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Research Article

HEAVY METALS INDUCE APOPTOSIS IN LIVER OF MICE

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ABSTRACT: Cadmium (C d) and zinc (Zn) are an industrial and environmental pollutant of aquatic system has attracted the attention of research's all over the world. In the present study the toxic effects of zinc (Zn) and Cadmium (C d) on the liver of male mice. Male Balb /c mice weighing 32-34 gm, 70 days old, were treated orally with (1-10 mg/kg body wt. $CdCl_2$ and 1-8 mg/kg body wt. $ZnCl_2$). The body weight, liver weight, histological examination of liver, along with DNA ladder for apoptosis was studied. Cadmium and zinc induced both a time, and dose dependent increase in apoptotic, severity of necrosis. Liver weight, body weight decreased with increase of dose. It has been concluded that cadmium and zinc caused necrotic effect in liver and apoptotic as well as decrease body weight and liver weight.

Key words: Cadmium, Zinc, and Apoptosis.

INTRODUCTION

Heavy metals occur naturally in the environment and are found in varying levels in the ground and surface water. Anthropogenic activities do, however, cause an increased discharge of these metals into natural aquatic ecosystems. Sometimes, aquatic organisms are exposed to unnaturally high levels of these metals. Histological study appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the liver. Exposure to heavy metals may cause histological change in liver. Zinc (Zn) is one of the most important trace elements in the body and participates in the biological function of several proteins and enzymes (Maity et al., 2008). Despite being an essential trace element, Zn is toxic to most organisms above certain concentrations (Ho,E, 2004). Therefore, heavy metals have been recognized as strong biological poisons because of their persistent nature, toxicity, tendency to accumulate in organs and undergo food chain amplification (Dinodia et al., 2002).

Among heavy metals, zinc and cadmium is used in various forms which eventually find its way into the river or sea. Excessive zinc enters the environment as a result of human activities such as mining, purification of zinc, lead and cadmium ores, burning of coal and burning of waste. Although, small quantities of zinc are required for the normal development and metabolism (Shukla et al., 2002), but if its level exceeds the physiological requirements, it can act as a the effect of two heavy metals, cadmium (Cd) and zinc (Zn), on the histology of the liver on the fresh water fish species Oreochromis mossambicus exposed to 5 and 10% concentrations of LC50 of Cd and Zn over both short and long term exposure was studied (Vandyk et al., 2007). The administration to laboratory animals is known to produce a number of toxic effect, such as hepatic and renal damage, and induce various tumors, including lung and kidney tumors and responsible for acute and chronic toxicity in humans(Rudolfs K and Sarfaraz, 2003). Therefore, the present study had the apoptosis as an important and currently unrecognized process occurring in acute Cd and Zn -induced hepatotoxicity.

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MATERIAL AND METHODS

Adult male mice, weighing 32-34gm of Blab /c, 70 days old. The animals were treated orally with different doses of cadmium chloride ($CdCl_2$) daily for 21 days, as follows (1mg/kg body wt., 5mg/kg body wt., and 10 mg/kg body wt) and zinc chloride ($ZnCl_2$) daily for 21 days, as follows (1mg/kg body wt., 5mg/kg body wt., and 8 mg/kg body wt . The animals were divided into four groups each group having 6 mice (one control group on normal diet and water). The body weight and liver weight from treated groups was taken along with control after 21 days.

The liver was cut into small pieces and fixed in Bouins fixative. Histological examination of liver was carried out by standard histological techniques. Sections of 7 µm thickness were cut and stained with hematoxylin: eosin (H/E) 7.The method Kerr J.F et al), used for apoptosis, which appears as a ladder in agarose gel electrophoresis was modified (Subramanian et al., 1994).

Statistical analysis: Results are reported as mean + SE. In experiments where the $CdCl_2$ and $ZnCl_2$ doses were varied, data was analyzed by using student's "t" test.

RESULTS

The signs of metal toxicity were observed with 10 mg/ kg body wt., and 8 mg/kg body Weight of administration of ($CdCl_2$, $ZnCl_2$).These included Shivering ,salivation and lacrimation.

The intake of feed and water by treated mice reduced as compared to control. Moreover, the decrease was dose dependent. The liver weight and body weight show significant decrease with increase of doses. (Fig. 1. Table 1 and 2).

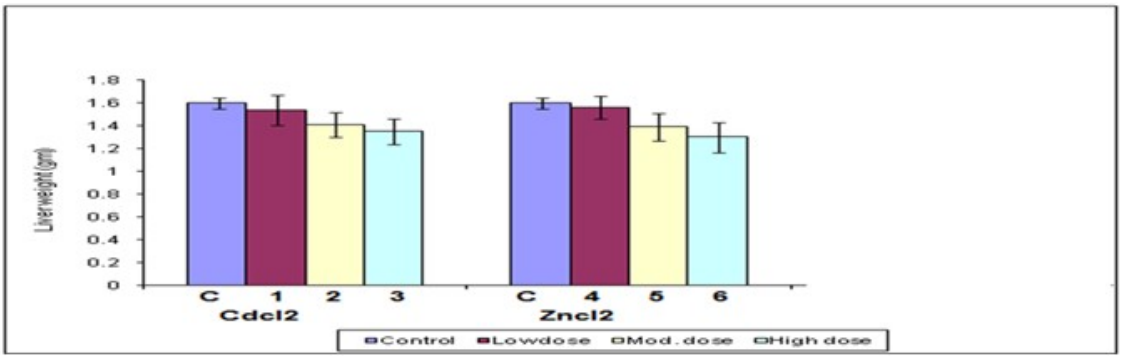

Figure 1: Effect of $CdCl_2$ on liver weight of male mice (values represent mean ± S.D., n=6 in each group.

Table 1: Effect of $CdCl_2$ on body weight of male mice

Group	Initial Body Weight (gm)	Final Body Weight (gm)	Percentage Change
Control	31.6±2.1	34.36±3.08	8.34
Low dose	32±3.3	30.84±2.8*	-3.62
Mod. Dose	32.4±2.2	29.6±2.19**	-8.64
High dose	31.6±3.07	28.6±2.06***	-9.49

Value represents mean ± S.D., n=5 in each group
The P-value was calculated between the test group and control group.
* Non-significant different from the control value p>0.05.
** Significant different from the control value p<0.05.
*** Highly significant different from the control value p<0.001.

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Table 2: Effect of $ZnCl_2$ on body weight of male mice

Group	Initial Body Weight (gm)	Final Body Weight (gm)	Percentage Change
Control	31.6±2.1	34.36±3.08	8.34
Low dose	32.8±2.9	31.56±1.4*	-3.42
Mod. Dose	31.2±3.03	29.4±3.0**	-5.76
High dose	32.0±2.1	28.3±2.5***	-11.56

Value represents mean ± S.D., n=5 in each group
The P-value was calculated between the test group and control group.
* Non-significant different from the control value p>0.05.
** Significant different from the control value p<0.05.
*** Highly significant different from the control value p<0.001.

Histological observation of liver after various doses of $CdCl_2$ and $Zncl_2$ treatment showed marked alteration (Figs 2, 3 and 4).

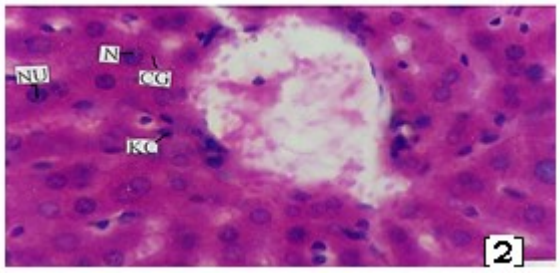
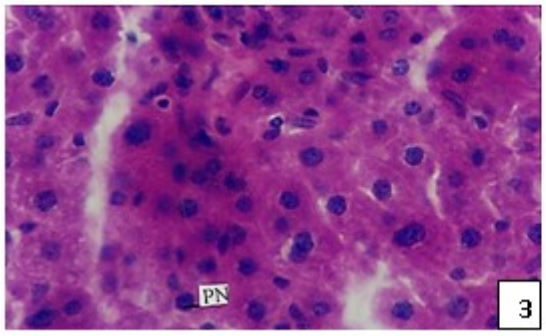


Figure 2: Showing central canal, cords of hepatocytes, sinusoids and kupffer cells Observe binucleated hepatocytes BF/H-E. x40. (N) nuclei,(NU) nucleoli,(KC) Kupffer cells and (CG) cytoplasmic granule.



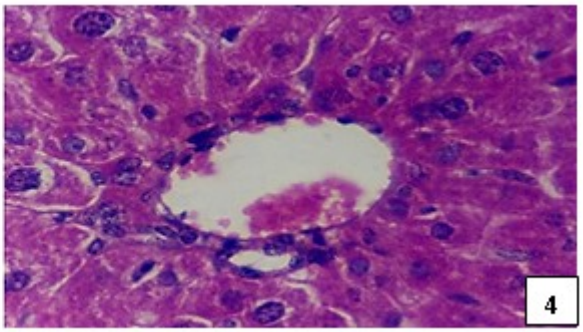


Figure 3, 4: Showing badly damaged hepatocytes, treated with 10 mg/kg body wt $CdCl_2$ (fig.3), and 8 mg/kg body wt $Zncl_2$ (Fig.4), hypertrophy of nuclei due to fragmentation in hypertrophied hepatocytes; blood in central canal. BF/H-E).x4, (PN) pycnotic nuclei.

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The metal treatment caused marked changes in liver such as swelling and massive fatty degeneration in hepatocytes and large vacuoles in cytoplasm. Cytoplasm of hepatocytes showed vacuoles and nuclei with pycontic and staining affinity of nucleus was comparatively poor, due to damage of the hepatic cells after treatment with $CdCl_2$ and $Zncl_2$. The damage of hepatic cells increase with increase of dose was observed. Apoptosis was observed at 10 mg/kg body wt., $CdCl_2$ and 8 mg/kg body wt. $ZnCl_2$ administration.

DISCUSSION

Necrosis as a feature of acute Cd and Zn hepatotoxicity is well established. This report characterizes apoptosis in the liver, and its relationship to liver necrosis, following acute poisoning. Several approaches were applied to investigate apoptosis in the liver following oral injection of Cd and Zn into mice. The result demonstrate that apoptosis is an important mode of eliminating damaged cells in Cd and Zn hepatotoxicity.

Liver necrosis resulting from Cd toxicity is well documented10. In the dose-response studies, focal, zonal and massive necroses were also observed and were dose-dependent. The dose dependence was reflected in the histology and decreased in weight of liver (Taxy, J.B and Peliosis 1978).

Zinc is generally regarded as one of the less hazardous metals [12], but frequently occurs in nature together with other metals of which cadmium is one of the most common. Previous studies have shown that, exposure to either cadmium or zinc caused histopathology of the kidney, epidermis13, and the liver(Morsey, M.G and Protasowicki.M 1990). In the dose-response studies, liver cell regeneration was dose- dependent. Apoptosis and liver cell regeneration are also observed in chronic Cd- induced hepatotoxicity(Habeebu S et al., 1997).

The mechanism by which cd induces apoptosis in the liver is unknown. Cd is rendered inert in liver by being complexed to metallothionein, alow- molecular- weight scavenger protein, rich in cysteine residues. Excess free cd in the cytoplasm binds to cellular organelles, including the nucleus, and disrupts their functions. Cd is genotoxic in vitro, causisng single- strand breaks in DNA(Coogan T.P., et al 1992), frame- shift mutation(Biggart, N.W and Murphy E 1988), and chromosomal aberrations(Hartwig A 1994).

The histological changes identified within the hepatocytes in this study may have been the result of various biochemical changes. The liver appears to be one of the most important sites for Zn accumulation in Channel punctatus findings(Senthil S et al., 2008). The high levels of Zn in liver can be ascribed to the bindings of Zn to metallothionein (MT) which was at highest concentration in liver(Kendrick.M.H et al 1992).

(Shetty et al 2007), reported that the determination of acute toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. In summary, we have presented evidence that apoptosis is an important and predictable event in acute Cd and Zn hepatoxicity. Apoptosis shows consistent time- course and dose- response patterns, and it precedes necrosis. While Cd and Zn – induced liver necrosis is sustained for at least 8-10mg/kg and also induced apoptosis. The early occurrence of apoptosis in acute cd hepatotoxicity suggests a role for apoptosis in the elimination of critically injured liver cells while attempting to preserve the structural and functional integrity of the liver.

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