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Effect of Cadmium on Oxidative Damage in the Liver Of Freshwater Heteropneustes fossilis(Bloch).

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ABSTRACT:- Environmental pollution is the major problem in developing countries including India. Among the major pollutants heavy metals are considered as the major toxicants that affect various organs of living beings. Cadmium is one of the most toxic heavy metal that induces a wide range of toxicity in various aquatic animals. Fishes are the major living being in aquatic habitat and very susceptible for water born contaminants including heavy metals. The main objective of the present study is to find out the mechanism of cadmium induced toxicity in the liver of fresh water teleost H.fossilis. Fishes were exposed to cadmium for various time periods and the liver damage was observed by histological and biochemical examinations. Our findings indicate that cadmium exerts toxic effect on the histology of liver and causes hepatic cell degeneration, necrosis and infiltration of blood cells. Cadmium also increases the lipid peroxidation with the increase in the exposure period. Furthermore, the cadmium exposure also causes the decline in the antioxidative defense mechanism of the liver significantly. We conclude that cadmium induces histological damages in the liver by the mechanism of increased oxidative stress and reduced antioxidant response.

KEY WORDS:- Cadmium, liver, histology, oxidative stress, lipid peroxidation , glutathion.

INTRODUCTION :-

Environmental pollution is the biggest threat to the existence of all the living organisms. Human beings, plants and animals are living under constant ecological stress in the modern world. Among all types of pollutants heavy metals are considered as the major toxicants. Cadmium is one of the most toxic heavy metal that causes adverse effects on organisms. It is a trace element which is not essential for living organism and is toxic to both plants and animals. (El- Sharaky et. al, 2007). It is mainly introduced into the environment through nickel cadmium batteries manufacturing plants and lead mining and processing units (Kumar et al. , 2009). Cadmium exerts toxic effect even in the minute amount. Cadmium has been found to accumulate in various organs. Its accumulation in liver has been reported by many researchers (Sumet and Blust, 2001 and Rangsayatorn et al, 2004).

Cadmium is known to cause various histopathological changes in liver (Rani and Ramamurthi , 1984 and Dangra et al, 2010). Cadmium can also alter the reduced glutathione (GSH) level and induces the expression of metallothioneins in the liver, which ultimately leads to lipid peroxidation (LPO) of cell membranes (Sevcikova et al, 2011). Effect of Cadmium exposure on the GSH level in fish is also on record (Kovarova et al., 2009; Cao et al., 2010; Jia et al., 2011).

The purpose of this study is to investigate the mechanism of cadmium induced histological damage and biochemical alterations in the liver of a fresh water teleost Heteropneustes fossilis (Bl.)

MATERIAL AND METHODS

Animals and treatments

A fresh water air breathing cat fish Heteropneustes fossilis is used for the present study. The average length and weight of fish are 15.5±2cm and 20±2 g, respectively. Fishes were treated with 0.01%$KMnO_4$ to remove dermal infection, if any. The fishes were acclimatized to laboratory conditions for 15 days and daily fed with dried and

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chopped prawns at the rate of 30mg/fish/day. Acclimatized fishes were divided into four groups as under.

Group I- Control

Group II- Treated with Cd for 15 days

Group III- Treated with Cd for 30 days

Group IV- Treated with Cd for 45 days

The Lc_{50} value of $Cdcl_2$ for catfish was 50.41 mg/lit. Experimental concentration of the metal was 0.20 mg/lit.

Water of all aquaria was changed on every 4th day and aquaria water was daily aerated for 30 min. Fishes from all the experimental groups were dissected on the completion of respective experimental periods and liver was taken out and processed for histology, lipid peroxidation assay and GSH assay.

Histological preparations:

For histology liver of H.fossilis from all the experimental groups was fixed in alcoholic Bouin's solution for 24 hrs. Material was then dehydrated by graded alcohols, cleared in xylene and embedded in paraffin wax. Paraffin sections of 6µ thickness were cut and stretched on albumenized glass slides. Sections were double stained with haematoxylene and eosin.

Qantitation of lipid peroxidation(LPO)

For lipid peroxidation assay TBARS test described by Ohkawa et al.,(1979) was used. LPO was determined by measuring thiobarbituric acid reactive substance (TBARS) in terms of malo-naldehyde equivalent (MDA) using the molar extinction coefficient of 1.56 x 105 min-1.cm-1 . Liver tissue was homogenized in 50 mM phosphate buffer (pH 7.7), centrifuged at 3,000 rpm for 15 min and the supernatant was used for the assay. 0.1 ml supernatant was mixed with 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml 20% glacial acetic acid, and 1.5 ml of 0.8% thiobarbituric acid (TBA). Then test tubes containing reaction mixture were shaken on vortex mixture and heated at 95 °C for 60 min in a water bath and then cooled under tap water before mixing with 1 ml distilled water and 5 ml mixture of n-butanol and pyridine (15: 1). The reaction mixture was centrifuged at 2,200 rpm for 10 min. The upper organic layer was isolated for the measurement of TBARS value based on absorbance at 532 nm. The results were expressed as nM TBARS/mg protein.

Qantitation of reduced glutathione(GSH)

GSH acts as an important antioxidant. GSH was measured by DTNB (Dithiobis) as described by Jollow et al.,(1974) that involves the spectrophotometric assessment of the 5-thio-2-nitrobenzoate in the presence of NADPH and glutathione reductase. Briefly, liver tissue was homogenized in 0.5 M ice cold metaphosphoric acid and centrifuged for 15 min at 16,000 rpm at 4 ºC. The 0.5 ml supernatant was mixed with 4 ml of ice cold 0.1 mM solution of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) in 0.1 M phosphate buffer (pH 8.0) and the optical density was observed at 412 nm . A standard calibration curve was prepared using GSH.

RESULTS

Effects of cadmium on liver histology

Fig. 1 shows the histology of control fish which exhibited normal histological architecture consisting of hexagonal hepatocytes, clear cytoplasm and a round nucleus enclosing a distinct nucleolus. Blood sinusoids with blood cells are dispersed among the hepatic cells. Wide spread histological changes in hepatic nuclei, hepatic cell boundaries and blood sinusoids were noticed in 15 days treated (group II) liver due to cadmium toxicity (Fig. 2). Structural damage in liver increased with the increase in exposure period of cadmium to 30 and 45 days (groups III and IV) leading to necrosis and degeneration. Blood cells infiltration was markedly observed following the cadmium treatment (Figs. 3 & 4).

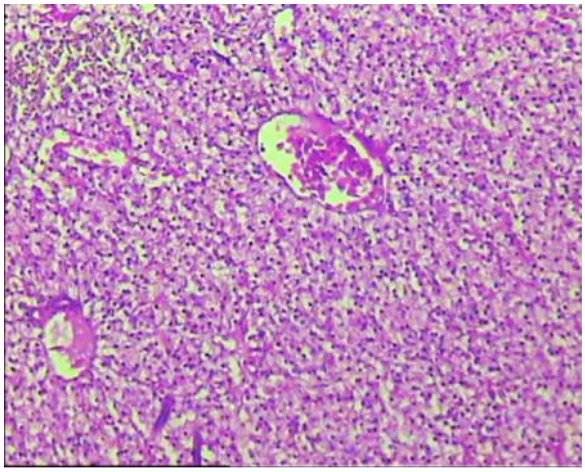

Fig: 1. shows Liver of control fish showing normal histological architecture.

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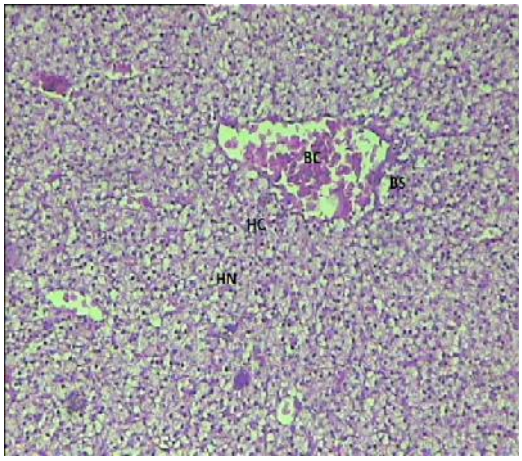

Fig: 2. 15 days cadmium treated liver showing damaged hepatic cell boundaries and blood sinusoids.

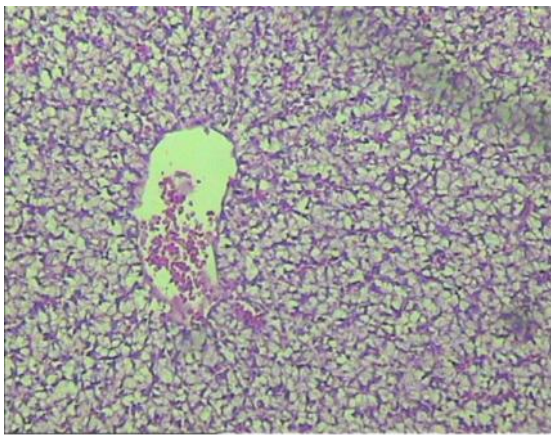

Fig: 3. 30 days cadmium treated liver showing damaged hepatic cells without nuclei and broken walls of blood sinusoids.

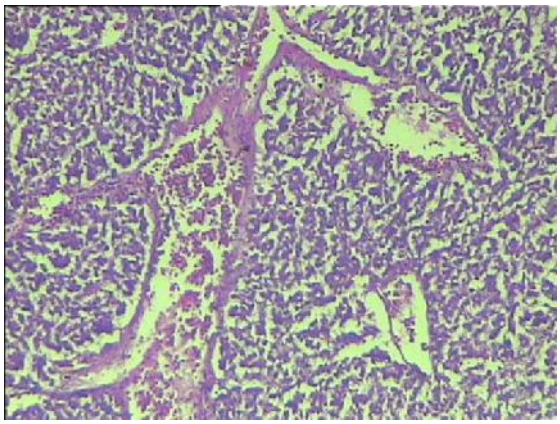

Fig: 4 .45 days cadmium treated liver showing severe hepatic damage with clumps of hepatic cells , elongated blood capillaries and infiltration .

Effect of cadmium on lipid peroxidation

LPO value in control liver was 1.115 nmTBARS/mg protein, which gradually increased in experimental groups due to the cadmium toxicity. The LPO value increased from 1.115 nmTBARS/mg to 1.859 nmTBARS/mg after 15 days of cadmium treatment. The value of LPO was further increased from 1.115 nmTBARS/mg to 4.090 nmTBARS/mg and 7.437 nmTBARS/mg in 30 and 45 days exposed groups, respectively.

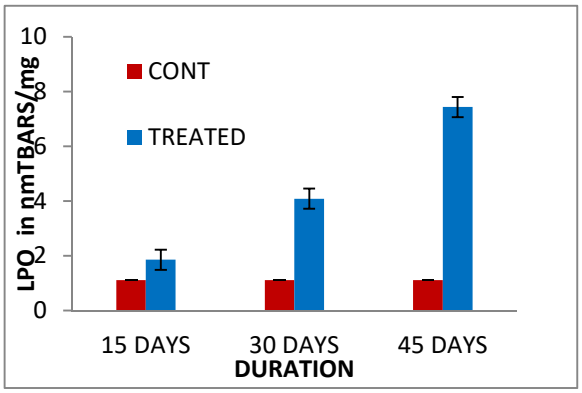

Fig : 5. showing values of LPO of 15, 30 and 45 days cadmium treated liver against control.

Effect of cadmium on reduced glutathione (GSH)

Reduction in the GSH content was noticed after the exposure of fish to cadmium. The GSH value of control liver was 92.666mg/gm tissue weight. In 15 days treated group reduction in GSH was found and the value was 88.0mg/gm which gradually decreases to 26.0 mg/gm and 2.666 mg/gm in 30 and 45 days, respectively.

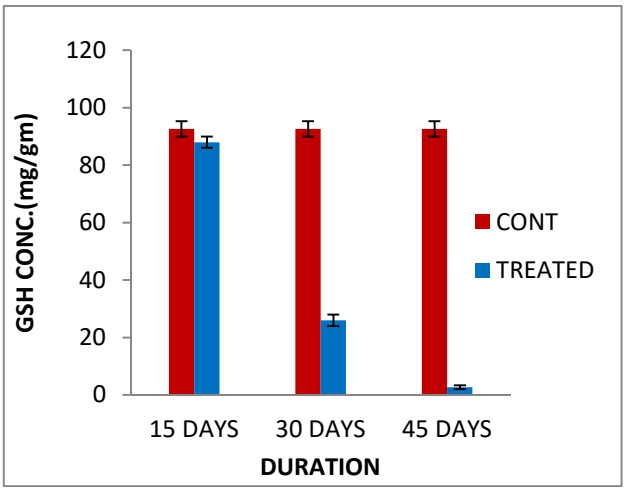

Fig :6 showing value of GSH in 15, 30 and 45 days cadmium treated liver against control.

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DISCUSSION

Cadmium is highly toxic heavy metal and causes adverse effects on the living organisms.

Several previous studies revealed that cadmium induces various histological changes in liver such as, elongation of blood vessels, congestion, vacuolar degeneration of hepatocytes and fatty changes in peripancreatic hepatocytes (Rani and Ramamurthi, 1989 ; Lie et al., 2009). Severe structural damage in liver leading to loss of regular compartmentation is also reported (Giari et al., 2007).various studies show peripheral shifting of nucleus and infilteration of blood cells (Camargo and Martinez, 2007 ; Mohamed 2009).

The results presented in this study show changes in hepatic nuclei, hepatic cell boundaries and blood sinusoids. In few hepatocytes hepatic nuclei were shifted towards cell boundaries. The images showing the hepatic degeneration after the cadmium treatment. Randomly aggregated nuclei are seen without hepatic cell boundaries. Blood capillaries exhibited with broken capillary wall causing infiltration of blood cells.

One of the mechanism by which the heavy metals can exert their toxic effect is through the increase in the oxidative stress (Jobling ,1995 ; Kim et al, 2010). Increase in oxidative stress causes the degenerative changes in the tissue as exhibited by the increase in the lipid peroxidation of cell membrane and the disruption of membrane bound activities as seen in fish liver in this study. Cadmium stimulates the peroxidation of lipid membrane. (Knight and Voorhees, 1990) . The end product of lipid peroxidation is malondialdehyde (MDA) (Viarango,1989) which is extremely toxic for the cell. Similar result of increased LPO was also reported by Vinodhini and Narayanan,(2009) in fish tissues. Jeane et al.,(2009) also noticed increased hepatic lipid peroxidation due to cadmium exposure . One of the most significant effect of cadmium is to reduce the antioxidant system in the tissue. Previous reports showed the alteration in the GSH level due to cadmium treatment (Kovarova et al., 2009; Cao et al., 2010; Jia et al., 2011). Our results showed that the level of GSH in liver was significantly declined by the exposure of cadmium.

In the present study the value of GSH decreses with the increase in the exposure period of cadmium. These results shows the decline in the antioxidant defense system in the tissue. Similar decline in GSH was also found in another study in the liver of animal treated with cadmium (Sevcikova et al., 2011).

CONCLUSION

The results of the present study indicate that the cadmium exerts the toxic effect on the histology of fish liver. It causes severe damage to the liver tissue, such as hepatic cell degeneration, necrosis and infiltration of blood cells. Cadmium also induces oxidative stress by increasing LPO with the increase in the exposure period. Cadmium exposure also caused the decline in the antioxidative defense mechanism by decreasing the level of GSH significantly. We conclude that cadmium causes toxicity in the liver by increasing the oxidative damage and declining endogenous antioxidants.

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