Protective Effect of Zinc (Zn) on the Histology and Histochemistry of Liver and Kidney of Albino Rat Treated with Cadmium

Alsayed Ali Mahran1, Husam Eldien H. Osman1, Ahmed M. A. Abd El-Mawla2,3 and Adel M. Attia4

1Department of Anatomy and Histology, Faculty of Medicine and Medical Sciences, Taif University, Taif 21974, Saudi Arabia
2Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt
3Department of Pharmacognosy, Faculty of Pharmacy, Taif University, Taif 21974, Saudi Arabia
4Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Taif University, Taif 21974, Saudi Arabia

Abstract

The protective effect of zinc (Zn) on the liver and kidney of albino rats exposed to intraperitoneal injection of cadmium chloride (CdCl2) was studied. Light microscopic examination for rats which were injected intraperitoneally with 0.16 mg CdCl2/kg of body weight for 8 weeks indicated severe histological changes in both liver and kidney. In liver, a blurred trabecular structure, vacuolar degeneration and increased density of nuclear chromatin with very compact nuclear structure were found in hepatocytes. Moreover, mononuclear cell infiltrations and necrosis of single cells were also observed. In the kidney tubules, degeneration and hypertrophy of epithelial cells and dilation in the glomeruli were also observed. The effects of cadmium on the ultrastructures of both organs were studied. There are ultrastructural damages appeared in both organs as 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. Zn partially alleviated the damage observed in both the liver and kidney and differences in histological structure has been observed between the Zn-Cd and the control groups. Our results demonstrate the protective effect of ZnCl2 in prevention CdCl2-induced significant toxic pathological changes in the liver and kidney of the albino rats.

Keywords: Zinc chloride; Cadmium chloride; Kidney; Liver; Histology; Histochemistry

Introduction

Cadmium (Cd) is known as a heavy and high toxic metal that widely distributed in the environment. It is present in trace levels in seawater and a broad range of animal and plant species [1-5]. It was reported that the maximum tolerance Cd level for domestic animals was 0.5 ppm. Dietary concentrations of one ppm Cd results undesirable effects, while concentrations of 5 ppm leads to adverse health effects [6]. Gastrointestinal absorption of Cd is affected by the diet and nutritional status [7]. Absorption of ingested Cd is only about 5% and after absorption it accumulates in the liver and then in the kidney [8,9]. Therefore, one of the most important health effects of chronic Cd exposure is the liver and kidney damage [2,10,11].

Zinc is one of the important nutrients that can reduce the toxicity of orally administrated Cd and shows its effect by competing with Cd for some transport system as well as for the binding sites in the metallothionin [2,9,12,13]. The aim of the present study was to investigate the histological and histochemical changes in the liver and kidney of albino rat exposed to intraperitoneal injection of Cd, and the protective effect of intraperitoneal injection of zinc on Cd-induced changes in both.

Materials and Methods

Animals

The present study was based on materials obtained from 60 adult albino rats of both sexes with body weigh from 220-250 gm. The experiment was conducted to 8 weeks, and the animals were randomly designed into three groups, each of 20 rats: two experimental groups and one control group. Each group was isolated in a single cage and kept in the same conventional condition of diet and environment in the animal house.

Chemicals

The cadmium chloride (CdCl2) and zinc chloride (ZnCl2) of analytical grade or higher purity were the substances used in this study. Each was soluble in physiological saline and was injected intraperitonealy at dose levels of 0.16 mg CdCl2/kg of body weight and 0.53 mg ZnCl2/kg boy weight [9].

Experimental design

The experiment was conducted for 8 weeks. The animals were randomly allocated into three groups: two experimental groups and one control group each include 20 rats.

Rats of experimental group 1 were injected intraperitonealy with cadmium chloride solution (0.16 mg CdCl2/kg of body weight) [9].

Rats of experimental group 2 were injected intraperitonealy with both zinc chloride solution (0.53 mg ZnCl2/kg of body weight) [9] after injection with cadmium chloride in an aforementioned dose.

Rats of control group were received injection of the same dose of normal saline. The injections were done into the peritoneum of used rats for eight weeks five times/week. The animals of each group were

*Corresponding author: Dr. Ahmed Abd El-Mawla, Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt, Tel: +20882411330; Fax: +20882332776; E-mail: Ahmedpha2000@yahoo.com

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sacrificed with inhalation of overdose of ether. The kidney and liver were excised and divided into small pieces for histological examination.

**Light microscopy**

Small slices of kidney and liver tissue were taken and fixed in 10% formalin for 24 hours, and were imbedded in paraffin. Five-micron-thick sections were routinely stained with hematoxyline and eosin [14].

**Histochemical study**

Some paraffin sections were stained with (a) periodic acid-Schiff’s method to demonstrate carbohydrates [15] and with (b) Mallory method to demonstrate the tissue fibroses [14].

**Electron microscopy**

Both liver and kidney were cut into small pieces 1 mm thick and fixed in 4% F.O.G in phosphate buffer solution (pH 7.2) for 3 h at 4°C, after which the tissues were removed and postfixed in buffered 2% OsO4 for one hour 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, collected on naked copper-mesh grids and stained with uranyl acetate for 1/2 hour and lead citrate for 20-30 min [16].

**Results**

**Light microscopic observations**

The Liver of a control rat should a normal structure where the liver appeared to be composed of hexagonal or pentagonal lobules with a central vein and peripheral hepatic triads or tetrads embedded in connective tissue. The hepatocytes are arranged in trabecules running radiantly from the central vein and the spaces between the cell cords called blood sinusoids which converged towards the central vein and lined by Kupffer cells. Also the hepatocytes are regular and contain a large spherical nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Some cells have two nuclei (Figure 1 and 2).

The Kidney of control rats, had normal renal structure of both (a) cortex which showed a normal structure of renal glomeruli.

The proximal convoluted tubules are lined with typical thick cubic epithelium. The distal convoluted tubules show considerably lower cubic epithelium. The tubules have a relatively regular distinct lumen. The glomerular capsule are lined with a flat epithelium, and (b) medulla in which, the collecting tubules are lined with the relatively low simple cubic epithelium. The thick descending and ascending parts of Henle’s loops are lined with simple cubical epithelium with small caliber, and a small amount of interstitial tissue can be seen normally in the cross-sections (Figure 3 and 4).

Light microscopic examination in the liver of rats treated with CdCl2 showed that there were degenerative changes in numerous hepatocytes; the cells were enlarged and had light and foamy cytoplasm filled with numerous vacuole-like spaces. The walls of the blood sinusoids were dilated and showed numerous Kupffer cells. In a few liver zones, the CdCl2 induced also hepatocytes necrotic changes which appeared as; a small, pyknotic cellular nucleus with condensed chromatin, lack of nucleolus and strongly acidophilic cytoplasm. Mononuclear cell infiltrates were also noted in hepatic areas (Figure 5 and 6).

Light microscopic examination in the kidney of rats treated with CdCl2 only showed that there were many areas of tubular damages.
ranged from mild to severe in the kidney were observed in all treatment animals. These renal damages appeared as hypertrophy and degeneration of epithelia of renal tubules with distinct of mononuclear cells infiltration. A few renal tubules showed single epithelial cells desquamated to their lumen. Also some vascular glomeruli were apparently enlarged, tightly filling the Bowman’s capsule with absence of the capsular spaces was observed. Moreover, hyperaemia of the kidney vessels was observed (Figure 7 and 8).

ZnCl₂ in combination with CdCl₂ caused a reduction of toxic effects of CdCl₂ on the liver were an absence of nucleus fragmentation and a decrease in the sinusoidal dilation, necrosis of some hepatocytes, and mononuclear cell infiltrations; was observed. In fact we noticed the presence of rare inflammatory sites in the sinusoids and some hepatocytes with light cytoplasm (Figure 9 and 10).

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Light microscopic examination also revealed a positive correlation between ZnCl₂ and CdCl₂ in the kidney tissues with marked reduction...
of the toxic effect on the kidney. However, some toxic effects of CdCl₂, as mild hyperaemia in the kidney vessels, some degenerative changes in the tubular epithelium and cystic dilatation were observed (Figure 11 and 12).

**Histochemical observations**

The light microscopic observations revealed that, the liver and kidney tissues of the control group should positive PAS reaction in the cells cytoplasm, more in the liver (Figure 13) than in the kidney (Figure 14) with PAS positive reaction in the brush borders of the proximal convoluted tubules. While the glomeruli were intensely positive to PAS reaction.

The Liver and kidney tissues of the rats exposed to CdCl₂ alone should, a marked reduction in PAS reaction in all vicinity of the liver particularly the hepatocytes (H). N.B. Bile duct (B). Hepatic artery (A), and blood sinusoid (S). Periodic acid-Schiff’s X 400.

The PAS reaction of both liver and kidney tissues of the rats exposed to CdCl₂ in combination with ZnCl₂ for eight weeks appeared convoluted tubules. While the glomeruli were intensely positive to PAS reaction.

The Liver and kidney tissues of the rats exposed to CdCl₂ alone should, a marked reduction in PAS reaction in both liver (Figure 15) and kidney (Figure 16) tissues particularly in degenerative and necrotic areas. The kidney tissue appeared more affected than the liver. The reduction in PAS reaction was more intensive in the renal tubules and glomeruli.

The PAS reaction of both liver and kidney tissues of the rats exposed to CdCl₂ in combination with ZnCl₂ for eight weeks appeared...
to have a moderate increased in intensity of PAS positive reaction in the liver (Figure 17) and kidney (Figure 18) tissues but not reach to the normal level.

Transmission electronmicroscopic observations

The Liver of a control rat showed that a normal ultrastructure; the cytoplasm of hepatocytes appeared to have a fine granular appearance due the presence of numerous free glycogen granules. The nuclei of the hepatocytes were oval or rounded and the nucleoplasm showed a fine granular component with a thin peripheral heterochromatin and euchromatin condensation. The mitochondria were numerous, usually round to oval in shape. The blood sinusoids appeared to be lined by flat endothelial cells or their extensions which were separated from the adjacent hepatocytes by the subendothelial space or space of Disse (Figure 19).

Transmission electron microscopic observations of kidney of the control rats showed the endothelial cells in the glomerular capillaries were very richly fenestrated with large pores which appeared to lack any trace of a closing diaphragm. The proximal renal tubular epithelium of control rat is characterized by a dense brush border, basal or central nucleus, apical endocytic vesicles, occasional lysosomes and elongate or round mitochondria. Cisternal profiles of rough ER located between the mitochondria (Figure 20).

In the experimental group 1 which exposed to CdCl₂ alone the transmission electron microscopic observation of the liver tissue revealed a histopathological changed in the hepatocytes, which appeared as thickening and enfolding of the cell membrane ,damage of the nuclear membrane ,regression of mitochondrial cisternae, deterioration of rough endoplasmic reticula, losses of glycogen particles, and proliferation of smooth endoplasmic reticula with condensation of the nuclear chromatin. The hepatocytes also appeared to be contains many cytoplasmic fat droplets and many vacuoles (Figure 21). The blood sinusoid appeared to be more dilated with many Kupffer cells seen in contact with its endothelial lining. The hepatocytes appeare to be contains many cytoplasmic fat droplets and many vacuoles (Figure 21).

The CdCl₂ induced a toxic ultrastructural changed in the kidney which observed as, enlargement of the glomeruli with narrowing of
the capillary lumen, swelling of the capillary endothelium, and loss of the fenestrae. Damage of the renal tubules was noted as focal loss of the brush border of the epithelial lining of proximal renal tubules, disturbance of the nuclear membrane, chromatin condensation, swelling of several mitochondria with regression of their crestae. The protein synthesis rough endoplasmic reticulum was degenerated. Increased number of lysosomes and cell death were also noticed (Figure 22 and 23).

Rats of experimental group 2 which exposed to CdCl₂ in combination with ZnCl₂ in this group, co-administration of ZnCl₂ practically prevented most of the ultrastructural pathological toxic effects of CdCl₂ on the liver tissues. In fact we observed the presence of rare sites of ultrastructural pathological changes in the blood sinuoids and hepatocytes (Figure 24). In the kidney, many of ultrastructural pathological changes caused by the toxic effect of CdCl₂ on the kidney tissues were noticed to be decreased in the kidney tissues of rats received ZnCl₂ in combination with CdCl₂ for eight weeks (Figure 25).

**Discussion**

Cd is used worldwide in modern industries. Therefore, Cd pollution was internationally recognized as the most important environmental health hazard that remains to be eliminated [17]. When Cd is taken into the human body through the oral route by the ingestion of contaminated water or food, it accumulates in the liver and kidneys and its half-life is very long, exceeding 10 yr [10,18].

Once Cd is absorbed into the liver from the digestive tract, it stimulates the synthesis of metallothionin (MT) in the organs and the forms MT-bound Cd (MT-Cd). The MT-Cd transfers to the kidneys via the blood stream. When MT-Cd reaches the kidney, it is filtered through the glomerular membrane and is reabsorbed in the proximal tubular cells. MT-Cd shows strong nephrotoxicity after pinocytosis through the glomerular membrane and is reabsorbed in the proximal tubular cells.

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**Figure 21**: Ultramicrograph of a section of liver of rat exposed to Cd Cl₂ for 8 weeks, showing two liver cells with heterochromatic nuclei (N), numerous elongated mitochondria (M). Some lipid droplets (F), many lysosomes (broken arrows) as well as many vacuoles (V) appeared in cells cytoplasm. A blood sinusoid (arrow) contains RBCs (*) is seen in between. The basal lamina appeared thick and infolded (CM). TEM mag. =8000X.

**Figure 22**: Ultramicrograph of a section of kidney of rat exposed to Cd Cl₂ for 8 weeks, showing cell of proximal convoluted tubule with rounded heterochromatic nucleus (N), basal elongated mitochondria (M), and thick enfolded cell membrane (CM) disorganized apical microvilli (Mc) around the tubular lumen (arrow). The protoplasm appeared to be contains numerous vacuoles (V) and lysosomes (broken arrows). TEM mag. =8000X.

**Figure 23**: Ultramicrograph of a section through juxtaglomerular complex of kidney of rat exposed to Cd Cl₂ for 8 weeks, showing (1) Macula densa (Md) with elongated nucleus (N), prominent nucleoli (n) and cytoplasm contains many mitochondria (M), vacuoles (V) and lysosomes (broken arrows). (2) Juxtaglomerular cell (J.G) has rounded heterochromatic nucleus (N), nucleolus (n), many mitochondria (M), and pale vacuolated protoplasm. The basal lamina (CM) of the seen cells is thick and enfolded. TEM mag. =6000X.

**Figure 24**: Transmission electron microscopic picture of a section of liver of rat exposed to Cd Cl₂ and ZnCl₂ for 8 weeks, showing, hepatocyte with euchromatic nucleus (N) and prominent nucleolus (n). Golgi apparatus (G) and rough endoplasmic reticulum (RE), numerous mitochondria are seen in less vacuolated protoplasm. TEM mag. =8000X.

**Figure 25**: Transmission electron microscopic picture of a section of kidney of rat exposed to Cd Cl₂ and ZnCl₂ for 8 weeks displays two cells of distal convoluted tubules (D1&D2) and one cell of proximal convoluted tubules (P) with euchromatic nuclei (N) and nucleolus (n), many mitochondria (M) and dark nonvacuolated protoplasm. Intact microvilli (Mv) surrounded the lumen (arrow) of the proximal convoluted tubule. TEM mag. =4000X.
In agreement with a large number of studies who noted similar or more pronounced changes in the hepatic and renal tissues under Cd effect [2,9,11,20-24]. Our results indicated significant CdCl2 toxic effects on the liver and kidney structures. As mentioned previously [20] early pathological changes in rat kidney obtained after 6 weeks of administration of 50 mg Cd/l in drinking water. After 12 weeks, they revealed signs of tubular necrosis, interstitial fibrosis and glomerular epithelial cell hypertrophy in small areas of the kidney cortex. In fact, it has been suggested that Cd disturbs membranes integrity [25,26] and generates reactive oxygen species [22,27] and involves cytotoxic and inflammatory mediators [28,29] in the liver and kidney.

In agreement with a large number studies [11,24,30-33], our results indicated a significant ultrastructural damages in CdCl2 contaminated liver, which observed mainly in hepatocytes as chromatin condensation, swelling of mitochondria with regression of mitochondrial cristae, disintegration of protein synthesis structures such as rough endoplasmic reticulum and increased number of lysosomes and ultimately cell death. Also the hepatocytes cytoplasm showed some intra cellular vacuoles and reduced glycerogen granules. These were evident in all animals of the Cd-contaminated group. Similar or more advanced changes in the liver and kidney structures under CdCl2 influence have been reported by others [11,24,30-35] who suggested that Cd inhibits protein synthesis and glycogen metabolism in the liver. Also reported data [36] postulated that, cell membranes were disintegrated and manifested by the occurrence of vacuoles in the cytoplasm, and in some cells the changes were less evident and dense mitochondria with distinct membranes were found.

In the present study, some pathological changes in kidney ultrastructure as narrowing of the capillary lumen and swelling of the capillary endothelium of the glomeruli, injured brush-border microvilli and swollen mitochondria in the proximal convoluted tubular cells were observed. These results were in agreement with that of [11,22,23,37].

The narrowing of capillary lumina contributes to the hypertension reported previously [38] in rats following cadmium intoxication. Hypertension may result from cadmium-induced changes in vasculature, the rennin-angiotensin system, or renal ion transport process. The initial effect of cadmium administration is on the integrity and permeability of the vascular endothelium; other necrotic changes occur secondary to this effect have been declared [39].

In the present study, 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 crestae, degranulation and disintegration of protein-synthesizing structures such as rough endoplasmic reticulum, increased number of lysosomes and ultimately cell death. These changes were attributed to the reduction of the surface density of microvillus membrane per unit cell volume to 19% in cadmium contaminated rats [15,40]. Cadmium also inhibits the 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 [41].

The mitochondrial swelling and degeneration of the mitochondria cristae observed in our study may reflect the disturbances in oxygen reduction processes taking place in the organelle [42]. Also, the changes in the nucleus, such as chromatin condensation indicate that this organelle is affected in a major way from cadmium exposure. Chromatin condensation suggests progressive inactivation of the nuclear component, probably due to inhibition of DNA repair and DNA methylation [43,44]. Furthermore, proliferation in the number of lysosomes in cells of the proximal tubules 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 [45].

In Zn co-treated animals, we noticed an improvement in the Cd-induced damage in the liver. Our results were in agreement with published data [46] which reported that the effect of co-treatment with Zn during Cd administration completely prevented the changes in renal function produced by the toxic metal in the rat, even though they did not find any significant difference in the renal Cd content. There is considerable information in the literature regarding the protective effect of Zn against the cellular toxicity caused by Cd. Previous studies [47-50] supposed that Zn protection is perhaps due to redistribution of Cd in the organism since Zn is able to induce synthesis of metallothionin in the liver and kidneys. However, another hypothesis must be proposed to explain the protective effect of Zn which appeared through our histological. Findings in spite of the further accumulation of Cd in the liver and kidney. In fact, in recent studies, Zn has been demonstrated to play an important rule in preventing the oxidative stress, apoptosis and necrosis induced by Cd [9,51-53].

In the present study there is considerable improvement in Cd induced toxic damages on both liver and kidney tissues in Zn supplementation with Cd for 8 weeks. Our results were in agreement with those previously reported [9,24,46,51,53] which postulated that, Zn play an important rule in preventing the oxidative stress, apoptosis and the necrosis induced by Cd. Moreover, in a plant study, Zn and Se have been shown to have similar characteristics in their antagonism against Cd. Soil enrichment with the two trace elements was helpful in antagonizing Cd absorption by plant roots [54]. In contrast to the present findings, it has been reported that, no significant effect of Zn on Cd toxicity of liver and kidney tissues, and they attributed this lack of positive effect of Zn to the lack of time for metallothionen synthesis caused by administration of Zn and Cd simultaneously [2].

Conclusion
Our results demonstrate the ameliorative effect of Zn supply on the Cd induced toxic structural changes in the liver and kidney tissues of the rats. These results validate the hypothesis that the metabolism and toxic action of Cd may be modulated by Zn supplementation.

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