day) by gavage, and a group treated with opuntia extract then treated with cadmium. After 10 weeks of treatment, animals from each group were rapidly sacrificed by decapitation. Blood serum was obtained by centrifugation. Bone toxicity was estimated by examining femoral length and weight, calcium, phosphorus, vitamin D3 and alkaline phsphatase (ALP) levels, oxidative status and DNA aspects in femur tissue. Results showed that cadmium could induce hypocalcemia, hypophosphatemia, Vit D deficiency, increase in ALP level, and decrease in femur weight and length. Also, an oxidative stress evidenced by statistically significant losses in the activities of catalase (CAT), superoxide-dismutase (SOD), glutathione-peroxidase (GPX) activities and an increase in lipids peroxidation level in bone tissue of cadmium-treated group compared with the control group. In addition, histological analysis in bone tissue of cadmium-induced rats revealed pronounced morphological alterations with areas of bone resorption and a loss of normal architecture of femur diaphysis bone as well as DNA fragmentation. However, administration of cactus extract attenuated cadmium-induced bone damage. The protective effect of the plant can be attributed to its antioxidant properties and the existence of phenolic acids and flavonoids, as highlighted by HPLC-based analysis. These findings indicate that *'Opuntia ficus indica'* extract, can be used as a new option in nutraceutical field.

Keywords: cadmium, 'Opuntia ficus indica', osteoporosis, oxidative stress, DNA damage

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1 Introduction

Cadmium (Cd) is a widespread environmental pollutant that is considered as one of the most toxic heavy metals. Due to its unique physico-chemical properties [1], cadmium has several industrial uses, such as paints and electrolysis industry [2], fertilizers and battery manufacturing [3]. Cd can be found in certain drinks, meat, grains as well as in cigarette. Therefore, important sources of Cd intake are via food or cigarette [4]. Nevertheless, prolonged cadmium exposure can induce several injuries, including renal dysfunction, liver damage, and cardiovascular diseases [5] [6] [7].

Bone is a specialized connective tissue, which forms the framework of the body. Femur is the longest bone provides mechanical support, protects bone marrow stromal cells and participates in metabolic homeostasis. Occupational exposure to cadmium has for long been associated with renal tubular cell dysfunction, osteomalacia with osteoporosis, hypercalciuria and renal stone formation. Cadmium may also act directly on bone. Hypercalciuria, which may progress to osteoporosis, has been taken as a sensitive renal-tubular biomarker of a low level of cadmium exposure [8]. Animal studies have shown cadmium to stimulate the formation and activity of osteoclasts, breaking down the collagen matrix in femur bone [9]. Even though the first epidemiological argument for Cd-induced bones effects was the clear-cut interference of low level Cd exposure with Calcium metabolism [10].

Different experimental studies have reported that ROS production is particularly involved in mineral tissue homeostasis and contributes mostly to bone remodeling by promoting bone resorption [11]. However, there are only a few studies that imply oxidative stress as a mechanism for Cd-induced osteotoxicity. In vivo observations suggested that cadmium exerts its toxic effects via oxidative damage to cellular organelles by inducing the generation of (ROS). Reactions of these ROS with cellular biomolecules have been shown to lead to lipid peroxidation, membrane protein damage, altered anti-oxidant system, DNA damage, altered gene expression and apoptosis [12]. If these ROS-mediated stress events are not balanced by repair processes, affected cells undergo apoptosis or necrosis [13].

As oxidative stress is one of the important mechanisms of cadmium-induced damages, it can be expected that the administration of some antioxidants should be an important therapeutic approach [14]. In this context, there has been a growing interest in bioactive compounds due to their side effects (such as liver, kidney, or immune system problems) when used for medicinal purposes [15] [16] [17] . Vitamin (C, D and E) can protect against Cd-induced liver and kidney injury [18] [19]. In addition, numerous studies have shown that natural products, such as green tea catechins, daidzein, quercetin compounds and emodin, are also valuable in protecting against Cd toxicity [14]. However, only a few studies focused on the treatments of Cd-induced osteoporosis. Recently, fullerene C_{60}, virgin olive oil and ginger may be valuable in protecting against Cd induced skeleton damage [20] [21] which suggests that natural products may be also valuable for the treatment of Cd-induced osteoporosis.

'Opuntia ficus indica' is a cactus species widely distributed in Latin America, South Africa, and the Mediterranean area. It is utilized in arid and semi arid zones as a fruit and forage crop [22] .This plant was proved successful in protecting deferent organs against oxidative stress in various experimental models [14]. According to several studies; *'Opuntia ficus indica'* yields high values of important nutrients such as minerals, carotenoids, fatty acids, and essential oil [23]. It is also very rich in vitamins

[24]. This desert plant can be used as an anti-inflammatry, hypoglycemic, antiviral and may protect against numerous chronic diseases, including cancer, and neurological diseases [25][26]. Furthermore, previous studies proved its hepatoprotective, immunoprotective, renal protective and antioxidant capacities against heavy metals toxicity [27][28]. However, thus far, therapeutic effects of *Opuntia ficus indica* extract against Cd-induced skeletal damages have not been investigated. This study evaluates the preventive effects of *Opuntia ficus indica* against skeletal damage induced by cadmium administration in female rats. For this purpose, in this study, animals were treated either with cadmium, or with Opuntia extract, or co-administration of cadmium and *Opuntia ficus indica* extract. Then, levels of serum calcium, magnesium, vitamin D3 phosphorus and alkaline phosphatase (ALP) were measured. Also, the oxidant/antioxidant status of femur tissue was assessed by measuring TBARS level and activities of antioxidant enzymes (SOD, GPX, and CAT). Finally, phytoscreening of the cactus extract was carried out to identify its bioactive compounds.

2 Materials and Methods

2.1 Chemicals

Cadmium chloride (CdCl2) was obtained from Merck (Darmstadt, Germany) and dissolved in sodium chloride (saline, 0.9% NaCl). Ascorbic acid, 2- thiobarbituric acid (TBA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), and aluminum chloride (AlCl3) were purchased from Sigma (Sigma, Aldrich). Gallic acid, rutin, (1)-catechin, and Folin–Ciocalteu reagent were purchased from Merck (Darmstadt, Germany). Commercial diagnostic kits were purchased from Biomaghreb (Tunisia). Gen Elute “Mammalian genomic DNA Miniprep Kit sufficient for 70 purifications” was purchased from Sigma Aldrich. All the other chemicals used were of the highest grade available and obtained from commercial sources

2.2 Preparation of ‘Opuntia ficus indica' extract

Young cactus cladodes of *Opuntia ficus indica* (2-3 weeks old) were collected from the local area of Elguettar, Gafsa City (Tunisia). They were identified by a botanist (Dr Lefi El Kadri) at the Faculty of Sciences of Gafsa, Gafsa University, Tunisia. Cactus cladodes were washed with water, chopped into small pieces (without removing the small leaves and spines), dried at 37°C, and then pressed using a handpress. 4 g of the obtained dry powder was added to 100 ml of distilled water; and centrifuged at 4,000 rotations per minute (rpm) for 15 min at 4 °C to remove any impurities resulting from the extraction process. Opuntia supernatant was stored at −20 °C until use.

2.3 DPPH radical-scavenging activity

The free radical-scavenging activity of cactus cladode aqueous extract was evaluated using the stable radical DPPH, according to the method of Brand- Williams [29]. Tests were conducted in darkness. Briefly, 1.5 ml of DPPH methanolic solution (0.1mM) was incubated with 1.5 ml of aqueous extract of cactus at varying concentrations (50–300 mg/ml) and allowed to stand for 30 min at 27°C. A control sample was prepared without any cactus extract. The absorbance was then measured at 517 nm. DPPH radical-scavenging activity (RSA), expressed as a percentage, was calculated using equation 1.

$$\text{DPPH scavenging effect (\%)} = [(A_{contol} - A_{sample})/A_{control}]\times 100 \quad \text{(Equation 1)}$$

Where:

$A_{control}$ is the absorbance of the control reaction, and
A_{sample} is the absorbance of *Opuntia ficus indica* extract. Ascorbic acid (AA) was used as standard.

2.4 Extraction of phenolic acids and flavonoids

The dried powder of cactus (1 g) was mixed with 10 ml of extraction solution (methanol 80%), agitated for 10 min and then centrifuged at 12000 x g for 5 min. An aliquot of supernatant (0.5 ml) was added to 0.5 ml of

acetone and agitated. The homogenate was then centrifuged (12000 x g for 5 min). A Speed-Vac device was used to dry the homogenate which was then used for HPLC analysis of phenolic acids and flavonoids [30].

2.5 Experimental conditions of high-performance liquid chromatography

Analyses by liquid chromatography were performed using a Varian Prostar HPLC equiped with a ternary pump (model Prostar 230) and a Prostar 330 diode array detector. The HPLC separation of the active compounds was carried out using C-18 reverse phase HPLC column (Varian, 150 mm × 4.6 mm, particle size 5µm). The mobile phase consisted of water: acetic acid (98:2 v/v) (A) and water: acetonitrile: acetic acid (58:40:2 v/v) (B).The elution gradient used was: 0-80% B for 55 min, 80-100% B for 15 min and 100-0% for 5 min. The flow rate was 0.9 ml/min and the injection 175 volume was 20 µl. The identifications were performed at 280 nm for phenolic acids and at 360 nm for flavonoids based on a comparison between the retention time as well as mass spectra of the peaks in the injected extracts and those of HPLC standard compounds.

2.6 Animals and treatments

36 male Wistar rats (6-8 weeks old), about 240 g body weight, were purchased from l'Institut Pasteur deTunis (IPT), Tunisia. They were kept for a two-week adaptation period under the same conditions of temperature (22°C), relative humidity (70±4%), and a dark/light cycle of 12 h. The animals were given standard pellets from SICO, Sfax, Tunisia, and tap water ad libitum. The experimental procedures were carried out according to the general guidelines on the use of living animals in scientific investigations (Council of European Communities, 1986) and approved by the Ethical Committee of Sciences Faculty of Gabes. After the adaptation period, the rats were divided into 4 groups of 9 each.

The treatments were carried out as follows:

Group 1 (C):	received (0.5ml/ 100 g of body weight b.w.) saline solution (NaCl 0.9%) subcutaneously (Control group).
Group 2 (Cd):	rats given cadmium (3.5 mg/kg b.w. daily by subcutaneous injection for 10 weeks) (dissolved in distilled water) and fed on rodent pellets. This concentration was chosen according to previous data [21].
Group 3 (CCE):	rats given '*Opuntia ficus indica*' extract daily via gavages at 100 mg/kg (b.w.) for 10 weeks.
Group 4 (CCE + Cd):	rats given 100 mg/kg (b.w) *Opuntia ficus indica* and then injected with cadmium at a dose of 3.5mg/kg (b.w) daily for 10 weeks.

To investigate the protective activity against cadmium hazards, '*Opuntia ficus indica*', at a dose of 100 mg/kg b.w, was administered to the animals by daily oral gavage. This dose was chosen based on previous reports which proved its efficiency in preventing Zearalenone-induced genotoxicity [22]. The rats were observed for water intake and physical signs of toxicity following treatment, and were weighed daily.

After 10 weeks, rats from each group were rapidly sacrificed by decapitation in order to minimize the handling stress. Blood samples were collected from jugular vein in dried tubes and centrifuged at 1500 g for 15 min at 4 °C. Serum samples were collected for biochemical analyses to determine the TC rate, HDL-chol, LDL-chol and TG. Liver, femurs were also quickly excised, and the surrounding muscles and connective tissues were removed. All femur samples were weighed and the femoral length was measured with a digital clipper. Some of them were intended for histological examination and the others were stored at -80°C for biochemical analysis. 100 mg of bone samples were taken from the femoral region and homogenized with 2 mL of 0.1MTris-HCl buffer (pH 7.2) using mortar and pestle according to Ramajayam et al. [11]. The homogenates were centrifuged at 10,000 tr/min for 30 min, at 4°C and the supernatant was used for biochemical assays.

2.7 Biochemical assays

2.7.1 Lipid peroxidation

The level of lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS), according to Yagi's method [31]. For the assay, 125 µl of supernatant (S1) were mixed with 175 µl of 20% trichloroacetic acid containing 1% butyl-hydroxytoluene and centrifuged (1000 × *g*, 10 min, 4°C). Then, 200 µl of supernatant (S2) was mixed with 40 µl of HCl (0.6 M) and 160 µl of thiobarbituric acid (0.72 mM), and the mixture was heated at 80°C for 10 min. Absorbance was measured at 530 nm. The amount of TBARS was calculated using an extinction coefficient of 156 $mM^{-1}cm^{-1}$ and expressed as nmoles/mg protein.

2.7.2 Superoxide-dismutase (SOD)

The total superoxide-dismutase (SOD) activity was determined by measuring its ability to inhibit the photoreduction of nitrobluetetrazolium (NBT) [32]. One unit of SOD represents the amount inhibiting the photoreduction of NBT by 50%. The activity is expressed as units/mg protein, at 25°C.

2.7.3 Glutathione-peroxidase (GPX)

Glutathione-peroxidase (GPX) activity was assayed using the method of Flohe and Gunzler [33]. The activity at 25°C was expressed as µmoles of GSH oxidized/min/g protein.

2.7.4 Catalase (CAT) activity

Catalase (CAT) activity was measured according to the method of Aebi [34]. The reaction mixture (1 ml) contained 100 mM phosphate buffer (pH = 7), 100 mM H_2O_2, and 20 µl (approximately 1-1.5 mg of protein) of liver. H_2O_2 decomposition was determined at 25°C by measuring the decrease in absorbance at 240 nm for 1 min. The enzyme activity was calculated using an extinction coefficient of 0.043 $mM^{-1}cm^{-1}$ and expressed in international units (I.U.), in µmoles H_2O_2 destroyed/min/mg protein.

2.7.5 Biochemical parameters

Protein content in tissue extracts was determined according to Lowry's method [35]. using bovine serum albumin as standard. Then, serum calcium, phosphorous, vitamin D_3, magnesium and alkaline phosphatase (ALP), levels were determined using specific kits (Spinreact).

2.8 Histological evaluation

The femurs intended for histological examination were taken and immediately sectioned at the midshaft of the diaphysis, then the segments were demineralized for 72 h in acetic acid (1.7 $molL^{-1}$) in accordance with Talbot et al. [36]. After that, they were fixed for 48 h in 10% formalin solution, embedded in paraffin, serially sectioned at 5 mm, and stained with hematoxyline-eosine for light microscopy examination [37].

2.9 DNA fragmentation by agarose gel electrophoresis

The modified phenol-chloroform method [38] was used for the extraction of bone DNA tissue. The bone tissue (200 mg) was lysed with a chaotropic saltcontaining buffer to ensure denaturation of macromolecules. DNA is bound to the spin column membrane and the remaining lysate is removed by centrifugation. A filtration column is used to remove cell debris, after washing to remove contaminants; the DNA is eluted with buffer into a collection tube. The pellet was rinsed with 70% ethanol, dried at room temperature for 2 h and resuspended in 200 lL of TE (20 mM Tris-HCl pH 8. 0.1 mM EDTA). Loading buffer was added to 10 lg of DNA for each treatment, and the samples were analyzed by electrophoresis on a 0.8% agarose gel (1 h at 80 V/ 30 mA) with a TBE running buffer (44 mM Tris-HCl,44 mM boric acid, 50 mM EDTA, pH 8). DNA was visualized by UV exposure after staining with ethidium bromide.

2.10 Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical significance was calculated using a one-way analysis of variance (ANOVA), followed by a Tukey post-hoc test. $P < 0.05$ was considered statistically significant.

3 Results and Discussion

3.1 Free radical-scavenging activity of CCE

Free radical scavenging activities of the *Opuntia ficus indica* cladode extract were measured using the DPPH assay (Figure 1). These activities increase up to 300 µg/ml in a dose-dependent manner. The effective concentration (EC50) of the *Opuntia ficus indica* extract was 100± 0.73 µg/ml. It was significantly lower ($p < 0.05$) than the ascorbic acid activity (70±0.35 µg/ml).

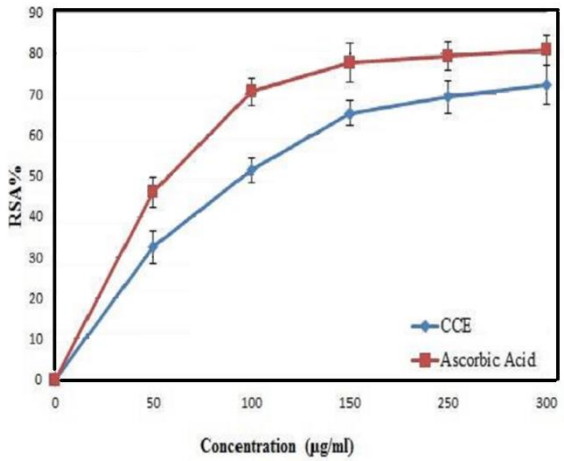

Figure 1. Free radical scavenging activities of the Opuntia ficus indica

3.2 HPLC analysis of cactus extracts

The HPLC analysis of cactus cladode extract (CCE) revealed the presence of phenolic acids and flavonoids (Figure 2; Figure 3). The compounds that were identified in the CCE were seven phenolic acids: gallic acid, catechic acid, epicatechic acid, vanillic acid, cinamic acid, coumarin and resveratrol with retention times of 6.820 min, 11.100 min, 22.345 min, 24.985 min, 28.1 min, 31.875 min and 36.2 min, respectively. The HPLC elution profile of flavonoids showed five main flavonoids: rutin, quercetin, kaempferol , apigenin, and lutiolin with retention times of 23.845 min, 25.512 min, 29.280 min, 34.742 min and 36.461 min, respectively.

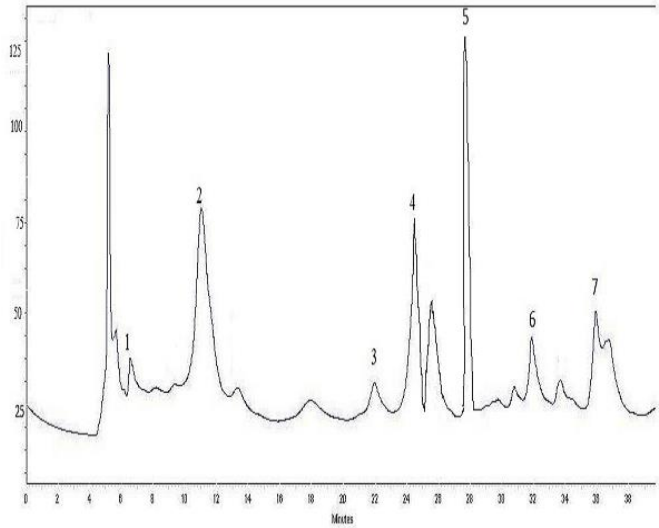

Figure 2. The HPLC analysis of cactus cladode extract (CCE)

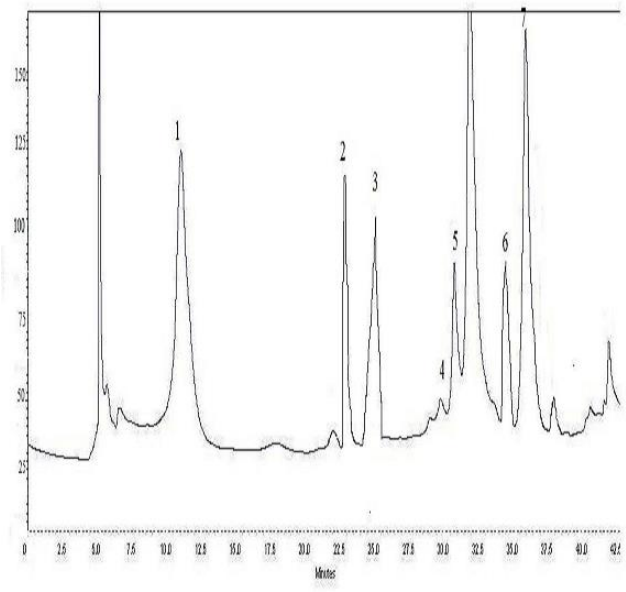

Figure 3. The HPLC analysis of cactus cladode extract (CCE)

Table 1. Body weight, femur weight, and length of rats.

Parameters studied	C	Cd	CCE	CCE+Cd
Initial body weight(g)	240.69±21.3	240.23±19.1	236.72 ±19.5	238.5±15.85
Final body weight (g)	361.92± 10.5	267.7 ± 11.70*	372.12±20.12++	337.75 ±18.42++
Weight of femur (g)	1.03±0.026	0.81±0.014*	1.02 ±0.02+	0.98±0.021+
Length of femur (cm)	3.18±0.071	3.01±0.054**	3.2±0.08++	3.10±0.12++

Values are the mean of 9 measurements ±SD.
*P < 0.05, **P < 0.01 compared with control group (C).
+P < 0.05, ++P < 0.01 compared with cadmium-treated group (Cd).

Table 2. Biochemical findings in control, Cd, CCE, and CCE +Cd groups of rats

Group	C	Cd	CCE	CCE +Cd
Calcium (mg/dl)	10.96±0.67	8.21±0.45**	11.12±0.74++	10.12±0.32++
Phosphorus (mg/dl)	9.63±0.38	6.25±0.61*	9.01±0.47++	8.75±0.73+
Magnesium (mg/dl)	3.1±0.58	3.03±0.24*	2.94±0.61+	2.97±0.42++
Vitamin D3 (Ug/l)	22.98±0.95	15.32±0.87**	24.15±0.52++	19.30±0.48++
ALP (U/l)	536.32±12.2	687.35±21.6**	512.36±26.1++	542.31±17++

Values are the mean of 9 measurements ±SD.
*P < 0.05, **P < 0.01 compared with control group (C).
+P < 0.05, ++P < 0.01 compared with cadmium-treated group (Cd)

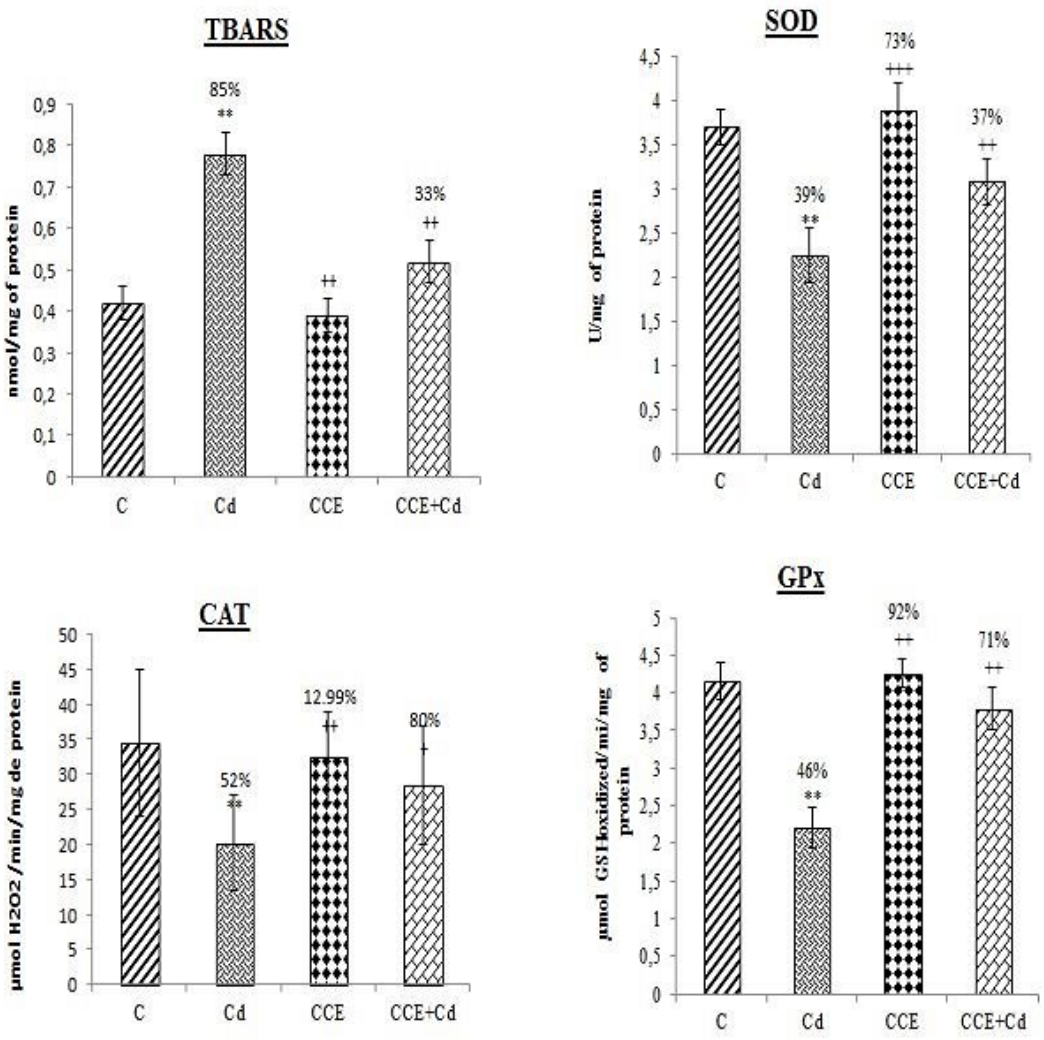

Figure 4. Activities of the SOD, CAT, and GPX enzymes that protect against oxidative stress

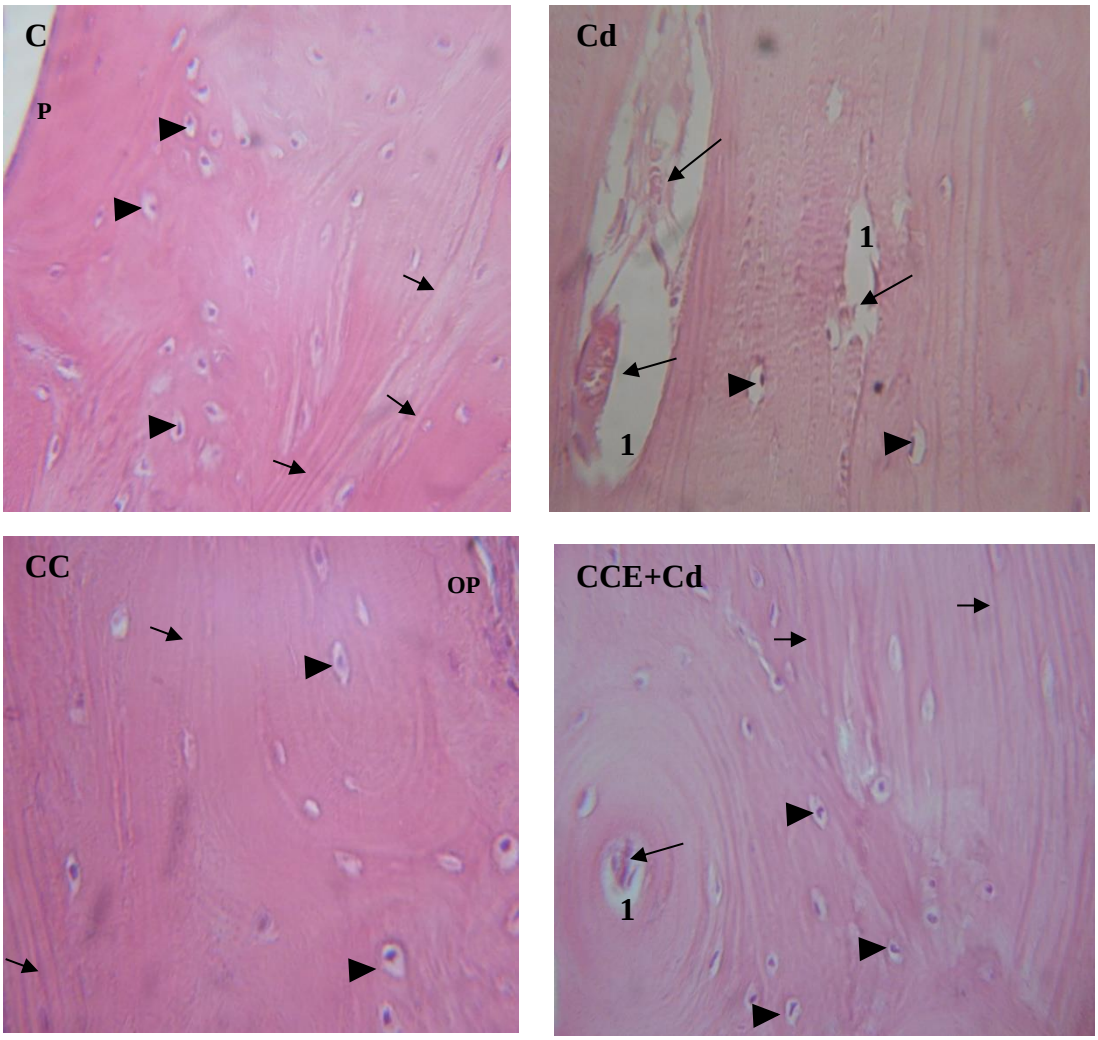

Figure 5. Examination of femur diaphysis section

3.3 Effects of treatments on body weight, femur weight and length

The results presented in Table 1 show that the total body weight, femur weight, and length were decreased by 26%, 21%, and 5%, respectively, in the Cd-treated group compared to the control group. However, the administration of CCE significantly ($P<0.01$) increased the body weight of rats compared with the cadmium-treated group. In contrast, CCE alone did not induce any change.

3.4 Biochemical assays

The exposure of rats to 3.5mg/kg of cadmium induced a significant decrease ($P<0.01$) in calcium (25%), phosphorus (35%), and vitamin D3 (33%) levels compared to control, and a significant increase ($P<0.01$) of ALP (28%), serum level compared to control, cadmium did not induce any change in the magnesium level (Table 2). Also, the results showed indicated that the pretreatment with CCE in combination with cadmium restored all these biomarkers to almost normal values.

3.5 Effects on lipid peroxidation

Lipid peroxidation expressed as TBARS increased significantly ($P \leq 0.05$) by 85% in rats exposed to cadmium compared to the control rats (Figure 4). However, CCE inhibited this effect.

3.6 Effects on antioxidant enzymes

Activities of the SOD, CAT, and GPX enzymes that protect against oxidative stress,

were reduced by -39%, -52%, and -46%, respectively, in the femurs of the Cd-treated group, as compared to the control group (C) (Figure 4). Treatment with *Opuntia ficus indica* cladode (100 mg/kg BW) extract alone did not affect this enzymatic activity. However, when combined with cadmium, all antioxidant enzyme levels were restored to their control values.

3.7 Histopathological findings

Examination of femur diaphysis section in the control group (C) revealed a periosteum and a cortical bone shell. The cortical bone was made up of compact bone consisting of outer, inner, and interstitial bone lamellae containing osteocytes with densely stained oval nuclei. The cortical bone showed sub-periosteal bone deposition appearing as a distinct dark line (Figure 5C). However, compared to the control group (Figure 5 Cd), the administration with a single dose of 3.5 mg/kg cadmium for 10 weeks revealed (1) loss of normal architecture of femur diaphysis bone; (2) areas of bone resorption; (3) cavitation with the presence of osteoclasts inside bone cavities (howship lacunaes); (4) irregularities in the surface of the cortical bone; and (5) few distinct lines of sub-periosteal bone deposition.

The group that was treated with a combination of CCE group showed normal compact bone architecture and density. Few sites of bone resorption were observed, with few numbers of osteoclasts and the presence of distinct lines of bone deposition compared to the Cd-treated group (Figure 5 (CCE+Cd)). Also, the cactus extract alone had no influence on the bone morphology (Figure 5 CCE).

3.8 Effect of treatments on bone DNA aspect

Results of femur diapyisis DNA damage are illustrated in Figure 6. It was noticed that the Cd-treated group showed marked increases in the DNA fragmentation (Figure 6, lane 2). On the other hand, simultaneous treatment of rats with cadmium and the *Opuntia ficus indica* extract showed moderate improvement in the DNA damage (lane 3). The DNA isolated from the control samples (lane 1) and the cactus extract (lane 4) group showed no specific differences.

Plants have been the primary source of medicines since life on earth. In lasts years, the use of medicinal plants has significantly increased in developed nations, due to their potential effects against environmental toxic compounds and several diseases [39]. In this line, our investigation was carried out to explore the protective effect of CCE on cadmium-induced bone toxicity and oxidative stress in adult female Wistar rats. Results showed that cadmium induced a significant decrease in the body weight, femur weight and length. This could be explained either by a slight decrease of feed intake by animals as reported by Mohamed al. [21] or by malabsorption of nutrients from the gastrointestinal tract f Cd-treated rats as reported by [40]. Similar results were observed by Chen et al. [41] which indicated a decrease in the body weight of Sprague Dawley rats that were subcutaneously injected with a dosage of 5 mg/kg of Cd. Other finding also demonstrated a significant decrease in the body weight of rabbits that were administered a dosage of 5 mg/kg of oral Cd for one month [42] [43]. The reduction in femur weight and length in the Cd-treated group observed could be due to the direct action of cadmium on several body mechanisms specially bone growth [44].

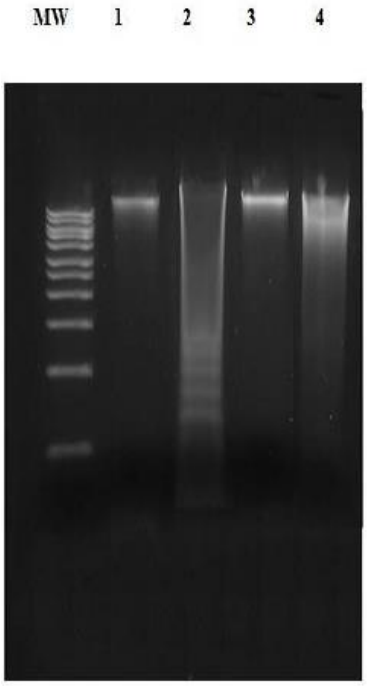

Figure 6. Femur diapyisis DNA damage

It is well known that the bone is a composite material consisting of collagen fibers and hydroxyapatite crystals containing inorganic components mainly calcium and phosphate. It is made up of different cell types: osteoblasts, (bone-forming cells) osteoclasts (bone-resorption cells), osteocytes, and bone lining cells. This is a dynamic tissue undergoing constant remodeling throughout life. As a result of its functionality of bone, several metals accumulate in bone [45]. In the current study, decreases in calcium, phosphorus and vitamin D3 levels, and an increase of ALP level compared to controls, were recorded. Animal studies have shown that long term Cd exposure induces an imbalance in the bone remodeling process, altering both formation and resorption and leading to the development of different bone pathologies [45]. Indeed, the first epidemiological argument for Cd-induced bone effects were the clear-cut interference of low level Cd exposure with calcium metabolism [46]. Earlier studies demonstrated that cadmium intoxication causes renal failure and osteoporosis and increases fracture risk [47, 48]. In fact, Cd has both mechanism of influencing the bone turnover [49]: it acts directly on bone cells through impairment of calcium homeostasis (compound responsible of mineralization process) and bone cell metabolism [50] and indirectly through kidney failure, increasing calcium and phosphorus excretion, and decreasing vitamin D synthesis. This insufficiency of vitamin D delays calcium uptake in the duodenum and active calcium reabsorption on the distal convoluted tubule and hence also bone loss [51]. Cd exposure may inhibit the production of 1, 25(OH) 2D, which subsequently diminish the calcium uptake in intestine [52]. In vivo studies in experimental animals have shown that chronic exposure to Cd decreases mineralization of vertebral bodies, altering their biomechanical properties and rendering them more susceptible to deformity and fracture [53] It is also well documented that Cd decreases expression of markers of osteoblastic differentiation (Runx2, osteocalcin), of extracellular bone matrix proteins (type I collagen), and of enzymes involved in the mineralization process (alkaline phosphatase-ALP) [52]. altering the bone formation and mineralization process.

The present work clearly demonstrates that chronic cadmium exposure increases lipid peroxidation level (TBARS), weakens the antioxidant defense mechanisms (SOD, CAT and GPx) leading to biochemical disturbances. In fact, Lipid peroxidation is one of the main manifestations of oxidative damage. Overproduction of reactive oxygen speacies (ROS) leads to increase lipid peroxidation and oxidative stress which play an important role in the toxicity of many xenobiotics [54]. ROS are involved in bone resorption with a direct contribution of osteoclast proliferation and simultaneously decreasing osteoblast activity [55]. Previous studies, in agreement with the present one, have clearly demonstrated that chronic cadmium exposure increases lipid peroxidation level and suppresses the antioxidant defence mechanisms in bone tissue. These results suggested that long term cadmium exposure induce an imbalance in the bone remodeling process, altering both formation and resorption and leading to the development of different bone pathologies [45]. In vivo experimental studies by Brzoska and colleagues [12] showed that Cd (5 or 50 mg Cd/L), when fed to male Wistar rats in drinking water for six months, weakened the antioxidative capacity of the bone tissue and led to oxidative stress. There was increased lipid peroxidation and H2O2 production as well as decreased activities of GPx, SOD and CAT. The accumulated ROS and oxidised lipids may affect the metabolism of bone tissue and these Cd-induced changes in the bone oxidative/antioxidative status can induce disorders in the bone marrow turnover leading to bone loss, such as osteoporosis and osteomalacia. . Smith et al. [56] proved in vitro in an osteosarcoma cell line, Saos-2, using 5–50 μM CdCl2 for 3–48 h that Cd-induced oxidative damage led to a decrease in RUNX2 expression resulting in osteoblast apoptosis suggesting RUNX2's anti-apoptotic role in osteoblasts. RUNX2 is an osteoblast transcription factor, which is known to play a protective role against osteoporosis in postmenopausal women.

Bone microstructure is an important factor to understand the mechanism of bone fragility. In our study, histological observations showed that treatment with cadmium can induce bone microstructure damage. In fact, the

results revealed: cortical bone resorption, fibrous osteodystrophy, decreased bone deposition, and an increase in the number of osteoclast cells compared to control. These findings indicated osteoporotic changes and micro-architectural deterioration of bone structure. Osteoporosis induction is also confirmed by the bone DNA aspect. In fact , in increase in TBARS level may alter the cellular membrane structure and then block cellular metabolism [54]. These factors may explain the negative effects of cadmium on femur bone. Besides membrane effects, lipid peroxidation can damage DNA and protein, which ultimately leads to bone degradation [43]. In this context, numerous reports investigated the effect of cadmium on bone properties and found that cadmium is a risk factor for the development of osteoporosis even at low doses by altering compact bone microstructure [51] [21].

Thus, femur of animals treated with cadmium showed DNA bone tissue lesions which are reported to be the consequence of cadmim-induced osteoporosis [55]. These findings are in agreement with those reported in an earlier study which proved that cadmium treatment modulated gene expression and signal transduction, reduced activities of proteins involved in antioxidant defenses [20]. Some other authors have demonstrated that Cd induced apoptic DNA lesions potentially leading to tumorigenesis [57].

On the other hand, the second part of this study was devoted to evaluation of the capacity of CCE to protect against cadmium-induced osteoporosis. To investigate the protective activity against health hazards of cadmium, '*Opuntia ficus indica'* extract at a dose of 100 mg/kg b.w, was administered to the animals by daily oral gavage. Thus, simultaneous administration of CCE (100 mg/kg of b.w.) to cadmium-treated animals partly or entirely neutralized the adverse effects of cadmium in this study. CCE ability to prevent oxidative damage induced by cadmium is certainly associated with the presence of several antioxidant compounds. It is well-known that phenolic substances found in CCE exhibit considerable free radical-scavenging activities by virtue of their reactivity as hydrogen- or electron-donating agents, as well as metal ion-chelating properties, preventing metal-induced free radical formation [58] [59]. As shown in Fig 1, the CCE was able to effectively reduce the stable free radical DPPH. These results suggested that the presence of phenolic compounds in CCE might be the main cause of their considerable radical-scavenging activity. These antioxidant properties could render CCE an excellent plant to protect cadmium-induced toxicity in vivo. This action could be explained by the ability of CCE to reduce the TBARS level in cell membrane by scavenging free radicals induced by cadmium. In accordance, previous investigations have shown that CCE is capable of protecting tissues against oxidative stress [60]. The administration of CCE had a potent protective effect on oxidative cadmium-induced damage in rats, as revealed by a significant increase in hepatic CAT, SOD and GPx activities. Several studies using the same extract showed its hepatoprotective, immunoprotective and renal protective effects against oxidative stress [27] [28] [61].

Also, the beneficial effect of CCE could be explained by the antioxidant capacity of its constituents. As can be seen in Figures 2 and 3, the HPLC analysis revealed the presence of five flavonoids (rutin, quercetin, kaempferol , apigenin, and lutiolin) and seven phenolic acids (gallic acid, catechic acid, epicatechic acid, vanillic acid, cinamic acid, coumarin and resveratrol), which are known to have beneficial effects as their responsibility in preventing the formation of reactive oxygen species [62]. Previous study indicated that the antioxidant properties of CCE are mainly due to vitamins and flavonoids, more particularly vitamin C and quercetin that has been reported to be a highly efficient radical scavenger [63].

The attenuation of femur damage was confirmed by DNA examination (Figure 6). CCE attenuated the histological alterations induced in cadmium-treated rats, which could be associated with CCE antiradical/antioxidant and metal-chelating capacities. This result is in consistent with study of Brahmi et al. [60], which showed that administration of CCE had an anti-genotoxic effect resulting in an efficient prevention of chromosomal aberrations and DNA fragmentation against cisplatin induced oxidative stress, genotoxicity and apoptosis in balb/c mice.

4 Conclusions

In conclusion, this report has proven that the present work, the CCE was found to possess excellent antioxidant activities based on various in vitro and in vivo assays. The different in vitro antioxidant tests proved that the CCE is rich in flavonoids, phenolic compounds as well as polysaccharide. Also, this study demonstrated that CCE could have a protective effect on cadmium-induced osteoporotic damages and oxidative stress in our experimental model. Future research is needed to carry out further biochemical investigations in order to isolate and clarify the mechanism behind the activity of this extract.

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6 Conflicts of Interest

The authors declare no conflict of interests. None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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