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Role of Zinc in the Protection Against Cadmium Induced Hepatotoxicity

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Abstract: The influence of Zinc (Zn) on Cadmium (Cd) intoxication was investigated in male rats. The exposure of the rats to Cd (2.2 mg kg⁻¹ CdCl₂, injected subcutaneously 4 times weekly for 2 months) caused alterations in ultrastructure of liver as manifested by deterioration of mitochondrial cristae, deposition of large amount of collagen fibrils and a hypertrophy of Kupffer cells accompanied by presence of large sized lipid droplets in their cytoplasm. On the other hand, the blood hydroperoxide level, concentrations of Zn, Cd and reduced Glutathione (GSH) in the liver were increased during the treatment period with Cd. On the other hand, blood GSH level and hepatic catalase activity decreased in the rats injected with Cd. Moreover, treatment with Cd produced significant increases in plasma Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), gamma Glutamyl Transferase (γ GT) and alkaline phosphatase activities and bilirubin concentration. Administration of Zn (2.2 mg kg⁻¹ ZnCl₂ injected s.c. 4 times weekly for 2 months) one hour prior to cadmium exposure ameliorated the toxic effects of Cd. Zn alleviated the elevation of hydroperoxide level, alterations in ultrastructure of liver and hepatic enzymes and Cd accumulation induced by Cd. We conclude that Zn could serve as a physiological antioxidant against cadmium-mediated oxidative stress.

Key words: AST, ALT, γ GT, bilirubin, GSH

INTRODUCTION

Heavy metals constitute serious threats to human health^[1]. Cadmium is a wide spread environmental pollutant, characterized by its toxicity in various organs^[2]. It induced serious peroxidation in membrane structures^[3]. The molecular mechanisms responsible for the toxic effects of Cd include interference with antioxidant enzymes^[4], alterations in thiol proteins^[5] and alteration in DNA structure^[6].

Hepatotoxic effects of Cd are evidenced by increased plasma levels of ALT, AST and alkaline phosphatase^[7]. Histological evaluation of liver damage induced by Cd exposure reveals that acute toxicity provokes parenchymal cell necrosis, infiltration of inflammatory cells^[8], hepatocellular swelling, sinusoidal congestion, pyknosis and karyorrhexis^[7]. These cellular changes may result in both apoptosis and necrosis. Moreover, release of apoptogenic proteins is likely related to calcium induced alteration of mitochondrial homeostasis which is also preceded by production of reactive oxygen species^[9]. On the other hand, Cd induced metallothionein synthesis plays an important role in the detoxification of heavy metals^[10].

Zinc (Zn), the most prevalent trace element in the body is involved in the structure and function of some enzymes collectively representing all major biochemical categories and therefore is essential for normal cell function and metabolism^[11]. Zn functions as a complex antioxidant. It has the ability to form coordinating bonds with electronegative atoms^[12]. It regulates metallothionein synthesis and can interact with radical of several compounds^[13]. Zn inhibited oxidative stress induced by cadmium^[14], alloxan^[15], malathione^[16] and chromium^[17]. Moreover, Zn deficiency results in increase of oxidative damage to cells^[18] as well as alterations in components of the antioxidant defense system^[19]. The present study was undertaken to investigate some of the protective mechanisms of Zn against Cd induced liver injuries correlating the ultrastructural changes of liver and biochemical markers.

MATERIAL AND METHODS

Animals: One hundred and twenty-eight male Wister rats were used in the present investigation. The age of rats was five months and their weights ranged from 180 to 220 g. The animals were maintained under standard

laboratory condition (12 h light, temperature $23\pm 1^\circ\text{C}$). They fed dry ration *ad lib*.

Chemicals: Cadmium as cadmium chloride (CdCl_2) and zinc as zinc chloride (ZnCl_2) were purchased from Merck (Dormstadt, Germany). The chemicals used were of the highest purity.

Experimental design: The animals were treated four times weekly for eight weeks. The rats were randomly divided into four groups of thirty-two animals each. The groups were: Group 1 served as control and received the equivalent volume of saline, Group 2 injected s.c. with 2.2 mg Cd kg^{-1} body weight in 0.1 mL saline, Group 3 injected s.c. with 2.2 mg Zn kg^{-1} 1 h prior to Cd injection (2.2 mg kg^{-1}) and Group 4 injected s.c. with zinc alone. Eight blood samples from Group 1, 2 and 3 were collected after 2, 4, 6 and 8 weeks and plasma were separated. The fourth group was used to determine the concentration of Zn in the liver only, while other parameters were not analyzed for this group. Eight animals from each group were sacrificed at the different time intervals and liver samples were kept frozen.

Electron microscopy: At the end of the experiment, tissue samples from the liver of all experimental groups except Group 4, were removed and fixed by immersion in 3% buffered glutaraldehyde (0.1 M sodium cacodylate buffer, pH 7.2) for at least 4 h at 4°C . Hepatic tissue specimens were then post fixed in 1% osmium tetroxide (OsO_4) in the same buffer for 2 h, dehydrated and transferred to epoxy resin mixture via propylene oxide. Ultrathin sections (70-80 nm) were cut using a diamond knife and double stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (JEOL 100 CX).

Biochemical assays

Blood hydroperoxide level: Blood levels of hydroperoxide were evaluated using free radical analytical system (IRAM, PARMA, Italy). The test is a colorimetric test that takes advantage of the ability of hydroperoxides to generate free radicals after reacting with some transitional metals. When buffered chromogenic substances (N, N-diethyl-Paraphenyldiamine) are added, a coloured complex appears. The complex can be measured by a spectrophotometer (absorbance peak: 505 nm). The test results are expressed in units called carr units. One carr unit corresponds to $0.08\text{ mg } 100\text{ mL}^{-1}$.

Cadmium and zinc content in liver cells: Cd and Zn content in liver cells was measured using atomic absorption spectrophotometry. Certified reference

solutions were used to generate standard curves for each element.

Hepatic catalase activity: Catalase activity was assayed according to method described by Aebi^[20].

Glutathione concentration: Blood glutathione was determined by colorimetric method of Beutler *et al.*^[21]. Hepatic glutathione concentration was evaluated using a dithionitrobenzoic acid reactivity method^[22].

Plasma liver enzymes and bilirubin: ALT, AST, alkaline phosphatase and γGT activities were determined by kinetic methods using bioMerieux kits, France. Moreover, total bilirubin concentration in plasma was evaluated colorimetrically.

Statistical analysis: The data were expressed as mean values \pm SE of 8 rats in each group and statistical difference between groups were assessed by students t-test.

RESULTS

Electron microscopy

Control animals: Hepatocytes of control animals revealed the normal ultrastructural features including the nucleus and cytoplasmic organelles (Fig. 1). The hepatocytes nuclei were regularly rounded having the normal proportion of euchromatin and heterochromatin with the latter being of uniform density. Mitochondria were of normal size and their cristae and matrices were morphologically normal concerning the regularity of the earlier and the density of the latter. The shape and extension of the Rough Endoplasmic Reticulum (RER) and its association with the mitochondria were normal. Kupffer cells were neither proliferated nor hypertrophied and their cytoplasm contained the normal amount of the tiny dense bodies reflecting its enzyme content. No deposited collagen fibrils were seen either within the hepatic lobules or at the vicinity of Kupffer cells and sinusoidal walls.

Cd-exposed group: The mostly affected organelle in the cytoplasm of the hepatocytes was the mitochondria. Large number of mitochondria showed marked swelling and this was accompanied with a reduction of the mitochondrial matrix density. The decrease in electron density was obvious in all affected mitochondria. Mitochondrial cristae were evidently deteriorated as evidenced by their fragmentation and even dissolution (Fig. 2). In the less affected mitochondria, the cristae were marginated and of decreased number. Considerable

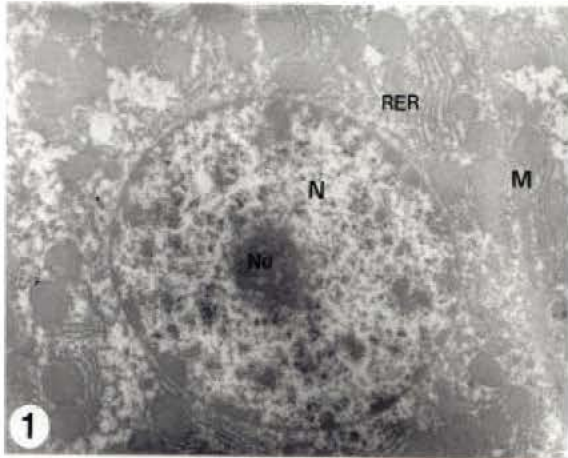


Fig. 1: Hepatocyte of a control non-exposed rat showing the normal ultrastructural features. The nucleus (N) is rounded having a prominent nucleolus (Nu) and regular chromatin. Cytoplasmic organelles including mitochondria (M) and RER are morphologically normal. Transmission electron micrograph. x 6700

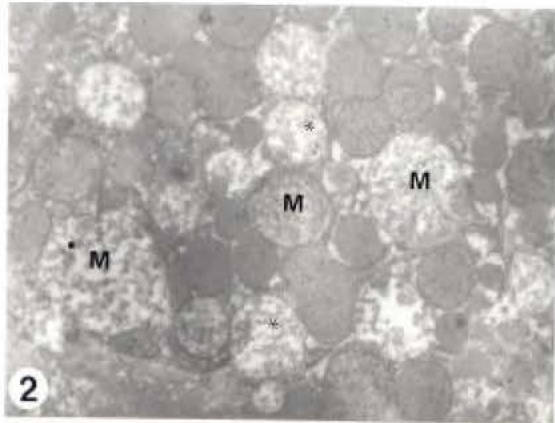


Fig. 2: Swollen mitochondria (M) in hepatocytes of a Cd-exposed rat. Mitochondrial cristae (*) are obviously deteriorated evidenced by their fragmentation and dissolution. Transmission electron micrograph. x 20.000

number of hepatocyte nuclei was smaller and had more condensed chromatin.

An outstanding feature in the hepatic tissues of the Cd-exposed animals was the deposition of large amount of collagen fibrils. The extension of these collagen fibrils in the form of bundles was noticed in the parenchyma on background of the degenerated organelles (Fig. 3). The existence of collagen fibrils was also noticeable in the vicinity of the proliferated Kupffer cells (Fig. 4). The

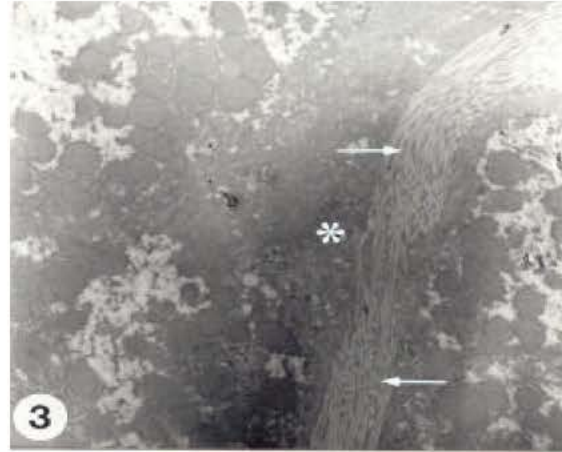


Fig. 3: Liver tissue of a Cd-exposed rat showing the extension of a collagen bundle (arrows) on the background of degenerated cytoplasmic organelles (*). Transmission electron micrograph. x 6700

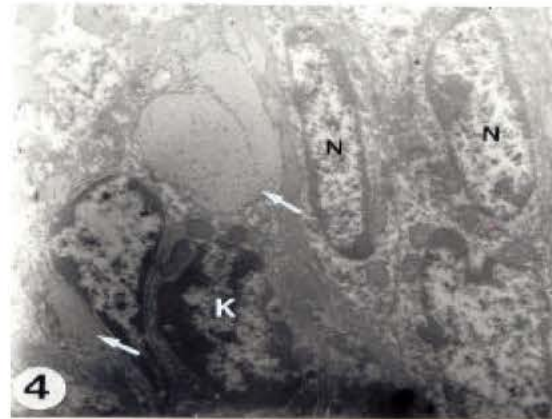


Fig. 4: Deposited collagen fibrils (arrows) in the vicinity of proliferated Kupffer cells (K). Note the nuclei (N) of the hyperplastic fibroblasts which yielded the collagen fibrils. Liver tissue of a Cd-exposed rat. Transmission electron micrograph. x 8000

proliferation of fibroblasts seemed to originate from the sinusoidal walls. The Kupffer cells at sites of fibrosis were occasionally necrosed as evidenced by the fragmentation of their nuclei (Fig. 5). Also, Kupffer cells were frequently hypertrophied since their nuclei were larger in size and their cytoplasm was impacted with large-sized lipid droplets (Fig. 6).

Cd+Zn-exposed group: Most of the cytoplasmic organelles revealed nearly normal ultrastructural morphology (Fig. 7). Although, some degenerated mitochondria did exist, but the number of the swollen

Table 1: Effect of subcutaneous administration of Zn and Cd on blood hydroperoxide level and hepatic cadmium concentration

Week post-treatment	Parameters					
	Hydroperoxide level of blood (Carr U.)			Hepatic cadmium concentration ($\mu\text{g g}^{-1}$)		
	Control	Cd	Zn + Cd	Control	Cd	Zn + Cd
2	323.10 \pm 16.62	484.80 \pm 13.25**	402.40 \pm 21.59***	0.89 \pm 0.16	374.20 \pm 13.93**	237.60 \pm 5.79***
4	331.60 \pm 13.22	562.70 \pm 34.42**	423.00 \pm 24.43***	1.07 \pm 0.08	772.80 \pm 33.42**	441.60 \pm 19.96***
6	325.20 \pm 12.28	593.61 \pm 21.63**	469.80 \pm 15.75***	0.94 \pm 0.06	851.20 \pm 41.58**	520.40 \pm 21.90***
8	305.90 \pm 11.66	697.50 \pm 11.26**	480.90 \pm 10.04***	1.05 \pm 0.07	1077.20 \pm 26.39**	642.20 \pm 30.00***

Values are means \pm SE, N = 8, **Significantly different from control, $p < 0.01$, ***Significantly different from Cd group, $p < 0.01$



Fig. 5: Large amount of collagen fibrils (arrows) are seen at site of the necrosed Kupffer cells which exhibit fragmented nuclei *. Collagen fibrils are also deposited at the sinusoidal wall (arrowhead). Liver tissue of a Cd-exposed rat. Transmission electron micrograph. x 8000

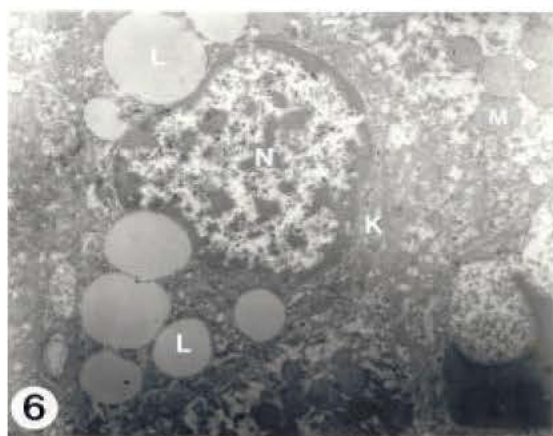


Fig. 6: Hypertrophied Kupffer cell (K) having larger irregular nucleus (N) and large-size lipid droplets (L). Mitochondria (M) in the neighboring hepatocytes are degenerated. Liver tissue of a Cd-exposed rat. Transmission electron micrograph. x 8000

mitochondria was relatively less and the cristae deterioration was of much less severity. The extension of the deposited collagen fibrils was noticeably limited to certain foci in the hepatic parenchyma (Fig. 8). There was still evidence of Kupffer cells hypertrophy, however, non of the hypertrophied cells revealed advanced degeneration or necrosis. Only few cytoplasmic lipid droplets were recognized in these cells (Fig. 9). No or little deposited collagen fibrils were discerned at the vicinity of the sinusoidal wall or nearby the Kupffer cells.

Biochemical assays

Hydroperoxide level: Cd administered subcutaneously (2.2 mg kg^{-1} 4 times weekly for 2 months) resulted in a significant increase of hydroperoxide level in blood ($p < 0.01$) by 50, 70, 83 and 128 % compared to control at 2, 4, 6 and 8 weeks post-treatment (Table 1). The hydroperoxide level in the rats receiving Zn (2.2 mg kg^{-1}) and Cd in combination increased significantly ($p < 0.01$) by 25, 28, 45 and 57%, respectively at the previous time intervals. It is evident that the hydroperoxide level of rats treated with Zn and Cd was significantly ($p < 0.01$) less than that of rats treated with Cd alone at the four time intervals.

Hepatic cadmium concentration: In Cadmium-intoxicated rats, hepatic Cd concentration (Table 1) showed a significant increase ($p < 0.01$) at all time intervals. Similar results were recorded in case of rats given Zn + Cd. The maximum cadmium concentration in the liver of rats treated with Cd alone (1077.20 \pm 26.39 $\mu\text{g g}^{-1}$) or Zn+Cd (642.60 \pm 30.00 $\mu\text{g g}^{-1}$) was observed 8 weeks post-injection. Hepatic cadmium concentration was lower in Zn + Cd treated rats than in Cd injected group.

Hepatic zinc concentration: As shown in Table 2, Cd administration (s.c.) increased the amount of zinc significantly ($p < 0.01$) in the rat liver at 2, 4, 6 and 8 weeks post-treatment. Similar results were observed in the rats treated with Zn + Cd or Zn alone at the time course. There was no significant difference in zinc concentration between rats injected with Zn + Cd and rats injects with Zn alone at all time intervals.

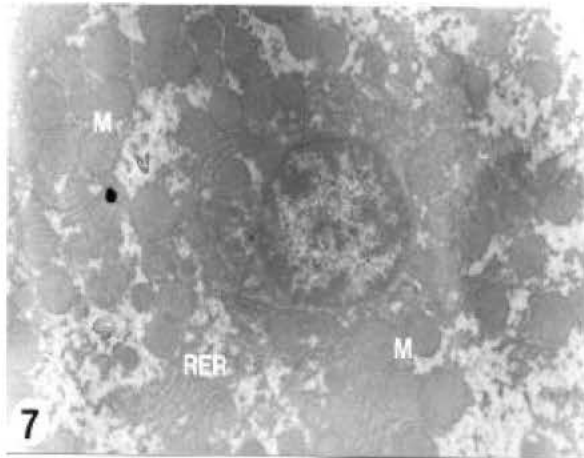


Fig. 7: Hepatocyte of a rat in Cd-Zn group. Most of the cytoplasmic organelles including mitochondria (M) and RER reveal the normal ultrastructural morphology. Transmission electron micrograph. x6700

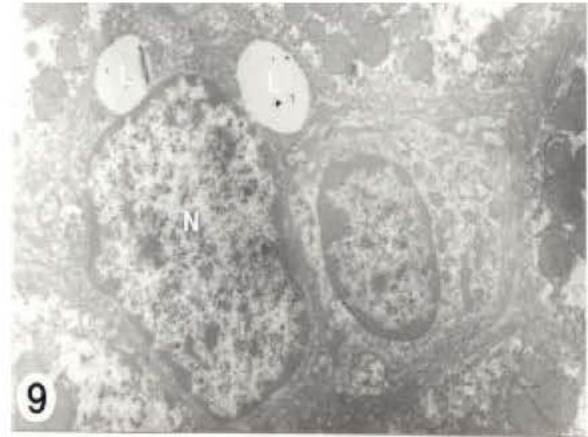


Fig. 9: Few lipid droplets (L) in a hypertrophied Kupffer cell (K) which has a large nucleus (N). Liver tissue of a rat in Cd-Zn group. Transmission electron micrograph. x 8000

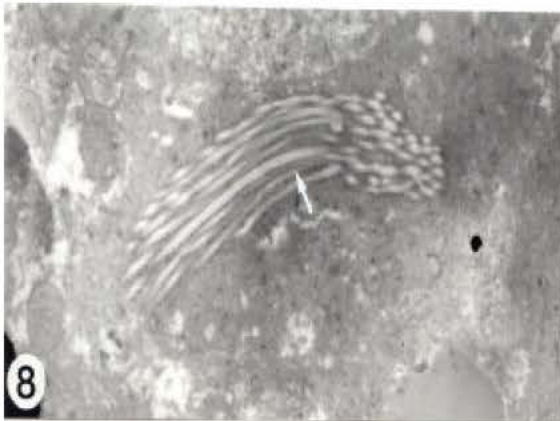


Fig. 8: Liver tissue of a rat in Cd-Zn group exhibiting a focus of collagen deposition (arrow). Transmission electron micrograph. x 8000

Hepatic catalase activity: The catalase activity (Table 2) was significantly ($p < 0.01$) decreased in liver of rats administered Cd alone by 8, 15, 18 and 29% at 2, 4, 6 and 8 weeks post treatment. In the animals treated with Zn + Cd, the enzyme activity decreased significantly ($p < 0.01$) after 8 weeks by 11% while it did not change significantly in other time intervals. However, the catalase activity of the rats administered Zn+Cd is significantly higher ($p < 0.01$) than that of the rats given Cd alone.

Reduced glutathione (GSH): Treatment of rats with Cd caused a significant decrease ($p < 0.01$) in blood GSH (Table 3) at all time intervals. Following Zn + Cd exposure,

blood GSH slightly decreased, but there was no significant change in other time intervals. The values of GSH of rats given Zn and Cd were significantly ($p < 0.01$) higher than those of rats administered Cd alone.

Cd administration tended to elevate hepatic GSH (Table 3) significantly ($p < 0.01$) at 2, 4, 6 and 8 weeks post-treatment by 24, 48, 63 and 108% respectively. Hepatic GSH increased significantly ($p < 0.05$) in the rats given Zn + Cd after 6 and 8 weeks from treatment by 21 and 19%. Moreover, liver GSH concentration of rats treated with Zn and Cd was significantly ($p < 0.01$) less than those of rats injected with Cd alone at all time intervals.

Hepatic enzymes activity in plasma

AST activity: AST activity was significantly ($p < 0.01$) increased in the rats treated with Cd (Table 4) by 24, 141, 330 and 322% at 2, 4, 6 and 8 weeks post treatment. The activity of AST also increased in the rats given Zn + Cd significantly ($p < 0.01$) by 52, 94 and 103% at 4, 6, 8 post week treatment, but no significant difference between this group and control was observed after 2 weeks from treatment. The activity of the enzyme of rats injected with Zn + Cd was significantly ($p < 0.01$) less than that of rats given cadmium alone at all time intervals.

ALT activity: A significant increase ($p < 0.01$) in ALT activity was observed in the rats given Cd alone (Table 4) at 4, 6 and 8 post week treatment. Moreover, the enzyme activity of Zn + Cd group elevated significantly ($p < 0.05$) after 8 weeks from treatment, but ALT activity was normal in other time intervals. The values of Zn + Cd group were significantly less than those of Cd group.

Table 2: Effect of subcutaneous administration of Zn and Cd on hepatic Zinc concentration and catalase activity

Parameters		Zn concentration ($\mu\text{g g}^{-1}$)				Catalase activity (U mg^{-1})		
Week post-treatment	Control	Cd	Zn + Cd	Zn	Control	Cd	Zn + Cd	
2	146.20±4.47	364.65±16.91**	413.15±13.59**	386.50±11.35**	364.68±5.57	337.29±6.06**	363.99±8.14*	
4	163.49±6.92	409.05±17.46**	485.55±10.64**	455.10±12.71**	380.20±8.54	323.56±7.07**	361.07±9.21**	
6	162.70±8.46	483.45±13.74**	477.90±17.04**	454.50±13.42**	376.59±8.19	308.43±11.79**	358.71±6.58**	
8	159.66±5.01	500.50±9.43**	503.40±5.30**	485.50±16.36**	372.70±7.36	266.15±10.40**	330.59±7.51***	

Values are means±SE, N=8, ***Significantly different from control, $p<0.01$, **Significantly different from Cd group, $p<0.05$, * $p<0.01$

Table 3: Effect of subcutaneous administration of Zn and Cd on blood and hepatic reduced Glutathione (GSH)

Parameters		Blood GSH ($\text{mg } 100 \text{ mL}^{-1}$)			Hepatic GSH ($\mu\text{mol g}^{-1}$)		
Week post-treatment	Control	Cd	Zn + Cd	Control	Cd	Zn + Cd	
2	42.60±2.38	25.60±1.15**	37.40±2.05**	9.00±0.43	11.20±0.39**	9.15±0.46*	
4	45.60±1.82	17.80±2.81**	45.10±3.04**	8.63±0.35	12.75±0.72**	9.30±0.76**	
6	44.50±1.41	19.70±0.68**	38.10±2.49**	8.90±0.61	14.50±0.45**	10.80±0.65**	
8	42.76±1.55	17.60±0.86**	36.11±2.14**	9.20±0.51	19.10±0.38**	10.92±0.42**	

Values are means±SE, N=8, ***Significantly different from control, $p<0.05$, ** $p<0.01$, *Significantly different from Cd group, $p<0.01$

Table 4: Effect of subcutaneous administration of Zn and Cd on blood AST and ALT activities (U/l)

Parameters		AST (U L^{-1})			ALT (U L^{-1})		
Week post-treatment	Control	Cd	Zn + Cd	Control	Cd	Zn + Cd	
2	49.77±2.32	61.57±2.54**	48.32±2.53**	24.43±1.22	25.11±1.47	23.19±1.41	
4	46.23±2.82	111.61±9.28**	70.35±5.84***	23.22±1.52	29.68±1.16**	24.07±1.68*	
6	52.69±5.32	226.63±18.19**	76.83±4.11***	26.42±1.34	42.78±1.77**	24.42±1.01**	
8	50.84±1.19	214.53±10.14**	103.31±3.41***	28.47±0.99	67.33±3.51**	32.69±1.26**	

Values are means±SE, N=8, ***Significantly different from control, $p<0.05$, ** $p<0.01$, *Significantly different from Cd group, $p<0.05$, ** $p<0.01$

Table 5: Effect of subcutaneous administration of Zn and Cd on blood alkaline phosphatase and γ -GT activities (U L^{-1})

Parameters		Alkaline phosphatase (U L^{-1})			γ -GT (U L^{-1})		
week post-treatment	Control	Cd	Zn + Cd	Control	Cd	Zn + Cd	
2	209.60±5.66	283.90±13.14**	193.26±5.34**	2.39±0.17	2.76±0.19	2.03±0.18	
4	219.76±9.87	295.53±24.05**	207.23±6.22**	1.95±0.18	4.33±0.46**	1.99±0.13**	
6	227.98±9.39	367.05±18.51**	202.85±6.31**	2.41±0.22	6.65±0.41**	2.21±0.25**	
8	240.77±9.62	512.17±31.78**	218.11±8.11**	2.56±0.19	14.74±0.67**	3.78±0.37**	

Values are means±SE, N=8, ***Significantly different from control, $p<0.05$, ** $p<0.01$, *Significantly different from Cd group, $p<0.01$

Alkaline phosphatase activity: The treatment of rats with Cd alone produced a significant increase ($p<0.01$) in alkaline phosphatase activity (Table 5) at all time intervals. The enzyme activity was not changed significantly in Zn + Cd group.

γ -GT activity: In Cadmium intoxicated rats, γ -GT activity (Table 5) increased significantly ($p<0.01$) at 4, 6 and 8 weeks, post-treatment, while the activity of the enzyme increased significantly ($p<0.05$) after 8 weeks from treatment in the rats given Zn + Cd. The activity of γ -GT of Zn + Cd group was significantly less than that of Cd group.

Table 6: Effect of subcutaneous administration of Zn and Cd on blood bilirubin concentration ($\text{mg } 100 \text{ mL}^{-1}$)

Week post-treatment	Treatments		
	Control	Cd	Zn + Cd
2	0.19 ± 0.04	0.18 ± 0.03	0.17 ± 0.01
4	0.14 ± 0.03	0.16 ± 0.02	0.12 ± 0.01
6	0.12 ± 0.01	0.74 ± 0.08**	0.10 ± 0.05*
8	0.11 ± 0.01	2.27 ± 0.2**	0.12 ± 0.008*

Values are means±SE, N = 8, **Significantly different from control, $p<0.01$, *Significantly different from Cd group, $p<0.01$

Bilirubin concentration: Bilirubin concentration (Table 6) showed a significant increase ($p<0.01$) in animals treated with Cd at 6 and 8 weeks post-treatment, while it did not change significantly in animals given Zn + Cd.

DISCUSSION

Liver is a target organ for Cd intoxication. Cd administration to experimental animals leads to morphological and functional hepatic changes^[8]. The results of the current study showed that Cd injection induced alterations in mitochondria as manifested by its marked swelling and deterioration of cristae. The cytotoxicity mechanism of cadmium likely involves mitochondria as a target. Initial effect of Cd appears to be caused by the binding of Cd to sulfhydryl groups in the mitochondrial membranes followed by depression of mitochondrial ATP. The respiratory chain undergoes inhibition and generation of reactive oxygen species causing mitochondrial lipid peroxidation^[23] and a decrease of total glutathione level in mitochondria^[24]. In the present study, varied sized cytoplasmic lipid droplets were noticed in the hepatocytes of rats exposed to Cd. This condition can be described as fatty degeneration of hepatocytes which was most probably related to the demonstrated mitochondrial damage. Also, mitochondrial damage leads to ATP depletion which results in compromising of all energy derived processes such as protein synthesis. Thus, lipid utilization in lipoproteins synthesis is ceased and the excess neutral lipids accumulate as micro-and macroglobules^[25]. In the present Cd-intoxicated cases, signs of Kupffer cells activation such as nuclear hypertrophy and presence of excess cytoplasmic debris were apparent. Considering the profound phagocytic capability of Kupffer cells and presence of degenerated hepatocytes, it is reasonable to interpret the Kupffer cell activation as a way to clean up the cell debris. Moreover, there is recent evidence that activated Kupffer cells contribute to Cd-induced hepatotoxicity through release of reactive nitrogen radical^[26]. Signs of hepatic fibrosis noticed in the present Cd-intoxicated cases were undoubtedly the result of increased formation of collagen fibrils. The present limited hepatic fibrosis was most likely a subsequent event to hepatocytes damage. The presently described ultrastructural changes may represent an evidence for the progressive hepatic damage which arises as a result of Cd accumulation in the liver tissue.

The observed increase in the hydroperoxide level of blood in rats treated with Cd may indicate oxidative stress which affects liver organelles. Cd participates in oxidation reactions associated with the generation of some reactive species which interact with membrane lipids of liver cells to produce lipid peroxides^[27]. Reactive oxygen species such as superoxide anion radical and hydroxyl radical provoke sever changes at cellular level leading to cell death because of their extreme reactivity. They attack

essential cell constituents such as proteins, lipids and nucleic acids. Also, they induce peroxidation of fatty acids^[28]. Lipid peroxides that accumulate due to peroxidation of lipids are known to be harmful to cells and tissue^[29]. Lipid peroxidation is a relevant consequence of acute and chronic exposure to cadmium and considered a potential marker for susceptibility of early and irreversible tissue damage^[30].

It is interesting to note that in the biological system, there is a defense mechanism against free radical induced deleterious effects through scavenging properties of various endogenous antioxidants. When the rate of free radical production is greater than the scavenging rate, oxidative damage likely occurs in cells and tissues. One of the effective antioxidant which participates in the prevention of oxidative damage is catalase. This enzyme degrades H₂O₂ into a molecule of oxygen and a molecule of water^[31]. In the present study, the observed decrease in hepatic catalase of rats exposed to Cd may explain the oxidative stress of liver. It has been proposed that the enhancement of lipid peroxidation by cadmium in rats is a consequence of a decrease in superoxide dismutase and catalase activities^[32]. The two enzymes are important antioxidants that protect from lipid peroxidation via elimination of reactive oxygen species.

Reduced Glutathione (GSH) is known as one of the endogenous antioxidants, being identified as a protector against the damaging effect of the free radicals^[33]. GSH can also form complexes with Cd to alter Cd distribution and excretion^[34]. The mechanism of Cd induced lipid peroxidation may involve a decrease in the level of GSH and the total pool of sulfhydryl groups which can induce peroxidation of lipids^[35]. Presently, a significant decrease in blood GSH of the rats given Cd alone at all time intervals was noted. This decrease may be due to inhibition of glucose 6-phosphate dehydrogenase by Cd causing a decrease of conversion of oxidized glutathione (GSSG) to GSH^[36]. On the other hand, exposure to Cd increased hepatic GSH level probably due to an increase in the activity of gamma-glutamylcysteine synthetase, which catalyzes the biosynthesis of GSH^[37]. It seems that Cd effect on liver GSH differs from one species to another. Karmakar *et al.*^[38] found that administration of Cd subcutaneously to mice decreases GSH and glutathione S. transferase activity in the liver. The present ultrastructural changes, hydroperoxide level, catalase activity and concentration of GSH indicate that Cd can induce oxidative stress during repeated administration.

Currently, injection of Cd to rats increased hepatic zinc concentration. Cd induces changes in homeostasis of Zn resulting in an increased retention of Zn in the liver which is due to metallothionein induction in this organ^[39].

Induction of metallothionein, a thiol rich protein, has been proposed to be an important adaptive mechanism in decreasing Cd toxicity^[40]. It seems that increased GSH and Zn in the liver of rats exposed to Cd can be a compensatory response to the oxidative stress, but this increase is not sufficient enough to overcome the harmful hepatic effect of Cd.

The relation between the hepatic tissue damage and elevation of the relevant serum enzymes is well documented^[41]. The observed increase in activities of plasma AST, ALT, alkaline phosphatase and γ GT is likely due to lipid peroxidation of biomembranes which causes leakage of cellular components^[42]. Exposure of hepatocytes to Cd stimulates cellular production of H_2O_2 which affects permeability barrier of plasma membranes^[43]. It seems that, the increase in the liver enzymes of the present study may be due to accumulation of Cd in hepatic tissue which enhances formation of lipid peroxidation.

Because free radicals are involved in wide range of harmful effects, scavenging of these radicals may protect the body cells. Here, we found that pretreatment of rats with zinc prior to Cd administration decreased blood hydroperoxide level significantly. Zinc provides an antioxidant function to cells by different mechanisms. Zn decreased reactive oxygen species and lipid peroxidation^[44]. The antioxidant action of Zn is thought to involve the antagonism of redox active transition metals such iron and copper; it competes with them for binding to cell membranes and making them more available for binding to metallothionein^[45]. Many studies have demonstrated that redox-active metals catalyzed the formation of HO radical leading to lipid peroxidation or protein oxidation^[46]. The protective effect of Zn may be in part, mediated by induction the synthesis of metallothionein (MT) and by providing thiol groups which can scavenge hydroxyl radicals and singlet oxygen^[47]. In the present work, the reduction of hepatotoxicity induced by Cd in Zn-pretreated rats can be attributed to decreased intracellular Cd accumulation leading to a decrease in production of reactive oxygen species and lipid peroxidation. Administration of Zn has been reported to decrease Cd accumulation in different species of experimental animals^[48,49]. Zinc supplementation could compete with binding sites for Cd uptake. The protective activity of zinc may be due to its ability to maintain normal GSH level and catalase activity. It appears that zinc has a direct antioxidant activity through scavenging of free radicals and indirect effects via prevention of the depletion of blood GSH level and hepatic catalase activity result from Cd treatment or by interfering with Cd transport into the cell rather than its activity to stimulate MT synthesis. The administration of zinc before cadmium treatment may help in minimizing the

damage to liver cells. We can conclude that our results confirmed the antioxidative activity of zinc, which seems to be connected with the enhancement of protective mechanisms against the toxic effects of cadmium.

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