Effects of Cadmium on Kidney Functions and Oxidative Stress In Albino Rats

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Abstract

Background: Cadmium (Cd) is widespread used in industry and extensively disseminated in the environment. As a result of the extensive use of cadmium (Cd) in industry and its extensive dissemination in the environment, numerous studies have focused on the identification of the early stages of Cd-induced renal injury in exposed persons. Following long-term low-level exposure, nearly one-third of the cadmium in the body will be found in the renal tissue. So, the aim of the present work is to evaluate the disturbed renal functions induced by cadmium exposure and its underlying mechanism. **Materials and Methods:** Twenty male rats were divided into two main groups, ten rats per each group. **Group 1** (n=10) was the Cd group in which rats were treated for 13 days with a daily dose of 1 mg CdCl2/kg body weight in sterile 0.9 % NaCl solution subcutaneously. **Group2** (n=10) was the vehicle control, they received equivalent volumes of sterile 0.9 % NaCl solution by subcutaneous injections. **Results:** cadmium administration led to a decrease in serum sodium and potassium level and increase their level in urine. It led to a significant decrease in creatinine clearance and a significant increase in the oxidative stress biomarker tissue malondialdehyde (MDA), as well as, and a significant decrease in serum total antioxidant capacity. Moreover, cadmium led to a significant decrease in glucose uptake by kidney (mg/gm wet tissue/h.) **Conclusion:** Cd has detrimental effects on kidney functions and renal handling of glucose and this effect is referred to its oxidative stress effect.

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Introduction

Cadmium (Cd) is widespread used in industry and extensively disseminated in the environment. It is considered as a harmful pollutant to the kidney that threatens all populations all over the world (1-3). With chronic exposure, cadmium accumulates in the epithelial cells of the pars convolute and pars recta of the renal tubules. When a critical cadmium concentration in renal tissue reaches 150-200 µg/g tissue, cadmium causes a generalized malfunction of the proximal tubule characterized by increased urine volume, presence of low molecular weight proteins in urine and occurrence of glucosuria (4, 5).

Numerous studies have focused on the identification of the early stages of Cd-induced renal injury in exposed persons (6,7). There is a possible relation between cadmium exposure and hyperglycemia. Following long-term low-level exposure, approximately, one third of the cadmium in the body will be found in the renal tissue, while at higher exposure, proportionately, more will be in the hepatic tissue. The accumulation in the kidneys is explained by the flow of cadmium via renal blood flow where cadmium bound to metallothionein (Cd-MT) is filtered with the glomerular filtration rate and reabsorbed into the tubular cells as other low molecular weight proteins. The bound-cadmium molecule is probably taken up into the tubular cells by endocytosis (8).

A continuous catabolism of Cd-MT takes place after reabsorption in the tubules and cadmium is split from the Cd-MT and bound to newly formed renal proteins in the tubules. Kidney damage is prevented until a stage is reached at which the kidneys can no longer produce enough renal proteins. At this stage, free cadmium ions will become very toxic (9). The aim of this work is to evaluate the disturbed renal functions induced by cadmium exposure and its underlying mechanism.

Materials and methods:

Experimental procedures with animals

Twenty healthy male Sprague Dawley Albino rats, 80 ± 5 days old with an average weight of 200 ± 50 grams old were used in the study. They were obtained from El Nile Center for research purposes, Mansoura, Egypt. The experimental protocol was approved by the Institutional Animal Ethics Committee of Mansura University. All rats were fed with chow diet and housed in policarbon cages and were exposed to a 12 hour light-dark cycle at a room temperature of 21-24°C and 50 - 60% relative humidity.

Study design

Rats were randomly divided into two groups:

Group1 (n=10): was the Cd group in which daily dose of 1 mg CdCl2/kg in 0.9 % NaCl solution was applied for two weeks subcutaneously to make sure that Cd2+ application was controlled and consistent. Cd was purchased from Sigma–Aldrich, St. Louis, MO.

Group 2 (n=10): was the vehicle control in which rats received equivalent volumes of sterile 0.9 % NaCl solution by S.C injections (10).

After two weeks, the animals were anesthetized with pentobarbital (0.6 ml/ kg) and

the blood collected by heart puncture and allowed to clot for 30 min. Serum was separated by centrifugation at 2500 r. p. m for 15 min and used for biochemical estimations. After that rats were sacrificed by cervical dislocation and the abdomen of terminated animal was cut open quickly then, the kidney was dissected and washed in Krebs Ringer Bicarbonate Buffered Solution, dry by filter paper then placed immediately in the incubation medium.

Assessment of renal functions

a. Determination of blood level of Na+, K+ and creatinine

b. Determination of proteins, Na+, K+ and creatinine level in urine

c. Creatinine clearance (Ccr)

d. Determination of glucose uptake by renal tissue

Creatinine clearance (Ccr)

Creatinine clearance is measured using urine flow in the 24 hours and the creatinine values measured in the urine (U cr) and serum (Pcr), and volume of urine per minute $= \dfrac{\text{volume of urine in 24 hours}}{24.\ 60}$ (11)

$$Ccr = \frac{V.Ucr}{Pcr}$$

(Where V is the volume of urine, Ucr is creatinine concentration in urine and Pcr is creatinine concentration in plasma)

Measurement of oxidative stress biomarkers

a. Determination of malondialdehyde (MDA) in renal tissue

b. Determination of serum total antioxidant capacity (TAC)

Kidney homogenate preparation

Samples of renal tissue were homogenized (1:10, w/v) in chilled phosphate buffer (0.1 M, pH 7.4) containing KCI (1.17%). The homogenate was centrifuged at 8000 r.p.m for 5 min at 4°C to separate the nuclear debris and supernatant was collected. The centrifuged homogenates were stored at -25°C until they were analyzed.

Measurement of oxidative stress biomarkers in homogenate

The assay for lipid peroxidation of the renal tissue was carried out (12). It was measured as malondialdehyde (MDA). Thiobarbituric acid reacts with malondialdehyde to yield a coloured product that can be measured at 535 nm. The results were expressed as nmol MDA formed/gram tissue. Kits used were commercially available colorimetric kits (BioDiagnostics, Dokki, Giza, Egypt).

Serum total antioxidant capacity (TAC)

Total antioxidant capacity (TAC) was estimated in serum [13]. The absorbance was measured at 340 nm against blank. Kits used were commercially available colorimetric kits (BioDiagnostics, Dokki, Giza, Egypt).

Incubation Procedures

Determined weights of renal tissue were put in 3 ml of the incubation medium with known glucose concentration then, placed in metabolic shaker at 37° C for two hours then determine the glucose

concentration in the incubation medium and glucose uptake was calculated.

Renal tissues glucose uptake

It was calculated as mg per gram wet tissue weight per hour. The glucose uptake was calculated using the following equation:

Glucose uptake $= \dfrac{M \times V \times 1000}{N \times 100}$

Where:

N= Net weight of tissue in Gm

V= volume in ml of the incubation medium in the flask (3 ml)

M= difference in the concentration of glucose per 100 ml of the medium between the control and the tissue bottles

Statistical Analysis

Data were tabulated, coded then analyzed using the computer program SPSS (**Statistical package for social science**) version 17.0. Descriptive statistics were calculated in the form of Mean ± Standard deviation (SD). Student t-test was used to compare between mean of two groups of numerical (parametric) data.

Results:

Table (1) shows that cadmium administration decreased serum Na+ level significantly from 144 ± 4.4 to 129± 2.3 (mmol/L.) (P < 0.001) and decreased serum k+ level significantly from 4.6±0.2 to 3.5±0.17 (mmol/L.) (P < 0.001) but significantly decreased serum creatinine level from 0.52 ± 0.08 to 3.21 ± 0.49 (mg %) (P < 0.001).

Table (2) shows that cadmium administration increased Na+ level in urine significantly from 58±3.2 to 67±2.5 (mmol/L.) (P < 0.001) and increased k+ level in urine significantly from 1.2 ±0.16 to 1.5 ±0.14 (mmol/L.) (P < 0.05) and, also, significantly increased creatinine level in urine from 93.5±2.2 to 157.5±3.5 (mg %) (P < 0.001). Moreover, it decreased creatinine clearance significantly from 1.26 ± 0.1 to 0.32 ± 0.03 (ml /minute) (P < 0.001).

Table (1): effect of cadmium administration on serum Na+ level (mmol/L), K+ serum (mmol/L.) and serum creatinine (mg %)

	Control	Cadmium	P
Na+ (mmol/L.)	144 ± 4.4	129± 2.3	< 0.001
K+ (mmol/L.)	4.6±0.2	3.5±0.17	< 0.001
Creatinine (mg %)	0.52 ± 0.08	3.21 ± 0.49	< 0.001

P: as compared with control group. Highly significant: P < 0.001

Table (2): effect of cadmium administration on urine volume (ml/24 hours), Na+ level in urine (mmol/L.), K+ level in urine (mmol/L.), protein in urine (mg/dl), urine creatinine (mg%) and creatinine clearance (ml /minute)

	Control	Cadmium	P
- Urine volume (ml/24 hours) - Na+ (mmol/L.)	9.71± 0.16 58±3.2	9.4 ± 0.2 67±2.5	< 0.05 < 0.001
-K+ (mmol/L.)	1.2 ±0.16	1.5 ±0.14	< 0.05
- Protein in urine (mg/dl) - Creatinine (mg %) - Creatinine clearance (ml /minute)	8.8 ± 0.29 93.5±2.2 1.26 ± 0.1	17.2 ± 0.54 157.5±3.5 0.32 ± 0.03	< 0.001 < 0.001 < 0.001

P: as compared with control group**.** Highly significant: P < 0.001

Table (3): effect of cadmium administration on tissue malondialdehyde (MDA) (nmol/g. renal tissue), serum total antioxidant capacity (µmol/ L.) and glucose uptake by kidney (mg/gm wet tissue/h.)

	Control	Cadmium	P
Malondialdehyde (MDA) (nmol/g. renal tissue)	11.3±0.47	22.7±0.4	< 0.001
Total antioxidant capacity (µmol/ L.)	6.42±0.23	3.9±0.24	< 0.001
Glucose uptake by kidney (mg/gm wet tissue/h.)	2.88±0.28	0.77±0.21	< 0.001

P: as compared with control group. Highly significant: P < 0.001

Table (3) shows that cadmium administration increased tissue malondialdehyde (MDA) (nmol/g. renal tissue) significantly from 11.3±0.47 to 22.7±0.4 (mmol/L.) (P < 0.001) and decreased serum total antioxidant capacity (µmol/ L.) significantly from 6.42±0.23 to 3.9±0.24 (mmol/L.) (P < 0.001) and, also, significantly decreased glucose uptake by kidney (mg/gm wet tissue/h.) from 2.88±0.28 to 0.77±0.21 (mg %) (P < 0.001).
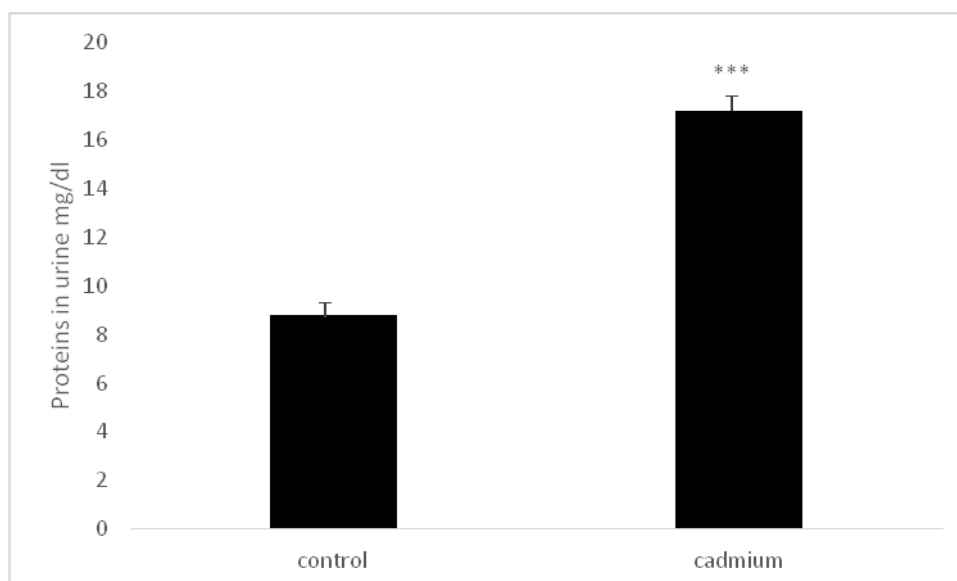

Figure (1): effect of cadmium administration at dose 1 mg CdCl2/kg body weight in sterile 0.9 % NaCl solution on protein in urine (mg/dl). (***) denote highly significantly different form control group
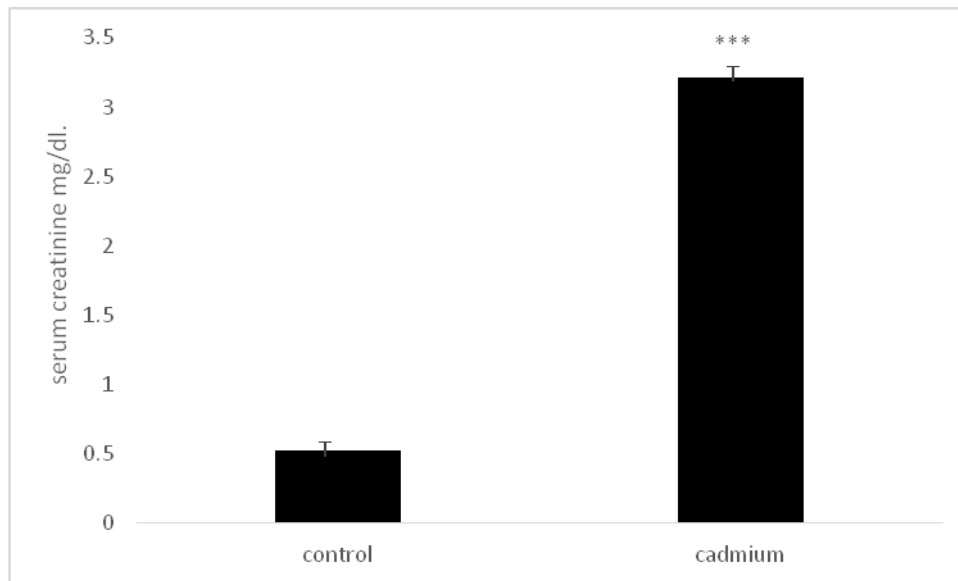
Figure (2): effect of cadmium administration at dose 1 mg CdCl2/kg in 0.9 % NaCl solution on serum creatinine (mg %). (***) denote highly significantly different form control group
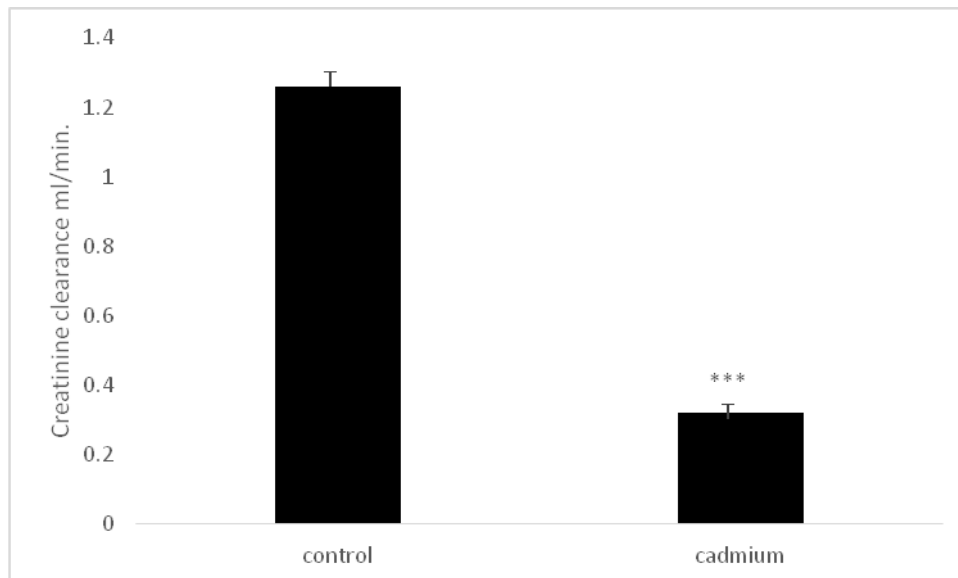

Figure (3): effect of cadmium administration at dose 1 mg CdCl2/kg in 0.9 % NaCl solution on creatinine clearance (ml /minute)**.** (***) denote highly significantly different form control group

Figures (1) shows that cadmium administration at dose 1 mg CdCl2/kg body weight in sterile 0.9 % NaCl solution led to a highly significant increase in proteins in urine as compared with control group.

Figure (2) shows that cadmium administration at dose 1 mg CdCl2/kg body weight in sterile 0.9 % NaCl solution led to a highly significant increase in serum creatinine as compared with control group.

Figure (3) shows that cadmium administration at dose 1 mg CdCl2/kg body weight in sterile 0.9 % NaCl solution led to a highly significant decrease in creatinine clearance as compared with control group.

Figure (4) shows that cadmium administration at dose 1 mg CdCl2/kg body weight in sterile 0.9 %

NaCl solution led to a highly significant increase in lipid peroxidation, expressed as MDA level, in the kidney homogenate as as compared with control group.
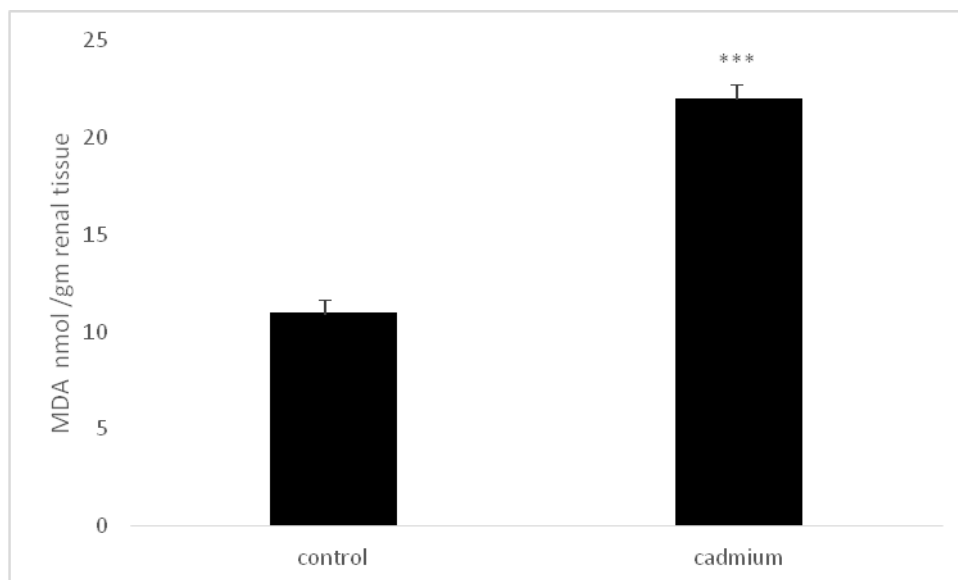
Figure (5) shows that cadmium administration at dose 1 mg CdCl2/kg body weight in sterile 0.9 % NaCl solution led to a highly significant decrease in glucose uptake by kidney (mg/gm wet tissue/h.) as compared to control.

Figure (4): Lipid peroxidation, expressed as MDA level, in the kidney homogenate of control rats, Cd Cl2 treated rats at dose 1 mg CdCl2/kg in 0.9 % NaCl solution. Results were expressed as mean ± S.D from 10 animals. (***) denote highly significantly different form control group
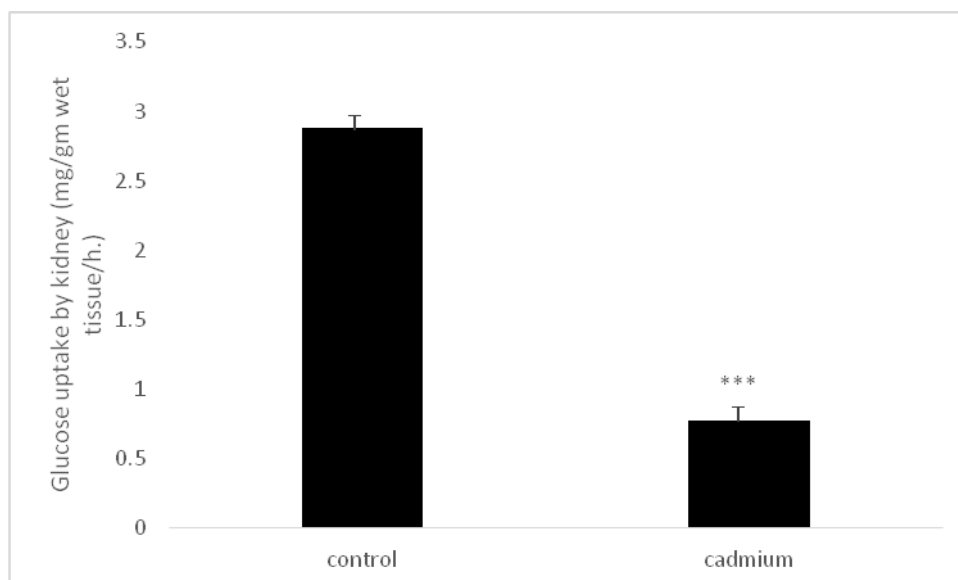

Figure (5): effect of cadmium administration at dose 1 mg CdCl2/kg in sterile 0.9 % NaCl solution on glucose uptake by kidney (mg/gm wet tissue/h.). *** Highly significant

Discussion

The main findings in the present study included: a) cadmium administration led to a decrease in serum sodium and potassium level and increase their level in urine b) cadmium led to a significant decrease in creatinine clearance   c) cadmium led to a significant increase in tissue malondialdehyde

(MDA) and a significant decrease in serum total antioxidant capacity d) cadmium led to a significant decrease in glucose uptake by kidney (mg/gm wet tissue/h.)

Cadmium is a widely spread heavy metal in the environment and it is considered as one of the major heavy metals pollutant in drinking water. It was demonstrated that cadmium is more highly precipitated in kidney. In animals subjected to cadmium via ingestion, the kidney is, by far, the main organ affected by cadmium. Complexes of Cd-MT (formed in hepatocytes in response to the entry of cadmium to the body) are released from necrotic liver cells and are delivered via renal blood flow to kidney, where it appears that they are taken up and induce apoptosis of pars convolute and pars recta (14).

Several studies report that cadmium induces apoptosis in different cell types, including the renal tubular epithelial cells (15). Moreover, It was demonstrated that the most important target tissue for chronic low-level exposure to Cd is the renal tissue (16) and is reflected in tubular necrosis and increase all of these chemicals in urine (Glucose, amino acids, calcium and proteins) (17).

In the present study we evaluated the detrimental effects of cadmium on kidney functions which are evidenced by the results in tables (1, 2 and 3). Table (2) shows that cadmium administration resulted in a highly significant decrease in creatinine clearance and a highly significant proteinuria compared to control group. Also, table (2) shows that cadmium administration resulted in a significant decrease in urine volume compared to control group. We assume that these detrimental

effects of cadmium could be explained by two possibilities: the direct effect of reabsorbed cadmium by renal tubules and the other possibility is its oxidative stress effect. In consistence with these assumptions, it has been demonstrated that cadmium is reabsorbed by the cells of proximal tubules due to the presence of transporters, metabolizing enzymes and sensors for cadmium and cadmium precipitated in these cells. Once cadmium enters the cells, it can displace other metals from metalloproteins and, thereby, affect cellular functions (18 & 19). Cadmium, also, induces oxidative stress, mostly by displacement of fenton metals, by damaging cell organells, or by decreasing GSH. The extent of this damage depends upon a number of different factors ranging from the cell type, acute or chronic mode of administration, and time of cadmium exposure (20). It was demonstrated that exposure of proximal tubular cells culture (1.25–40 μM CdAc2 for 12 h) to cadmium in rats led to loss of cell viability (most probably apoptotic). Cell toxicity was also noticed in renal tubular epithelial cells exposed to CdCl2 for 24 h. This cell toxicity was reduced by antioxidant therapy (21). Also, it was demonstrated that the ability of the antioxidant *N*-acetylcysteine to partially reverse apoptosis implicates a definite role of oxidative stress in the apoptotic mechanism induced by Cd (22). The ROS production, in these cells, can be the result of mitochondrial alterations as Cd-exposure change the mitochondrial membrane potential. 5 μM Cd helped ROS formation in the proximal tubular cell line, WKPT-0293 Cl.2. This further led to the degradation of Na+/K pump through proteolytic pathways (23).

In the present study, cadmium administration resulted in a highly significant decrease in creatinine clearance compared to control group, the decrease in creatinine clearance is parallel to the oxidative stress induced by cadmium (Figure 4 and 5). In the present study we reported that cadmium administration resulted in a highly significant decrease in glucose uptake by kidney (mg/gm wet tissue/h.) compared to control group. It has been demonstrated that cadmium decreased both glucose uptake and expression of sodium glucose transporter-1 (SGLT1) in primary cultures of rat renal cortex cells at concentrations of cadmium that did not cause cell death. This marked inhibitory effect may be attributed to the oxidative stress-induced by cadmium (24).

It has been demonstrated that cadmium stimulates the production of intracellular reactive oxygen species ROS such as hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide due to an inhibitory effect on mitochondrial electron transport (25). As a result of this inhibition, the electron transport chain becomes highly reduced; electrons are transferred directly to available oxygen and lead to enhanced formation of reactive oxygen species. Reactive oxygen species may lead to cellular damage when the rate of its generation exceeds the rate of its decomposition by antioxidant defense systems, such as the enzymes superoxide dismutase and catalase. The oxidative stress induced by cadmium in a biological system may be due to increased lipid peroxidation, which may be attributed to alterations in the antioxidant defense system (26 and 27). Lipid peroxidation is known to play an important role in cadmium induced renal injury and malondialdehyde (MDA)

is one of its end products. Thus, measurement of MDA can be used to assess lipid peroxidation. In the present study, table (3), the MDA content in renal tissue showed a highly significant increase by cadmium administration as oxidative stress biomarker as compared to control group. Also, table (3) and figure (4) show that cadmium administration resulted in a highly significant decrease in serum total antioxidant capacity (TAC) compared to control group. These findings are in agreement with several reports demonstrating that cadmium induces oxidative stress in cells by increasing lipid peroxidation (28). Also, it was reported that the renal impairment is the main effect observed upon chronic cadmium exposure and the proximal tubules of the kidney are the primary target (29).

CONCLUSION

This study demonstrates that cadmium exposure leads to oxidative damages in renal tissue. The decrease in glucose uptake by kidney may be attributed to the oxidative stress-induced by cadmium. So, further studies are needed to investigate miscellaneous drugs and hormones which may protect kidney against cadmium induced- oxidative stress.

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