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Protective Effect of Zinc, Selenium, Vitamin C, E and Epicatechine on Cadmium–Induced Toxicity and Disturbances in the Kidney, Liver, Bone, Lipid Metabolism and Oxidative Stress in Rats.

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ABSTRACT

The aim of the present study is to investigate the protective effect of zinc (6.13 mg /kg bw), selenium (0.4 mg /kg bw), vitamin C (100mg /kg bw), vitamin E (40 mg /kg bw) and epicatechine (4.5 mg /kg bw) on cadmium – exposed rats (1.23 mg /kg bw), 5 times per week for 30 days. Zn^{+2} significantly decreased the elevated level of urea in serum, but creatinine has still been at a higher level. Serum renal markers, β_2 microglobulin (β2 MG) concentration and the activities of N-acetyl –β-D glucosaminidase (NAG) and alanine aminopeptidase (AAP) were significantly decreased by cadmium and did not return to the normal level when using the antioxidants.While epicatechine increased the NAG activity again. Bone resorption marker, C-telopeptide of collagen-alpha-(1) chain (CTX-1) was increased by Cd and declined again by Zn^{+2} and all antioxidants except Se. The oxidative stress biomarkers, serum glutathione peroxidase (GPx) activity was decreased by cadmium and improved by all protective factors in the present study, while the elevated level of malonaldehyde (MDA) was ameliorated by Zn only. The protective factors, except vitamin C, decreased the elevated level of AST, in cadmium-induced toxicity. In addition, Se, vitamins C and E improved the lowered level of albumin. The used protective antioxidants improved the elevated levels of cholesterol, LDL-C and triglycerides in serum occurred by cadmium toxicity. Our results suggest that Zn^{+2} and some antioxidants may protect against cadmium-induced toxicity.

Keywords: Cadmium; zinc; antioxidants; bone resorption; nephrotoxicity; hepatotoxicity.

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INTRODUCTION

Cadmium metal has specific properties that make it suitable for a wide variety of industrial applications [1]. In the earth's crust, cadmium appears mainly in association with ores containing zinc, lead and copper in the form of complex oxides, sulfides and carbonates [2]. Exposure to cadmium occurs through intake of contaminated food, water, or by inhalation of polluted air [3]. Occupational exposures are found in many industries such as electroplating, welding, smelting, pigment production, cement production and battery manufacturing. Other respiratory exposure to cadmium can occur through inhalation of cigarette, car or fuel oil smoke [4] especially in big cities.

Cadmium concentrations in tested Nile Delta–ground water samples from industrial areas, Hilwan and El-Tebbin , were considerably high (0.01 - 0.038 mg /L) [5] compared with the permissible cadmium content in drinking water standards (0.003 mg / L) [6].The cadmium levels in Cairo City air ranged between 0.01-2 $\mu g/m^3$ with an average of 0.05 $\mu g/m^3$ [7] while the measurement data from northern Europe for the period 1980-1988 were reported as being around 0.1 ng/m^3 in remote areas ,0.1-0.5ng/ m^3 in rural area , 1-10 ng/m^3 in urban areas and 1-20 ng/m^3 in industrial areas[8] . Celery, parsley and spearmint, cultivated in Egypt Nile valley, contained the highest mean level of cadmium (2.44 µg /g) [9]. In addition, barely grain, potato, spinach, green bean and pea contained 0.012, 0.32, 3.9, 0.4 and 0.043 mg/kg dry weight cadmium [10]. The average concentrations of cadmium in most food must be less than 0.02 µg /g [11].

Cadmium is a potent carcinogenic environmental pollutant that has been lastly linked to breast cancer [12], pancreatic cancer [13], carcinomas in the lung [14], sarcomas [15] and testicular tumors [14]. Northeast Nile Delta region exhibits a high incidence of early – onset pancreatic cancer. It is well documented that this region has one of the highest levels of cadmium pollution in Egypt [13].

Cadmium has been reported to induce nephrotoxicity [16], hepatotoxicity [17] ,cytotoxicity [18], mortality [19], teratogenesis [20], foetal toxicity ([21], testicular toxicity [22], bone lesions (osteoporosis and osteomalacia) [23], oxidative stress [24], and disturbances in lipid metabolism [25].

Cadmium has an indirect role in the generation of various free radicals [26]. Antioxidants such as vitamin C [27], vitamin E [28], epicatechine [29] and selenium [30], in addition to zinc [31] are important for preventing the damage caused by reactive oxygen species or cadmium toxicity [26].

Thus, our objective in this study was aimed to investigate the protective effect of Zn, Se, vitamins C, E and epicatechine on cadmium–induced toxicity in the kidney, liver, bone as well as alteration in lipid metabolism and oxidative stress.

MATERIALS AND METHODS

Chemicals

All chemicals, solvents and reagents were of analytical grade purity (Sigma, St. Louis, Mo, USA).

Animals

A total of 35 male albino rats, (Spraque Dawely strain), weighing from 100 to 120g were obtained from Helwan Station for Experimental Animals, Helwan, Cairo, Egypt. The animals were housed in stainless steel cages and raised in the animal house of Biochemistry Department, Faculty of Agriculture, Cairo University. The rats were kept under normal healthy laboratory conditions (temperature was adjusted at 22 $\pm$ 2^{o}C with humidity of 50 $\pm$ 10% in July and 12-hour light-dark cycle). The animals were adapted on free access of re-distilled water and fed basal diet for two weeks before the initiation of the experiments. The protocol conforms to the guidelines of the National Institute of Health (NIH).

Diets

All animals were fed on a standard diet containing vitamin and salt mixtures that were recommended by [33] and [34], respectively.

Experimental procedure

The experiment continued for 30 days and at the end of experiment, the animals were fed-deprived for 12 hours. Rats were killed by decapitation and the blood sample of each rat was collected in dry clean centrifuge glass tube and was centrifuged at 3000 rpm for 15 min. to separate the serum. The clear-non haemolyzed serum was then pipetted into epindorff tubes and stored at –20^{o}C until biochemical determination of urea [35], creatinine [36], N-acetyl-β-D-glucosaminidase activity **(**NAG) [37], beta-2-microglobulin [38], alanine aminopeptidase activity (AAP) [39], C-telopeptide of collagen alpha-1(I) chain(Ctx) [40], malondialdehyde [41], glutathione peroxidase activity [42], albumin [43], total protein [44], aspartate aminotransferase activity (AST), alanine aminotransferase activity (ALT) [45], alkaline phosphatase activity(ALP) [46], gamma glutamyl transferase activity [47], total cholesterol [48], triglycerides [49], HDL-cholesterol [50] and LDL-cholesterol [51].

Experimental design

Thirty rats in 6 groups (5 in each group) were subcutaneously injected with Cd^{+2} solution (1.23 mg /kg bw) as cadmium chloride (13% of LD_{50}, 15.2 mg $CdCl_2$/kg of albino rats) [52] five times every week. The groups received cadmium chloride were subcutaneously injected in the same time and volume with (1) 6.13 mg Zn^{+2}/kg bw as zinc chloride solution, (2) 0.4 mg/kg bw sodium selenite solution, (3) orally administered 100 mg/kg bw vitamin C, (4) 40 mg/kg bw vitamin E (as α- tocopherol acetate), (5) 4.5 mg /kg bw epicatechine, (6) saline solution (positive control). The total volume injected or orally administered by gavage for each rat did not exceed 0.5 ml once a day. Negative control group (5 rats) was also performed in the same time. The administered Zn^{+2} was 5-fold of Cd^{+2} injected [53].

Statistical analysis

All the data were expressed as mean ± SE .Differences among the experimental groups were assessed by one way ANOVA followed by protected least significant difference Fisher's test.

RESULTS

Protective effect of Zn, Se and antioxidants on Cd-induced toxicity in the kidney

Table (1) shows the effect of Cd^{+2} and some antioxidants on serum urea levels. These data illustrate that the urea concentration in serum was significantly increased by 47.6% by injection of Cd^{+2}. Administration of both $ZnCl_2$ and $CdCl_2$ improved the renal tubular filtration and decreased the serum level of urea to the normal level. In addition injection of Cd^{+2} into experimental rats 3-fold increased serum creatinine value and it was still higher even after administration of zinc chloride or the antioxidants.

Data in Table (1) show also that cadmium administration significantly decreased serum NAG activity in rats by 17.6% and the concentration of β2-MG in serum was also decreased from 2.25 to 1.38 µg /ml that may be due to severe damage of nephrons .The same trend was observed in alanine aminopeptidase (AAP) activity in serum of rats.The activity of serum alanine aminopeptidase was significantly decreased by 49.9 %. Zinc and antioxidants did not restore the enzymes activities in serum or β_2-MG concentration to the normal level except epicatechine which increased NAG activity again.

Protective effect of Zn, Se and antioxidants on Cd-induced damage in the bone

Carboxy-terminal cross –linking telopeptide of type 1 collagen (CTX-1) in serum was measured as bone resorption marker in rat subcutaneously injected with cadmium chloride. Clear evidence for bone resorption in cadmium –treated rats was illustrated in the present data. CTX-1 was slightly increased (19.3%), it means that the bone resorption happened by Cd^{+2}. Selenium did not play any effective role in bone resorption but Zn^{+2}, and organic antioxidants did.

Protective effect of Zn, Se and antioxidants on Cd –induced oxidative stress

Exposure to cadmium may affect on the antioxidant defense system of red blood cells and lipid peroxides concentration in blood, therefore the oxidative stress of cadmium as well as the possible protective roles of zinc, selenium, vitamins C, E and epicatechine were studied. Cadmium significantly increased serum malonaldehyde (MDA), as a measurement of lipid peroxidation , from 2.43 to 3.11 µmol /l. Zinc had a protective role against cadmium –induced lipid peroxidation and restored the serum malonaldehyde level to the normal value ,but the other antioxidants did not. From the present data, it was shown that exposure to cadmium induced a significant decrease in glutathione peroxidase (GPX) activities in blood. Zn, vitamins C, E and epicatechine, had benefical effects on Cd-induced decrease in GPX activities .Selenium improved the GPX activity.

Protective effect of Zn, Se and antioxidants on Cd-induced toxicity in the liver

In the present study, the investigators estimated, in a rat model of moderate chronic human exposure to cadmium, whether zinc, selenium vitamins C, E and epicatechine consumption may prevent Cd-induced liver injury. For this purpose, the hepatic functions of the rats that received Cd^{+2} and specific concentrations of antioxidants were evaluated. Table 1 shows the decreased serum albumin level by the exposure to Cd. In addition, Se improved albumin levels in serum, but the concentration of serum albumin was not returned to the normal value. Vitamin C also restored the decreased serum albumin to the normal level. Selenite anion, zinc cation, antioxidant vitamins C, E and epicatechine, as a polyphenol, significantly decreased the elevated level of serum aspartate aminotransferase activity (AST). In addition, the elevated serum alanine aminotransferase activity (ALT) was not influenced by the previous protective factors. Very little increase was observed in serum alkaline phosphatase activity after injection of cadmium with protective antioxidants and Zn. Gamma glutamyl transferase (GGT) activity in serum is a good marker for liver function test, therefore it was measured in cadmium–induced liver injury, in experimental rat model, to evaluate the protective effect of some organic and inorganic antioxidants, as shown in Table 1. The present data indicate that exposure of rats to cadmium slightly, increased GGT activity in serum. The concentrations used of epicatechine, vitamins C, E, Se and Zn in rats administered 1.23 mg Cd/kg bw severely decreased the serum GGT activity to be below the control value.

Protective effect of Zn, Se and antioxidants on Cd-induced alteration of lipid metabolism

The hepatotoxicity and oxidative stress in chronic exposure to cadmium in human result in great attentions in lipid metabolism [54]. Therefore, a rat model of human exposure to cadmium was used to study lipid metabolism in the presence of many supplementations (Zn^{+2}, selenite, vitamins C, E and epicatechine) which may prevent Cd-induced alteration. Thereby, the serum level of cholesterol, triglycerides, high-density lipoprotein –cholesterol (HDL-C) and low- density lipoprotein –cholesterol (LDL-C) were measured in rats received both cadmium and antioxidants. Data in Table 1 show the elevated levels of serum cholesterol by injection of cadmium into rats .The serum cholesterol level were increased by 50.7 % and the administered Zn and antioxidants restored the elevated cholesterol level in serum to the normal value. Serum triglycerides were 50%, increased by administration of Cd^{+2}. Doses of antioxidants significantly decreased the elevated value of triglycerides in serum. High-density lipoprotein cholesterol (HDL-C) in serum was not differed than the control value, while cadmium blus Zn or vitamin C significantly increased serum HDL-C level. The present data illustrate also that cadmium significantly induced serum LDL-C and the bioelement and antioxidants used in the present study lowered the elevated level of LDL-C.

Discussion

As the risks of cadmium –induced kidney disease have become increasingly apparent, much attention has been focused on the development and use of sensitive biomarkers of Cd nephrotoxicity. In kidney injury, several damages have been occurred in renal tubule leading to excretion of some proteinous molecules in urine such as N-acetyl-β-D-glucosaminidase (NAG), β_2-macroglobulin (β2-MG), alanine aminopeptidase (AAP) and retinol binding protein (RBP). Therefore, the presence of high concentrations of these protein molecules in urine is good biomarkers for nephrotoxicity [55]. Unfortunately, there are many difficulties in collection of 24-hour- urinary sample, variation of urinary creatinine levels depending upon daily dietary protein and muscular dystrophy and complexity of determination of these biomarkers in urin. Care in collection of urinary samples is

essential and depending on which test is to be performed. Different buffers may be incorporated in each urinary test [56]. The fall in glomerular filtration rate and the increase urinary protein biomarker may result in decreasing of this biomarker in blood, therefore determination of these biomarkers in blood may be useful. In the present study, the proteinous biomarker of cadmium–induced renal dysfunction was measured in serum of experimental rats.

Table 1: Serum urea, creatinine, NAG, β-2-MG, AAP, CTX1, MDA, GPx, albumin, total protein, ALT, AST, ALP, GGT, total cholesterol, TG, HDL-C and LDL-C in rats subcutaneously injected with $CdCl_2$ in combination with $ZnCl_2$, Na selenite, vit C, vit E and epicatechine, five times at week for 30 days.

Parameter	Control	Cd^{+2} (1.23 mg/kg bw)						
		Saline solution	Zn^{+2} 6.13mg/kg Bw	Se 0.4mg/kg bw	Vitamin C 100mg/kg bw	Vitamin E 40mg/kg bw	Epicatechine 4.5mg/kg Bw	LSD
Urea(mg/dl)	31.12 ± 1.86 ^{c}	45.92 ± 2.75 ^{b}	33.34 ± 0.38 ^{c}	57.39 ± 2.5 ^{a}	49.77 ± 1.73 ^{b}	50.43 ± 0.17 ^{ab}	49.73 ± 4.8 ^{b}	6.99 ***
Creatinine (mg/dl)	0.74 ± 0.20 ^{c}	2.21 ± 0.16 ^{ab}	2.08 ± 0.11 ^{ab}	1.75 ± 0.18 ^{b}	2.49 ± 0.32 ^{a}	2.17 ± 0.25 ^{ab}	1.83 ± 0.28 ^{b}	0.64
NAG(U/L)	9.85 ± 0.21 ^{a}	8.17 ± 0.2 ^{b}	6.77 ± 0.001 ^{d}	7.29 ± 0.05 ^{cd}	8.32 ± 0.46 ^{b}	7.87 ± 0.03 ^{bc}	9.56 ± 0.47 ^{a}	0.77 ***
B-2-MG (µg/ml)	2.25 ± 0.34 ^{a}	1.38 ± 0.01 ^{b}	1.48 ± 0.002 ^{b}	1.40 ± 0.01 ^{b}	1.58 ± 0.03 ^{b}	1.56 ± 0.05 ^{b}	1.75 ± 0.09 ^{b}	0.38 ***
AAP(U/L)	58 ±7.36 ^{a}	20.5 ± 0.64 ^{bc}	18.13 ± 2.1 ^{b}	15.8 ± 0.74 ^{b}	23.2 ± 0.02 ^{bc}	28 ± 0.5 ^{c}	28 ± 0.08 ^{c}	8.2 ***
CTX1 (ng/ml)	60.6 ±1.90 ^{b}	72.3 ± 4.75 ^{ab}	22.65 ± 1.24 ^{d}	76.92 ± 1.34 ^{a}	35.74 ± 7.20 ^{c}	23.31 ± 5.38 ^{d}	30.30 ± 4.03 ^{cd}	11.5 ***
MDA (µ mol/l)	2.43 ± 0.12 ^{c}	3.11 ± 0.12 ^{a}	2.55 ± 0.22 ^{bc}	2.85 ± 0.14 ^{abc}	3.01 ± 0.14 ^{ab}	3.07 ± 0.20 ^{a}	2.64 ± 0.16 ^{abc}	0.48 *
GPx(U/L)	342.12 ± 8.68 ^{b}	164.48 ± 7.48 ^{e}	313.26 ± 8.72 ^{c}	286.69 ± 7.2 ^{d}	363.3 ± 5.28 ^{b}	304.14 ± 6.88 ^{cd}	398.61 ± 7.76 ^{a}	22.02 **
Albumin (g/dl)	4.81 ± 0.075 ^{a}	3.85 ± 0.07 ^{c}	3.45 ± 0.026 ^{e}	4.44 ± 0.09 ^{b}	4.83 ± 0.03 ^{a}	4.01 ± 0.05 ^{ce}	3.06 ± 0.15 ^{e}	0.235 ***
Total protein (g/dl)	7.42 ± 0.29 ^{a}	7.35 ± 0.2 ^{a}	7.67 ± 0.00 ^{a}	7.43 ± 0.34 ^{a}	6.94 ± 0.25 ^{ab}	7.39 ± 0.58 ^{a}	6.31 ± 0.32 ^{b}	0.916
AST(U/L)	71.16 ± 1.99 ^{e}	102.87 ± 3.12 ^{a}	77.12 ± 3.21 ^{de}	92 ± 1.75 ^{bc}	94.62 ± 2.60 ^{ab}	81.93 ± 6.73 ^{cd}	73.83 ± 2.95 ^{de}	10.44 ***
ALT(U/L)	43 ± 1.88 ^{b}	52.66 ± 1.70 ^{a}	52.2 ± 2.12 ^{a}	51.65 ± 1.84 ^{a}	50.5 ± 2.12 ^{ab}	49.8 ± 0.43 ^{a}	47.8 ± 1.86 ^{ab}	5.25 **
ALP(U/L)	31.23 ± 0.81 ^{b}	33.7 ± 0.54 ^{a}	31.76 ± 0.15 ^{b}	32.06 ± 0.34 ^{b}	31.83 ± 0.45 ^{b}	31.6 ± 0.25 ^{b}	31.8 ± 0.18 ^{b}	1.31 *
GGT(U/L)	4.95 ± 0.27 ^{b}	5.80 ± 0.03 ^{a}	3.28 ± 0.04 ^{c}	3.47 ± 0.44 ^{c}	2.32 ± 0.0 ^{d}	2.12 ± 0.04 ^{de}	1.6 ± 0.03 ^{e}	0.57 ***
Total cholesterol (mg/dl)	84.72 ± 6.54 ^{b}	127.65 ± 0.45 ^{a}	86.46 ± 1.27 ^{b}	69.73 ± 0.44 ^{c}	94.52 ± 6.42 ^{b}	87.61 ± 6.92 ^{b}	93.375 ± 5.76 ^{b}	13.8 ***
TG (mg/dl)	58.55 ± 3.31 ^{c}	92.76 ± 6.3 ^{a}	76.64 ± 1.11 ^{b}	67.76 ± 6.07 ^{bc}	72.37 ± 6.06 ^{b}	72.21 ± 1.23 ^{b}	72.36 ± 4.19 ^{b}	12.75 ***
HDL-C(mg/dl)	37.06 ± 1.68 ^{cd}	34.27 ± 3.26 ^{c}	51.27 ± 2.94 ^{a}	30.09 ± 2.66 ^{bd}	47.3 ± 2.08 ^{ac}	35.47 ± 3.33 ^{d}	32.28 ± 1.96 ^{d}	7.73 ***
LDL-C (mg/dl)	31.35 ± 2.4 ^{b}	47.23 ± 0.16 ^{a}	31.9 ± 0.46 ^{b}	25.8 ± 0.16 ^{c}	34.97 ± 2.4 ^{b}	32.4 ± 2.6 ^{b}	34.55 ± 2.11 ^{b}	5.09 ***

NAG, N-acetyl-β-D-glucosaminidase activity**; β-2-MG**, beta-2-microglobulin; **AAP**, alanine aminopeptidase activity; **CTX1,** C-telopeptide of Collagen alpha-1 (I) chain; **MDA**, malondialdehyde; **GPx,** glutathione peroxidase activity; **ALT,** alanine aminotransferase activity; **AST,** aspartate aminotransferase activity; **ALP**, alkaline phosphatase activity; **GGT**, gamma glutamyl transferase activity and **TG,** triglycerides.
Each value represents the mean ±SE. The mean values with different small letter within a row indicate significant differences (p<0.05).
* Significant at p<0.05 ** significant at p<0.01 *** significant at p<0.001

The kidney is highly susceptible to chemical injury as a result of its high blood flow. Chemicals and minerals also concentrate in the tubular fluid as a result of reabsorption, and as renal transport is transcellular. Elements have the potential to accumulate in the tubular cells [57]. In addition, the kidney is a major metabolic organ, where the chemicals may be biotransformed into more toxic derivatives [58]. Currently, urea and creatinine clearance are used as benchmarks for renal damage, but these tests are insensitive because some of kidney function is lost before changes occur in the value of each test [55]. Therefore, new early and sensitive biomarkers of nephrotoxicity have to be found.

Many circulating high-molecular-weight proteins (albumin, IgG and transferrin), circulating low-molecular-weight proteins (β_2, α_1, α_2 microglobulin, ribonuclease, retinol binding protein and lysozyme), lysosomal proteins (N-acetyl-β-D-glucosaminidase, β galactosidase, α-glucosidase, β-glucuronidase, arylsulphatase, glutathione-5-transferase and cathepsin), proximal tubular enzymes (alanine aminopeptidase, γ-glutamyl transferase, leucine aminopeptidase, intestinal and total non- specific alkaline phosphatase), distal tubular proteins (Tam-Horsfall protein and π- glutathione-S-transferase), glomerular structural proteins (fibronectin and laminin fragments), prostanoids (thromboxane β_2, prostaglandin $F_{2\alpha}$, 6 keto prostaglandin $F_{1\alpha}$ and prostaglandin E_2) and others (Epidermal growth factor and clara cell protein) can be used as urinary markers of site-specific renal insult.

In the present study, one protein from each category was used as early sensitive biomarker of renal failure, IgG was instance of high-molecular-weight proteins, β_2-microglobulin was instance of low-molecular-weight proteins, N-acetyl-β-D-glucosaminidase (NAG) was instance of lysosomal proteins and alanine aminopeptidase was example of proximal tubular enzymes.

Some chemicals or minerals may cause damages for kidney alone or can damage both kidney and liver. Then the early biomarkers of kidney and liver may be conflict. Albumin (circulating high-molecular weight proteins), total non-specific alkaline phosphatase and γ-glutamyl transferase (proximal tubular proteins) are urinary kidney markers. In the same time these compounds are blood liver markers. Increasing the blood concentrations of albumin, alkaline phosphatase and γ-glutamyl transferase activities means hepatic disorder while increasing their concentrations activities in urine means kidney injury. Thus in kidney failure the blood levels of the previous proteins may be decreased while, in hepatic disfunction the blood concentrations or activities of the above compounds must be increased .In both renal and hepatic insults, the total blood concentrations of albumin , alkaline phosphatase and γ-glutamyl transferase activities depend upon the degree of damage in both kidney and liver.

In the present study the blood level of N-acetyl- β-D glucosaminidase activity, β_2- microglobulin and alanine aminopeptidase activity were significantly decreased by subcutaneous injection of Cd^{+2} into rats. It means that increasing the urinary markers of site-specific renal insult, such as IgG (circulating high- molecular–weight protein), β_2-microglobulin (circulating low-molecular-weight protein), N-acetyl-β-D-glucosaminidase (lysosomal protein) and alanine aminopeptidase activities (proximal tubular protein) may result in significant decreases in their blood concentration and activities. Therefore, serum tests may be good indicators like urinary tests of renal damage and have the potential to indicate the initial site and severity of the damage.

The present data indicated that the declined level of early sensitive renal markers in serum after injection of Cd^{+2} were not significantly changed by administration of Zn^{+2}, selenium, vitamins (C and E) and epicatechine, except serum NAG which was increased after administration of vitamins C, E and epicatechine (organic antioxidants) .Therefore, the activity of AAP and the concentration of β_2-microglobulin may be good serum indicator as early sensitive biomarker of renal damage.

Cadmium decreases parathyroid hormone stimulation of adenylcyclase, inhibits hydroxylation of 25-hydroxy vitamin D_3, increases urinary calcium excretion, decreases gastrointestinal calcium absorption and affects both bone mineralization and bone collagen [59]. Therefore, carboxy- terminal cross- linking telopeptide of type -1 collagen (CTX -1) is an early serum biomarker of bone resorption [60] and osteoporosis. The bone resorption by cadmium may be ameliorated by Zn administration [23], as shown in our data. In addition vitamins C, E and antioxidant epicatechine may also decrease the bone resorption rate caused by cadmium administration.

Although, the oxidative numbers of Cd and Zn cannot be changed in living cells, they play an important role in the oxidative systems [61]. In addition, there are antagonistic interactions between the oxidative stress of Cd and Zn [29]. Several investigators showed that Zn can reverse the Cd –induced oxidative stress [62] and [54] in many organs and cells. This relationship is not fully understood till now [20]. But, some evidences indicated that metallothionein biosynthesis (a thiol-rich low molecular weight protein involved in the detoxification of heavey metals) was increased in the presence of Cd [63]. The increase in metallothionein biosynthesis may decrease the glutathione (GSH) biosynthesis because of consumption of –SH groups in metallothionein biosynthesis [62] and induce the oxidative stress [64]. It is also well known that GSH level is a good indicator of oxidative stress [20]. Cadmium may also react with active SH group of GSH. GSH may also be

consumed in the scavenging of free radicals generated by Cd. Finally, the GSH/GSSG ratio may be decreased resulting in great changes in the redox state [65].

Decreasing the GSH level and GSH/ GSSG ratio, as reported before could decrease GPx activity and increase lipid peroxidation measured as malonaldehyde [20] as shown in the present results. The reduction in the activity of GPx might be due to depletion of Se by Cd [66] as Se is an essential component of GPx or due to the formation of chemical complex between Cd and Se at the active site of GPx [67]. Therefore, administration of Se into rats injected by Cd restored Gpx activity to the normal level, as shown in Table 1.

Vitamins C, E and epicatechine are good acceptors of free radical produced by high rate of lipid peroxidation and oxidation stress [68]. Therefore, administration of these antioxidants decreased GPx activity, as shown in our results.

Serum albumin, gamma glutamyl transferse and total non-specific alkaline phosphatase activities are excellent indicators for liver function test and they are changed during hepatic failure [69], but they also act as urinary markers of renal damage [70] and [71]. Therefore, the albumin concentration, γ-glutamyl transferase and alkaline phosphatase activities in serum are not good markers in circumstances of liver and kidney injury together, as shown in cadmium - exposured subjects [72].Thus, the concentration of albumin and the enzymes activities of serum in this state are a resultant of both renal and hepatic disorders.

The significant increases in serum AST and ALT activities are in agreement with many investigators [31] who stated that AST and ALT activities are elevated in serum during liver damage. This increase may be due to alterations in liver transaminases of rats [73], hepatic membrane permeability [74], [75] and threshold values [76]. Zn, Se, vitamins C, E and epicatechine had a protective role against the increase in AST activity but not ALT activity in Cd-exposed rats.

Our interest has been focused on the possibility of protective effect of Zn, Se, vitamins C, E and epicatechine on the Cd-induced disorders in the body status of lipids. Data of several authors [74] and [76] indicated that the exposure to Cd altered the serum values of the very important measured indices of the body status of lipid. The alterations in the blood concentrations of cholesterol, triglycerides and LDL-C showed that Cd, even at small exposure, result in changes in the lipid profile of human – rat model. It may be due to that Cd induces oxidative stress [77] in the body. In addition, the increased blood concentrations of lipid peroxide (LPO), F_2 – isoprostane (F_2–isoP) and oxidized LDL (ox LDL) provide evidence for enhanced lipid peroxidation due to exposure to Cd [25].

CONCLUSION

Our results suggest that Zn^{+2} and some antioxidants have a protective role against Cd-induced toxicity in the kidney, liver, oxidative stress**,** damage in the bone, changes in the lipid profile.

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