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Acute Effect of Cadmium Treatment on the Kidney of Rats: Biochemical and Ultrastructural Studies

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Abstract: The present study was designed to explore the nephrotoxic effect of intraperitoneal acute administration of $CdCl_2$ (2.5 and 5 mg kg^{-1} b.w.) in rats. A number of toxicological parameters in kidney were examined including malondialdehyde (MDA) and endogenous antioxidants, e.g., catalase (CAT), superoxide dismutase (SOD) and Glutathione Peroxidase (GPx). The parameters that indicate tissue damage such as serum urea and creatinine were also determined, along with the ultrastructural changes of kidneys. A correlation was found between the dose and the intensity of changes. The results demonstrated that cadmium administration increased renal MDA but decreased CAT, SOD and GPx activities. In parallel, serum creatinine and urea elevated. The glomerular ultrastructural changes observed in cadmium-treated rats included narrowing of the capillary lumen and swelling of the capillary endothelium with occasional loss of fenestrae. The mesangium was wide with increased mesangial matrix. Loss of homogenous appearance of basement membrane displaying ondulation and thickening in many areas and deterioration of the slit membrane structures formed by the podocytes were also noted. The effects of cadmium on proximal cell ultrastructure were focal loss of brush border, nuclear membrane damage, chromatin condensation, swelling of the mitochondria with regression of mitochondrial cristae, degranulation and disintegration of protein-synthesizing structures such as rough endoplasmic reticulum, increased number of lysosomes and ultimately cell death.

Key words: Acute cadmium, oxidative stress, ultrastructure, kidney, rat

INTRODUCTION

Heavy metal intoxication, especially by lead, cadmium, arsenic and mercury, constitutes series threats to human health (Wenneberg, 1994; Hu, 2000). Cadmium (Cd) is one of the most toxic substances in the environment with a wide range of organ toxicity and long elimination half-life (20-30 years) (Jarup *et al*., 1998). It binds to cysteine residues of proteins, including metallothionein and reduced glutathione (GSH) in an intracellular complex and is known to be stored primarily in the kidneys and liver (Friberg, 1984; Pop and Rall, 1995; Klassen *et al*., 1999).

Metals, especially transition metals, act as a catalyst in the oxidative reaction of biological macromolecules; thus metal toxicities might be associated with oxidative tissue damage. Although cadmium is not a redox-active metal such as iron, copper and chromium, it has been shown to stimulate the production of Reactive Oxygen Species (ROS) due to an inhibitory effect on mitochondrial electron transport (Stohs *et al*., 2000). 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 ROS (Ochi *et al*., 1987; Tatrai *et al*., 2001). ROS may lead to cellular damage when the rate of its generation surpasses the rate of its decomposition by antioxidant defense systems, such as the enzymes catalase (CAT), superoxide dismutase (SOD) and Glutathione Peroxidase (GPx) (Mates *et al*., 1999; Datta *et al*., 2000).

One of the most important effects of free radicals is oxidation of polyunsaturated fatty acids (PUFAs). In particular, hydroxyl ($OH^{\bullet}$), peroxyl ($RO^{\bullet}$) and alkoxyl ($ROO^{\bullet}$) radicals play important roles in the oxidation of PUFAs (Sarkar *et al*., 1995). As a result of free radical attack, lipids are oxidized and hence membranes are damaged (Sarkar *et al*., 1998). Malondialdehyde (MDA), a well known secondary product of lipid peroxidation after exposure to ROS and free radicals, may be used as an indicator of cell membrane injury (Jacob and Burri, 1996). It has been reported that administration of cadmium via different routes causes increased lipid peroxidation in membranes of erythrocytes and tissues such as the liver, kidney, brain and testes where MDA is used as an

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indicator of oxidative damage (Gutteridge, 1995; Stohs *et al*., 2001). However, conflicting results have been reported on the activities of antioxidant enzymes in oxidative stress induced by cadmium in various organs (Casalino *et al*., 2002). On the other hand, few investigators have studied the ultrastructural effects of acute cadmium exposure on the kidneys of experimental animal models (Bomhard *et al*., 1986; Rehm and Waalkes, 1990; Condron *et al*., 1994) and have reported that the proximal tubule is the primary site of damage. In view of these findings, we decided to evaluate the oxidative stress after acute cadmium treatment and its relationship with the ultrastructural changes in kidneys of rats. This research also aimed to test the hypothesis that certain aspects of cadmium-induced renal dysfunction are the result of glomerular, rather than classic tubular, injury.

MATERIALS AND METHODS

Chemicals: Cadmium chloride ($CdCl_2.H_2O$, 99% pure) was purchased from Sigma Chemicals (St Louis, MO, USA).

Animals and treatment: All experiments were performed on male laboratory rats (18 animals) weighting 140-160 g housed in metallic cages. The animals had free access to standard laboratory chow and water *ad libitum*. They were maintained at a temperature of 25±2°C and under a 12 h light/12 h dark cycle. After one week of acclimatization, rats were randomized into three groups (6 for each group): group I (control) injected with 0.5 mL of physiological saline ip; group II (Cd 2.5) administrated 2.5 mg kg^{-1} b.w. Cd^{2+} ip and group III (Cd 5) administrated 5 mg kg^{-1} b.w. Cd^{2+} ip. The Cd intoxication protocol was chosen based on published research (Casalino *et al*., 2002; Yalin *et al*., 2006). The animals were scarified under light ether anesthesia 24 h after the injection.

Biochemical study: Whole tissues of the kidneys were obtained by dissection, washed with physiological saline and kept frozen at -20°C until analyzed. Tissues were minced and homogenized (10% w/v) in ice-cold 50 mM potassium phosphate (pH 7.5), 1 mM EDTA. Homogenates were centrifuged at 10,000 x g for 15 min at 4°C and the clear supernatants were used for analytical assays of lipid peroxidation and antioxidant enzymes (CAT, SOD and GPx). Blood samples were taken from abdominal aorta into tubes; sera were separated and stored at -20°C until creatinine and urea were assayed.

Lipid peroxidation (LPO) was measured by thiobarbituric acid (TBA) method (Ohkawa *et al*., 1979) that determines aldehyde formed by degradation of hydroperoxide, including malondialdehyde (MDA).

The activity of catalase (CAT) was assayed by the method of Aebi (1984). The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. The enzyme activities are given in U mg^{-1} protein. Superoxide dismutase (SOD) activity was determined, based on the ability of enzyme to inhibit the phenazine methosulphate-mediate reduction of nitroblue tetrazolium dye (NBT) (Nishikimi *et al*., 1972). 1.5 Unit/assay of the purified enzyme produced 80% inhibition of the NBT reduction rate. Glutathione Peroxidase (GPx) activity was expressed in the presence of GSH and hydroperoxide substrates (Wendel, 1980). The activity was expressed as loss of reduced GSH min^{-1}. GPx activity was expressed in units (1 unit is the enzyme quantity that oxidizes 1 μmol GSH min^{-1} in the above system at 25°C). Tissue protein content was determined according to the method developed by Lowry *et al*. (1951) using bovine serum albumin as a standard.

Creatinine was determined according to Henry *et al*. (1974). In this method, creatinine reacts with picrate to form a coloured complex. The rate of formation of the complex is measured photometrically at 492 nm. A totally enzymatic procedure was used for the determination of urea using the coupled urease/glutamate dehydrogenase (GLDH) enzyme system according to Tietz (1995).

Ultrastructural study: Kidney tissues (cortex) were cut into small pieces 1 mm thick and fixed in $_4F_1G$ in phosphate buffer solution (pH 7.2) for 3 h at 4°C, after which the tissues were removed and postfixed in buffered 2% OsO_4 for 2 h at 4°C. Postfixed tissues were rinsed in the buffer and dehydrated at 4°C through a graded series of ethanol. Then they were embedded in epon-araldite mixture in labeled beam capsules. Ultrathin sections (50 nm thick) were cut using glass knives, collected on naked copper-mesh grids and stained with uranyl acetate for ½ h and lead citrate for 20-30 min (Reynolds, 1963). The sections were examined and viewed using Jeol 100CX TEM.

Statistical analysis: Data are expressed as means±SE. The results were computed statistically (SPSS software package, version 10) using one-way analysis of variance (ANOVA). Post-hoc test was performed for inter-group comparison using the LSD. Values of $p<0.05$ were considered statistically significant.

RESULTS

Biochemical results: One-way ANOVA of lipid peroxidation and antioxidant enzymes (CAT, SOD and GPx), yielded a significant main effect of Cd^{2+} ($p<0.05$).

Table 1: Effects of acute cadmium administration on biochemical parameters indicative of oxidative stress in kidney of rats

Parameters	Control group	Cd 2.5	Cd 5
Lipid peroxidation (nmoles MDA mg^{-1} tissue)	0.053±0.002[a]	0.070±0.003[b]	0.074±0.003[b]
Catalase (U mg^{-1} protein)	8.63±0.52[a]	7.470±0.97[a]	4.630±0.46[b]
Superoxide dismutase (U mg^{-1} protein)	7.10±0.50[a]	5.900±1.04[a]	3.470±0.30[b]
Glutathione peroxidase (U mg^{-1} protein)	4.17±0.30[a]	3.500±0.46[a]	1.930±0.18[b]

Values represent the mean±SE for three rats, values within rows with no common superscripts are statistically different ($p<0.05$), according to LSD-multiple range tests

Table 2: Effects of acute cadmium administration on biochemical parameters indicative of kidney function of rats

Parameters	Control group	Cd 2.5	Cd 5
Creatinine (mg dL^{-1})	0.52±0.06[a]	1.10±0.07[b]	1.62±0.12[c]
Urea (mg dL^{-1})	30.00±2.51[a]	64.60±4.82[b]	74.80±6.98[b]

Values represent the mean±SE for five rats, values within rows with no common superscripts are statistically different ($p<0.05$), according to LSD-multiple range tests

Post-hoc comparisons indicated that 2.5 and 5 mg kg^{-1} Cd^{2+} increased ($p<0.05$) MDA level in the kidney (32 and 40% higher, respectively than the corresponding control values) (Table 1). Post-hoc comparisons also demonstrated that 5 mg kg^{-1} Cd^{2+} intoxication caused a significant decrease in the activities of CAT (46%), SOD (51%) and GPx (54%) as compared to the control group ($p<0.05$); whereas rats exposed to 2.5 mg kg^{-1} Cd^{2+} did not present a significant change in enzyme activities than the control ($p>0.05$) (Table 1). On the other hand, post-hoc comparisons revealed that acute cadmium exposure, at the two doses tested (2.5 and 5 mg kg^{-1}), significantly ($p<0.05$) enhanced the concentrations of creatinine (about 2- and 3-fold, respectively) and urea (about 2- and 2.5-fold, respectively) in serum of rats (Table 2).

Electron microscopic observations: The ultrastructural study was restricted to the glomerlular epithelium and the proximal segment of the tubular system. Electron microscopic examination of kidney sections showed that cadmium caused a dose-dependent alteration in renal morphology that was pronounced in the proximal tubular epithelial cells. In the examination of the glomerular structure belonging to the control group (Fig. 1), podocytes were observed to lie in the urinary space and they remained in contact with the basement membrane by extensions of their cytoplasm known as foot processes. The endothelial cells in the glomerlular capillaries were very richly fenestrated with large pores which appeared to lack any trace of a closing diaphragm. The mesangial cells had indented nucleus and were found in between the adjacent capillaries where the endothelial cells were not covered with podocytes and their basement membrane was very thin or absent. In the examination of the glomerular structure belonging to group II administrated 2.5 mg kg^{-1} Cd^{2+} (Fig. 2), narrowing of the capillary lumen was quite evident and the endothelial cells of the glomerlular capillaries were swollen and occasionally lost their fenestrae. In some of the podocytes, the nuclei were irregularly outlined. Foot processes were disorganized and many of the filtration slits were closed. The basement membrane become ondulated and thickened from place to place. The mesangial tissue showed many alterations. The content of mesangial matrix appeared to be increased and some mesangial cells exhibited clefted or segmented nuclei. The characteristics of the glomerular damage in rats exposed to 5 mg kg^{-1} Cd^{2+} (group III) (Fig. 3), although enhanced, were similar to those described for group II. Occasional pyknotic nuclei in the endothelial cells of the glomerlular capillaries were encountered and the fenestrate of the cytoplasm could not be traced up regularly. Ondualtion, thickening and irregularities in the homogenous appearance of the basement membrane were commonly noted. Foot processes were occasionally lost leaving fragmented parts in their vicinity and frequently exhibited focal adhesion forming blunted sheath of the cytoplasm. The mesangium was wide with increased mesangial matrix. The mesangial cells showed encroachment on the capillary lumen which became narrow.

The proximal renal tubular epithelium of control rat had dense brush border, basal or central nucleus, apical endocytic vesicles, occasional lysosomes and elongate or round mitochondria (Fig. 4). Cisternal profiles of rough ER lied between the mitochondria. Proximal tubular epithelial cells of rats exposed to 2.5 mg kg^{-1} Cd^{2+} (group II) showed focal loss of the brush border (Fig. 5a). Mitochondria were swollen and their loss of cristae was evident. Other giant mitochondria were many times larger than those described above. Their matrices were less electron dense and the number of cristae was not extensive. Nuclei showed chromatin condensation. Some nuclei appeared with more or less normal heterochromatin content but contained one or two terminalized nucleoli which appeared as well defined electron dense masses. RER dilation and detachment of ribosomes were frequently observed (Fig. 5b). Intracytoplasmic folding dilations and thickenings in the basement membrane attracted attention. Also, the lateral intercellular space became more wide than normal. Furthermore, apoptotic proximal tubular epithelial cells were seen. They were characterized by cell shrinkage, or separation of the nuclear envelopes, membrane blebbing and heterochromatin condensation and marginalization (Fig. 5c). Proximal tubular epithelial cells exhibited marked lesions in rats exposed to 5 mg kg^{-1} Cd^{2+} (group III). They

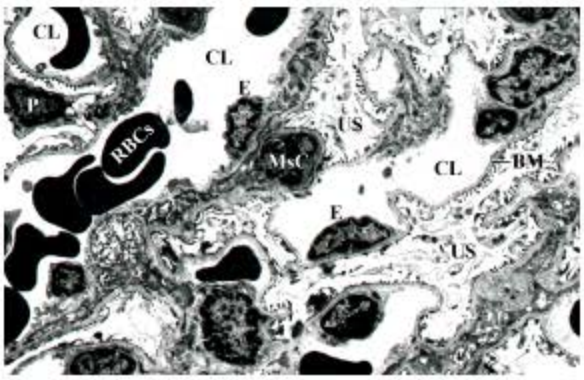

Fig. 1: The glomerular structure in the control group showing urinary space (US), podocytes (P) with foot processes, basement membrane (BM), capillary lumen (CL) lined with endothelial cells (E) and containing red blood cells (RBCs), mesangial cell (MsC), X3000

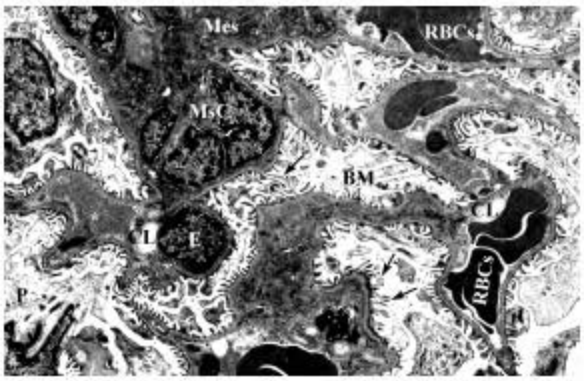

Fig. 2: The glomerular structure belonging to group II administrated 2.5 mg kg^{-1} b.w. Cd^{2+} showing podocytes (P), disorganized foot processes (arrows), thickening and ondulation in the basement membrane (BM), narrowing of the capillary lumen (CL) with compressed erythrocytes (RBCs) and homogenous coagulum (*), swollen endothelial cell (E), widening of mesangium with increased mesangial matrix (Mes), mesangial cell (MsC) with clefted nucleus, X3000

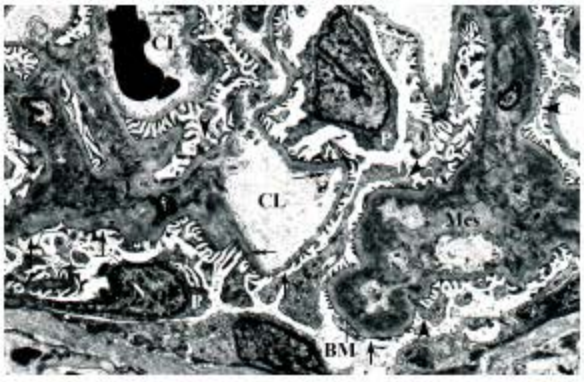

Fig. 3: The glomerular structure belonging to group III administrated 5 mg kg^{-1} b.w. Cd^{2+} showing podocytes (P) with irregular nuclei (N), arrows demonstrate loss of foot processes, arrowheads indicate fusion of foot processes forming blunted sheath of the cytoplasm. Note also, expansion of the mesangial area (Mes), increased thickness of basement membrane (BM) nearby the mesangium. CL: Narrow capillary lumen with marked loss of endothelial fenestrae (double head arrows), X3000

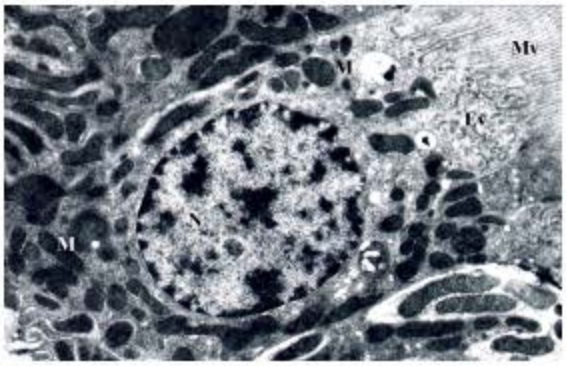

Fig. 4: The proximal tubular epithelium of the control group showing luminal border lined with closely packed microvilli (Mv), nucleus (N), apical endocytic vesicles (Ev) and numerous mitochondria (M) with thin, transverse and interdigitating cristae, X7500

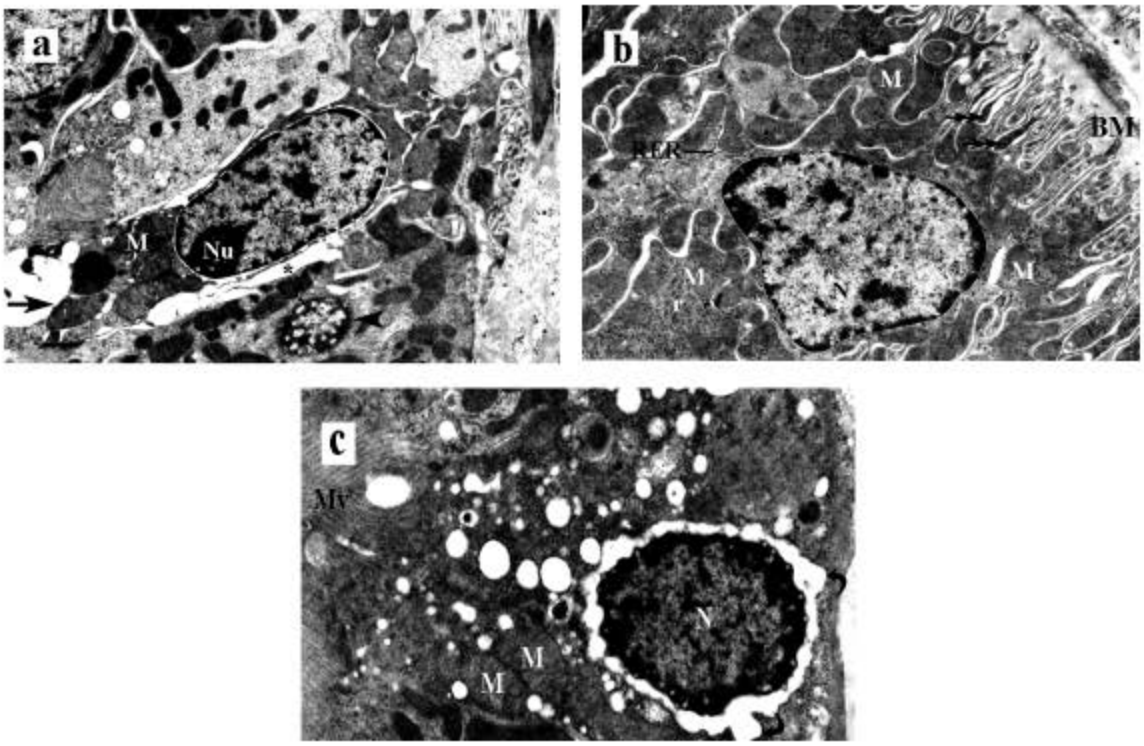

Fig. 5: The proximal tubular epithelium belonging to group II administrated 2.5 mg kg^{-1} b.w. Cd^{2+} showing: (a) loss of microvilli at certain site (arrow), degenerated nucleus (arrowhead), terminalized nucleolus (Nu), swollen mitochondria (M) and wide intercellular space (*), X5000; (b) thickenings in the basement membrane (BM) which appears hazy and dilations of intracytoplasmic foldings (double head arrows) could be observed. Note also, slight dilation of RER with ribosome (r) detachment, swollen mitochondria (M) with indistinct cristae. N: Nucleus, X7500; (c) apoptic cell with nucleus (N) exhibiting separation of the nuclear envelopes, membrane blebbing (curved arrows) and heterochromatin condensation and marginalization. Mv: Microvilli, M: Giant mitochondria, X7500

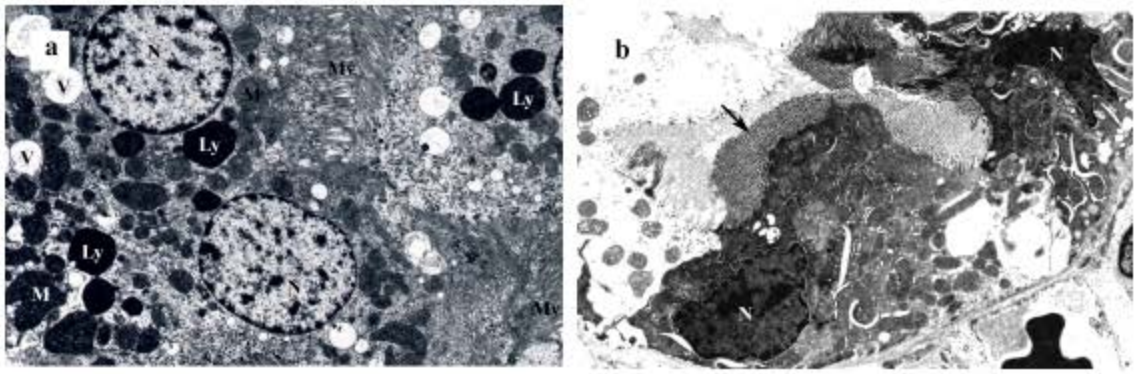

Fig. 6: The proximal tubular epithelium belonging to group III administrated 5 mg kg^{-1} b.w. Cd^{2+} showing: (a) disorganized apical microvilli (Mv), increased number of lysosomes (Ly), cytoplasmic vacuolation (V) and mitochondria (M) with swollen cristae. N: Nucleus, X4000; (b) epithelial cells undergoing apoptosis. Note shrunken cytoplasm, nuclear irregularity (N) and chromatin condensation. The microvilli are still intact (arrow), X4000

displayed large number of cytoplasmic vacuoles and numerous lysosomes (Fig. 6a). Considerable swelling and disruption of the inner mitochondrial cristae were noted for most cells. The brush border was markedly disorganized and frequently destroyed. Electron microscopic analysis of the kidneys of group III confirmed the presence of more apoptotic cell populations in the proximal tubular epithelial lining compared with group II. They exhibited shrunken cytoplasm, convoluted nuclei with invaginations in their nuclear membranes and chromatin condensation (Fig. 6b).

DISCUSSION

In the present study, an increase in renal MDA levels was observed following Cd treatment. Increased lipid peroxide levels after 24 h cadmium administration (2.5 mg kg^{-1} body weight) was previously demonstrated in the liver, kidney and blood of rats (Casalino *et al*., 2002; Ogjanovic *et al*., 2003). El-Maraghy *et al*. (2001) found an increase in MDA levels following single-dose Cd administration (0.5-2 mg kg^{-1}) in liver and kidney of rats. Similar result was also reported by Yalin *et al*. (2006) in rats following acute dose of Cd (5 mg kg^{-1} body weight).

Antioxidant enzymes like CAT, SOD and GPx form the first line of defense against ROS and the decrease in their activities contribute to the oxidative insult on the tissue. SOD detoxifies superoxide radicals and thus provides cytoprotection against free-radical-induced damage. Reports about SOD activity in Cd-treated rats are contradictory, some studies report an increase (Zikic *et al*., 1998; Ogjanovic *et al*., 2003) and some others report a decrease (Hussain *et al*., 1987; Stajn *et al*., 1997; Sarkar *et al*., 1998; Yalin *et al*., 2006) in activity. Casalino *et al*. (2002) demonstrated that SOD activity is strongly inhibited by cadmium, probably by interacting with metal moieties of SOD (Cu, Zn, or Mn) and thus reducing its activity. Alternatively, cadmium may alter the protein conformation by interacting with the enzyme, thereby altering its functional activity (Nagaraj *et al*., 2000). The alterations in SOD activity may depend on several factors such as Cd dose, Cd exposure time, type of Cd administration and the state of the animal (Yalin *et al*., 2006). In this study, a significant reduction in SOD activity of kidney was observed in Cd group compared to the control group.

CAT, which catalyzes the conversion of hydrogen peroxide to water and molecular oxygen, was found to be decreased in the kidney of the Cd group. This comes in agreement with Yalin *et al*. (2006). Sarkar *et al*. (1995) have shown that there was no significant change in CAT activity in kidney of rat after acute Cd exposure. However, Ogjanovic *et al*. (2003) have observed an increase in the CAT activity in the blood of rats.

GPx, a hydrogen peroxide-degrading enzyme, which requires selenium for activity was decreased in Cd-poisoned rats. Loss of GPx activity can be correlated to depletion of selenium by cadmium (Zumkley, 1988).

Acute exposure to Cd has been shown to decrease the activities of almost all enzymes (Yang *et al*., 1996; Sarkar *et al*., 1998), whereas chronic exposure causes an increase in the activities of antioxidant enzymes, as a result of cell adjustments (Kostic *et al*., 1993; Shukla *et al*., 2000). Koizumi and Li (1992) have

suggested that acute Cd exposure can increase oxidative stress by producing superoxide anions. In this study, the acute effect of Cd was investigated and found to have a significant effect on lipid peroxidation and oxidative stress.

In this study, the ultrastructural alterations occurring in the kidney were sudden responses to acute cadmium, with signs of cellular injury. Both glomeruli and tubules seemed to be sensitive to acute cadmium toxicity. The glomerular ultrastructural changes observed in cadmium-treated rats included narrowing of the capillary lumen and swelling of the capillary endothelium. The mesangium was wide with increased mesangial matrix. The narrowing of capillary lumina contributes to the hypertension reported by Puri and Saha (2003) in rats following acute cadmium intoxication. Hypertension may result from cadmium-induced changes in vasculature, the rennin-angiotensin system, or renal ion transport process. Nolan and Shaikh (1986) indicated that the initial effect of acute cadmium administration is on the integrity and permeability of the vascular endothelium; other necrotic changes occur secondarily to this effect.

Impaired glomerular filtration was indicated in the present study by increases in serum creatinine and urea. Ultrastructural findings stressing this functional pathology were indicated by failure to trace the fenestrate belonging to endothelial cells of the glomerlular capillaries, loss of homogenous appearance of basement membrane displaying ondulation and thickening in many areas and deterioration of the slit membrane structures formed by the podocytes. Uriu *et al.* (1993) also found decreases of Glomerular Filtration Rate (GFR) and Renal Blood Flow (RBF) in rats after acute administration of cadmium chloride.

The effects of cadmium on proximal cell ultrastructure were focal loss of brush border, nuclear membrane damage, chromatin condensation, swelling of the mitochondria with regression of mitochondrial cristae, degranulation and disintegration of protein-synthesizing structures such as rough endoplasmic reticulum, increased number of lysosomes and ultimately cell death. Condron *et al.* (1994) indicated that the surface density of microvillus membrane per unit cell volume was reduced by 19% in acute cadmium-dosed rats. Cadmium inhibits vacuolar H^+-ATPase and endocytosis in proximal tubule brush border of rat kidney and this may inhibit endocytosis of filtered proteins and impair vesicle-mediated recycling of some membrane transporters, thus contributing to the loss of reabsorptive capacity of the proximal tubules (Herak-Kramberger *et al.*, 1998). In the proximal tubule, Cd^{2+} has been shown to decrease phosphate and glucose transport by inhibiting the NaPi and the Na/glucose cotransporters, respectively. In the terminal segments, Cd^{2+} exerts a blocking effect on ion channels such as the Epithelial Calcium Channel (ECaC) and the renal outer medullary K^+ channel (ROMK) (Barbier *et al.*, 2005). Acute Cd^{2+} injection results in renal losses of Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{-2} and water in rats (Barbier *et al.*, 2004).

The mitochondrial swelling and lysis of cristae may reflect the disturbances in oxy-reduction processes taking place in the organelle (Thevenod, 2003). The mitochondria are a source of reactive oxygen species and a target of excessive ROS generation which plays important factor in cadmium-induced apoptosis (Pulido and Parrish, 2003). Excess ROS increases the mitochondrial membrane permeability and damages the respiratory chain resulting in increased ROS production (Chen *et al.*, 2001). The disruption in the mitochondrial membranes causes the release of cytochrome c from the mitochondria, which initiates events leading to apoptosis. Although the exact mechanism is undefined, ROS are thought to play a role in TNFR and Fas receptor-mediated apoptosis (Kranmer, 1999).

The dilation of rough endoplasmic reticulum and detachment of ribosomes is one sign of cell injury, causing the cell to stop functioning and leading to cell lysis (Cheville, 1994). These results indicate that cellular proteins are likely targets of cadmium action. Acute cadmium suppresses functional activity of tRNA, which effect may account for the decreased activity of the whole translation process during intoxication with cadmium (Ivanov *et al.*, 2002). Changes in the nucleus, such as heterochromatin condensation and marginalization, which was found in the present study, indicate that this organelle is affected in a major way from cadmium exposure. Heterochromatin condensation and marginalization suggest progressive inactivation of the nuclear component, probably due to inhibition of DNA repair and DNA methylation (Waisberg *et al.*, 2003). Furthermore, proliferation in the number of lysosomes in proximal cells is typical of heavy metal exposure. It has been suggested that this alteration may represent a cellular response to heavy metals such as cadmium. The increased number of lysosomes, a result of the attempt to digest these heavy metals or toxic substances, is considered a general manifestation of injury. The sequestration of damaged organelles in lysosomes is a mechanism of cellular repair and follows all types of sublethal injury (Cheville, 1994).

This study has been carried out with the intension of analyzing the toxic mechanism of acute cadmium induced

nephropathy in rats, with a view to providing a basis for understanding pathology in humans. A very important finding of this study is that a single injection of Cd acts on the whole kidney, especially on the proximal tubules, even at low accumulation in this organ (Swiergosz-Kowalewska, 2001). It confirms the hypothesis that humans environmentally exposed to cadmium, especially smokers, are at risk of renal dysfunction. Acute poisoning as a result of exposure to cadmium has increased in industrialized countries. The salts of cadmium are used as pigments and stabilizers in plastics. Reported cases of acute cadmium poisoning in human are well documented (Lucas *et al*., 1980; Barnhart and Rosenstock, 1984; Yates and Goldman, 1990; Seidal *et al*., 1993).

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