HEPATOTOXICITY ON ACUTE EXPOSURE TO CADMIUM CHLORIDE IN MICE

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Abstract : Objective: A dose-response study to investigate the effect of acute cadmium exposure on oxidative stress and histopathological alterations in liver.
Methods: Male Balb/c mice were administered intraperitoneal doses of 0.2, 0.4 and 0.8 mg $CdCl_2$/kg body weight and sacrificed 18 hours post exposure. Oxidative stress was quantified spectrophotometrically by measuring the levels of GSH, total thiols and MDA in liver homogenate. Histology of liver was evaluated under the light and Scanning electron microscope.
Results: A dose-response decrease in glutathione and total thiol content with a corresponding increase in MDA levels indicative of lipid peroxidation was observed in the liver of Cd treated mice, as compared to controls. Histopathological alterations were observed only in livers of mice exposed to 0.8 mg $CdCl_2$/kg body weight. Light microscopy showed basophilic depositions, Kupffer cell hyperplasia and lobular inflammation while electron micrographs revealed similar features along with crystalline deposits on hepatocytes, pronounced damage to the endothelial cell fenestrations and bile canalculi.
Conclusions: In the present study, it was seen that on acute Cd exposure, with increase in $CdCl_2$ dose there were significant changes in the biochemical parameters indicative of oxidative stress, while a certain amount of Cd load was required for histopathological changes to take place in hepatic tissue.

***IndexTerms* - Cadmium, acute exposure, liver, oxidative stress, scanning electron microscopy, light microscopy.**

I. INTRODUCTION

In recent years, metal toxicity has become a major concern in view of their steadily increasing concentration in the environment. One such metal is cadmium (Cd, Group II B metal) that has significantly increased in environment due to various human activities, such as mining, burning of fossil fuels and use in industry. Cadmium is a non-essential element that predominately binds to metallothioneins (Hamer, 1986) in the human body and is toxic at very low doses. In the absence of any mechanism for its excretion in humans, Cd accumulates in tissues and has a very long biological half-life. In the earliest studies on Cd toxicity, it was reported that the half-times of Cd were 6 to 38 years and 4 to 19 years in the human kidney and liver, respectively (Kjellström 1978).

Human exposure to Cd occurs primarily by inhalation or ingestion. Depending upon the particle size ten to fifty percent of inhaled cadmium dust is absorbed with negligible absorption through skin contact. Cadmium exposure from ingestion of contaminated food and water accounts for about five to ten percent absorption and has been associated with long-term health effects. Amongst smokers, cigarette smoke is the largest source of Cd exposure that accounts for 2-4 µg of Cd per packet per day (Friberg, 1983; Nordberg *et al.*, 2007; Abernethy *et al., 2010*).

The mechanism of Cd toxicity at molecular and cellular levels has been studied in great detail along with the toxicokinetics and toxicodynamics studies (Jarup, 2009). It has been reported that Cd in the bound and conjugated form is non-toxic rather the released ionized form of Cd^{2+} is responsible for its cellular toxicity (Jacobo-Estrada *et al.*, 2017). Toxic manifestations of short and long term Cd exposure are seen as hepatotoxicity and renal tubular dysfunction, respectively (Dudley, 1982; ATSDR, 2003). Nevertheless, at moderately low levels of oral exposure, cardiovascular (Akahori *et al.,* 1994), hematological (Ogoshi *et al.,* 1989), neurological (Valois & Webster, 1989), and testicular effects (Pleasants *et al.,* 1992) have also been reported.

Cadmium ions exert toxic effects in the organisms at tissue, cellular, subcellular and molecular levels. The possible factors reported to be involved in Cd induced toxicity include alterations in the activities of various enzymes (Jamall & Smith, 1985), induction of oxidative stress (Yiin et al., 2000; Beytut *et al.,* 2003; Ikediobi *et al.,* 2004) or lipid peroxidation of cell membranes (Shaikh *et al.,* 1999), interference with the normal actions of Zn^{2+}, Se^{2+}, and other essential metals (Nath *et al.,* 1984; King *et al.,* 1998), triggering apoptosis (El Azzouzi *et al.,* 1994; Ishido *et al.,* 1995; Habeebu *et al.,* 1998), genotoxicity (Karmakar *et al.,* 1998; Jimi *et al.,* 2004), altering gene expression (Matsuoka & Call, 1995; Matsuoka & Igisu, 1998; Wang & Templeton, 1998; Spruill *et al.,* 2002; Xu *et al.,* 2003) and either mimicking or antagonizing various physiologic actions of Ca^{2+} (Sutoo *et al.,* 1990; Chen & Smith, 1992). Histological evaluation of acute Cd toxicity manifests itself in the form of liver injury characterized by hepatocellular swelling, sinusoidal congestion, pyknosis and karyorrhexis (Dudley *et al.,* 1982). Dudley *et al.* while studying time-course Cd induced hepatotoxicity reported early cellular changes in the rough endoplasmic reticulum and nucleus which are followed by mitochondrial swelling, appearance of fibrillar material within the cytoplasm which may result in both apoptosis and necrosis reported by Habeebu *et al.* (1998). There have been reports that Cd may injure hepatocytes as a result of cytotoxic mediators such as reactive oxygen and nitrogen species (ROS, RNS) and cytotoxic proteins, released by Kupffer cells and other

inflammatory cells or ischemia resulting from extrusion of damaged endothelial cells into sinusoidal space which alters hepatic microcirculation (Liu *et al.,* 1992; Rikans & Yamano, 2000).

Despite these studies many other aspects of toxic mechanisms of Cd are not well understood and need to be elucidated. Therefore, the present dose-response study was undertaken to investigate the relationship between pro-oxidant status and histopathological alterations in liver of Balb/c mice following short-term exposure to Cd. In absence of any reports on the correlation between the oxidative stress and ultrastructural studies following acute Cd exposure in mice liver, this study shall provide an insight into dose-response hepatotoxicity following acute Cd exposure.

II. MATERIALS & METHODS

A. Chemicals

All chemicals were of analytical grade specifications and obtained from HIMEDIA Ltd, India. Cadmium chloride ($CdCl_2$) was obtained from Sigma Chem. Co., St. Louis, MO, USA.

B. Animals and Treatments

Four to six week-old Balb/c male mice weighing 20-25 grams were procured from the Central Animal House, Panjab University, Chandigarh. Mice were kept in cages, given food and water *ad libitum* and allowed to acclimatize for 7 days maintained, at 12 hour light/dark regime prior to experimental use.

The mice were divided into four groups of 7 mice each, one of which served as the control group which was given the vehicle (normal saline). The other three groups T-I, T-II and T-III were injected intraperitoneal (ip) $CdCl_2$ doses of 0.2, 0.4 and 0.8 mg/ kg body weight (bw) respectively and sacrificed by cervical dislocation 18 hours after treatment.

C. Hepatic biochemical estimations

The anti-oxidant status of liver was assessed spectrophotometrically (Infrared Spectrophotometer, Hitachi, 330) using the standardized techniques for estimation of reduced glutathione (Beutler, 1963), total thiols (Sedlak & Lindsay, 1968) and protein estimation (Lowry *et al.,* 1951). The lipid peroxidation in liver homogenate was estimated by measuring the tissue malondialdehyde (MDA) level (Beuge & Augst, 1978).

D. Histopathological studies

Tissue from liver of mice were fixed in 10% buffered formalin and processed routinely. The blocks were embedded in paraffin wax. Sections of 5-6 µm thickness were cut by rotary microtome, stained with Haematoxylin-eosin (H&E) stain and examined under light microscope (Leica DC 100, PC I Interface Digital Camera).

E. Scanning electron microscopy

The liver slices were washed with phosphate buffer and fixed in 4% gluteraldehyde in phosphate buffer. They were then dehydrated in ascending acetone grades and critical point dried through transitional fluid amyl acetate. The dried samples were fixed on metal stubs with double adhesive tape for gold sputtering. The stubs so prepared examined using JEOL JSM 6100 Scanning Electron Microscope.

F. Statistical analysis

Significance between pair of means for control and treated groups was determined by Student's t-test. The data were expressed as mean ± standard error of seven mice and the level of significance considered were $P < 0.05$.

III. RESULTS AND DISCUSSION

Dose-response hepatic toxicity of Cd was studied in Balb/c mice following acute exposures to 0.2, 0.4, 0.8 mg $CdCl_2$/kg bw, 18 hours after ip injection, as compared to control group that was injected normal saline. To investigate whether the Cd doses of the present study caused hepatotoxicity resulting from oxidative stress, the levels of hepatic GSH, total thiols and MDA were measured in the liver homogenates of treated mice.

The control GSH and total thiol levels in the liver were 0.91 ± 0.02 and 33.8 ± 2.8 nmoles/mg protein, respectively. Both GSH and total thiol levels deceased steadily following exposure to increasing doses of Cd. Total thiols showed a significant decrease of 1.7 and 4.97 fold for groups T-II and T-III exposed for 18 hours to doses of 0.4 and 0.8 mg $CdCl_2$/kg bw, respectively with a corresponding 2.6 fold decrease in GSH for group T-III as compared to the controls (Fig. 1 A). The basal MDA levels in the liver was 0.29 ± 0.028 nmoles/mg protein, while on exposure to Cd the MDA levels increased significantly with 1.5 and 1.9 fold increase for groups T-II and T-III exposed to 0.4 and 0.8 mg $CdCl_2$/kg bw, respectively for 18 hours (Fig. 1 B).

The rise in MDA levels concurrently corresponds with decrease in GSH and total thiols, indicating the depletion of reduced glutathione and total thiols, due to increased lipid peroxidation measured in terms of MDA level.

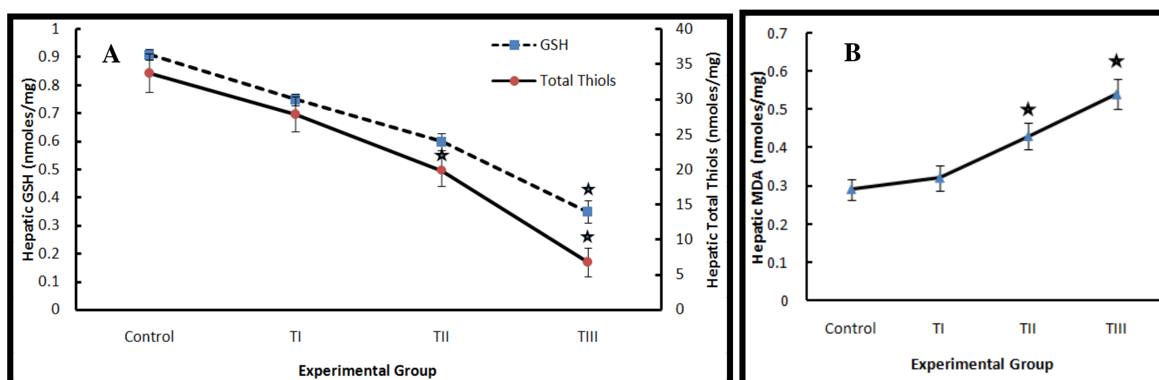

Fig. 1: Dose-response effect of Cd administration on hepatic GSH, Total Thiols (A) and MDA (B) levels. The mice were injected i.p. 0.2, 0.4, 0.8 mg $CdCl_2$/kg bw for 18 hours to groups T-I, T-II and T-III, respectively and saline for control group. Data expressed Mean ± SE (n=7). Level of significance $P < 0.05$.

Earlier reports have also indicated that acute Cd exposure can increase oxidative stress by producing superoxide anions and exhaustion of GSH stores (Shimizu, & Morita 1992; Sarkar *et al.,* 1995; Rana & Verma 1996). Waisberg *et al.* (2003) had reported that Cd cannot directly generate free radicals and rather is involved in indirect formation of ROS and RNS. An increase in lipid peroxidation in various rodent tissues and hepatic cell lines has been reported following exposure to Cd (Manca *et al.,* 1991; Sarkar *et al.,*1995; El-Maraghy *et al.,*2001; Xu *et al.,* 2003; Ikediobi *et al.,* 2004).

Biochemical studies indicated that oxidative stress was highly significant in mice of group T-III exposed for 18 hours to 0.8 mg $CdCl_2$ /kg bw. Therefore, the hepatic tissue of the control and treated groups were further investigated to identify histopathological alterations following acute Cd exposures to correlate the level of oxidative stress and histological changes.

Liver from Cd treated groups did not exhibit any macroscopic alterations in morphology of hepatic tissue of mice following acute exposure for 18 hours. At microscopic level, doses of 0.2 and 0.4 mg $CdCl_2$/kg bw did not result in any significant changes liver histology as compared to the control group. On the other hand, exposure of 0.8 mg $CdCl_2$/kg bw resulted in marked alterations in the histology of the liver as observed under the light and scanning electron microscopes.

Light micrographs of liver sections of group T-III mice exposed to 0.8 mg $CdCl_2$/kg bw, revealed signs of cell injury seen as multiple foci of lobular inflammation by mononuclear cells (Fig. 3 A), Kupffer cell hyperplasia (Fig. 3 B) and depositions of basophilic material (Fig. 3 C) as compared to the control untreated mice (Fig. 2 A). The histopathological changes observed were diffuse and not localized to any specific area suggesting that Cd acts as a general hepatotoxin. These changes were further investigated at finer scale using SEM as it aids to study the morphological and topographical changes at the ultrastructural level. The mesothelial layer of the liver showed hyperplasia (Fig. 4 A) which may be due to the inflammation observed under the light microscope (Fig. 3 A) in group T-III as compared to the normal features in the control group (Fig. 2 B). Topographically diffused, crystalline deposits were observed on the liver surface (Fig. 4 B) along with Kupffer cells covering necrotic hepatocytes (Fig. 4 C). Kupffer cell hypertrophy and hyperplasia is reported to occur following uptake of foreign material, and has been associated with inflammation as a consequence of the phagocytic activity of these cells (Harada *et al.,* 1999). The crystalline deposit observed could be deposits of Cd on the hepatocytes after the Cd load exceeds the body's capacity to handle the excess toxic metal and Kupffer cells were unable to engulf Cd during the 18 hours duration of exposure in the present study. Bhattacharyya *et al.* (1996) and Karmaker *et al.* (2002) have also reported that certain amount of Cd load is required for architectural alterations to occur.

Endothelial cells lining the sinusoidal wall of liver have characteristic fenestrations (Fig. 5 A) with well defined porosity of bile canalculi (Fig. 5 B) as seen in control mice. In group T-III loss of the characteristic fenestrations of the endothelial cells (Fig. 6 A) and loss of porosity and blockage of the bile canalculi was observed (Fig. 6 B) as a result of blebbing of endothelial cells.

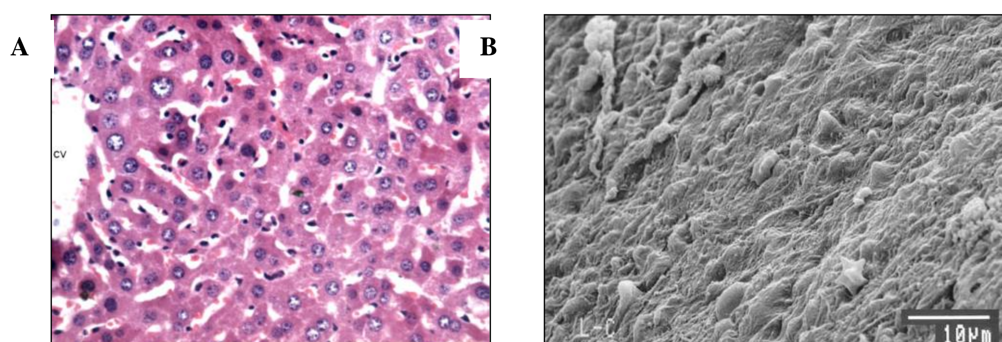

Fig. 2: Micrographs of liver of control mice.

(A) Histology of liver is composed of hexagonal or pentagonal lobules with central veins (CV) and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabeculi running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. H & E × 280.

(B) The mesothelial layer of liver is normal under SEM (10 μm).

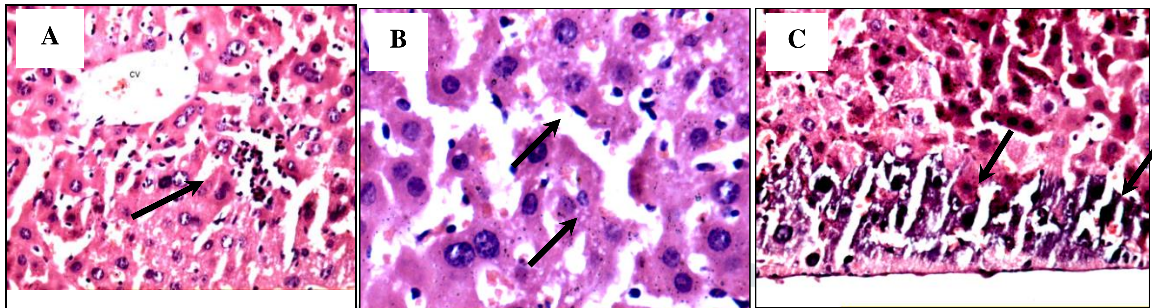

Fig. 3: Light micrograph of liver of mice exposed to 0.8 mg $CdCl_2$/kg bw for 18 hours.

(A) The trabecular pattern is blurred with lobular inflammation around the central vein (CV) and accumulation of mononuclear cells in the vicinity of the sinusoids. H &E × 280.

(B) Sinusoids showing Kupffer cell hyperplasia (indicated by arrows). H & E × 550.

(C) Deposition of basophilic material within the hepatocytes and sinusoids. H & E × 550.

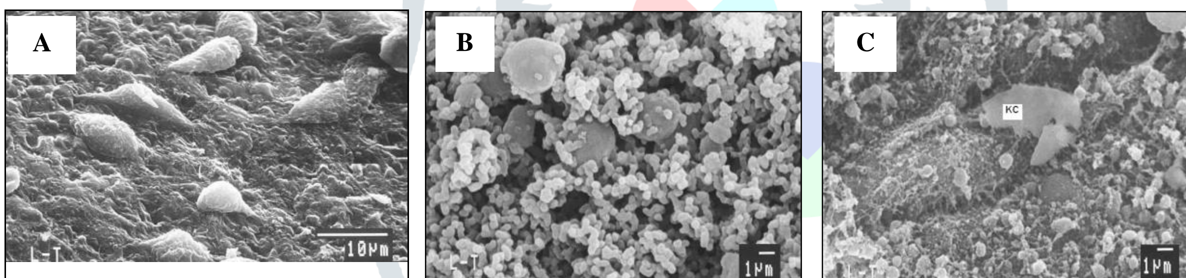

Fig. 4: Scanning electron micrographs of liver of mice exposed to 0.8 mg $CdCl_2$/kg bw for 18 hours.

(A) The mesothelial layer of the liver showed hyperplasia (SEM 10 μm).

(B) Crystalline deposits on the mesothelial layer of the liver (SEM 1 μm).

(C) Kupffer cell (KC) lying over the surface of necrotic hepatocyte (SEM 1 μm).

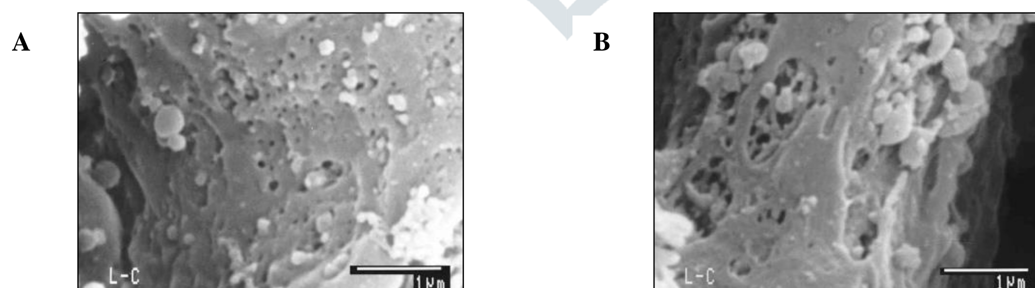

Fig. 5: Micrographs of liver of control mice.

(A) Sinusoids are lined with endothelial cells with characteristic fenestrations having well defined clear porosity (SEM 1 μm).

(B) Hepatocytes have bile canalculi showed marked porosity (SEM 1 μm).

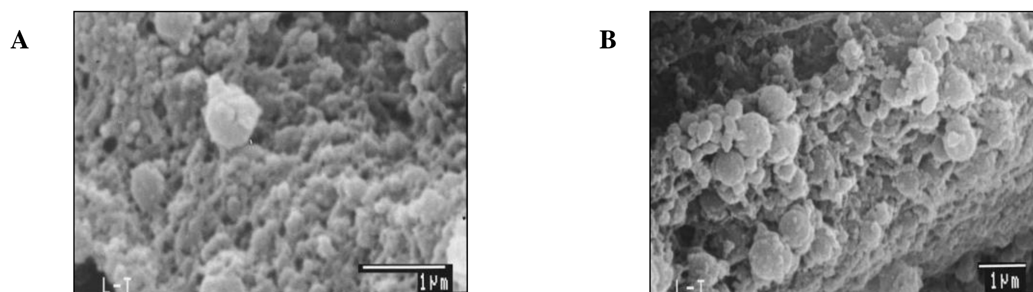

Fig. 6: **Scanning electron micrographs of liver of mice exposed to 0.8 mg $CdCl_2$/kg bw for 18 hours.** The characteristic fenestrations of endothelial cells are lost (A) and Bile canalculi of the hepatocytes are blocked (B) due to blebbing of the surface epithelia (SEM 1 µm).

Kupffer cell and mononuclear cell activation as observed on light and electron micrographs (Figs. 3 A & 4 C) along with inflammation (Fig. 3 B) may result from release of endogenous inflammatory mediators such as ROS following acute Cd exposures.

Based on the results obtained in the present study, it is postulated that a plausible mechanism of Cd induced acute hepatotoxicity results from a buildup of a high Cd load in the liver, which subsequently overwhelms the anti-oxidant system. This in turn causes activation of Kupffer cells and mononuclear cells that further increase ROS causing lipid peroxidation, inflammation and finally apoptosis or necrosis. Also excess Cd may be deposited within or on the surface of hepatocytes as deposits of basophilic material (Fig. 3 C) and crystalline deposits (Fig. 4 B) that were seen on light and electron micrographs. The blockage of bile canalculi and endothelial cell fenestrations lining sinusoidal lumen (Figs. 6 A & B) as observed in electron micrographs may also contribute to Cd-induced hepatotoxicity resulting from ischemia (Liu *et al., 1992*) and subsequent loss of function.

IV. CONCLUSION

The results of the present study indicate that hepatic anti-oxidant systems try to handle the oxidative stress induced due to increase in Cd burden during acute exposures. The depletion of the anti-oxidants results in increased lipid peroxidation and consequently significant histopathological alterations in liver were observed on light and electron micrographs for the highest dose in the present study. It is therefore concluded that oxidative stress increased in a dose-dependent manner following short-terms exposures of Cd, while a high Cd load brought about histological changes in hepatic tissue. Thus, histopathological alterations occur subsequent to oxidative damage.

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