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Research Article

ACUTE TOXICITY IMPACT OF CADMIUM SULPHATE ON BEHAVIOUR AND HISTOPATHOLOGICAL CHANGES IN LIVER OF OREOCHROMIS MOSSAMBICUS (CICHLIDAE)

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ABSTRACT

In this study the behavioural changes and the toxic effects of Cadmium Sulphate on the histology of liver of Cichlid fish *Oreochromis mossambicus* was investigated. The purpose of study was to investigate whether metal (Cd) concentration and exposure period influence the degree and nature of histological changes in the liver of treated fish (*Oreochromis mossambicus*). Data obtained from the toxicity tests were obtained using the probit analysis and the 96h LC_{50} value was calculated as 600 mg/l. The abnormal behaviours were observed in the lethal concentration exposed and the histopathological changes in the liver for sub-lethal concentrations were characterized by Hepatocyte, vacuolation, increase in the sinusoidal spaces and Haemorrhage.

INTRODUCTION

Fishes are very sensitive for monitoring the changes in their surrounding environment including an increase in pollution. Health of a fish thus may reflect a dependable estimation of the health status of an aquatic ecosystem. Heavy metals are present in runoffs from mining activities and industries which use these metals in various processes. The increased efforts of humans to promote industrialization have brought them into close contact with rare heavy metals. Among many other heavy metals, Cadmium is widely used in industrialized societies and thus growing the risk of artificially increasing the amount of this element in the environment (Fleischer *et al*.,).

Cadmium is used in the manufacture of pesticides and fertilizers and use of these products increases the levels of cadmium in the environment (Petering *et al*., 1971). The concern about cadmium as an environmental contaminant has resulted due to the acceptance of the valuable metallurgical properties of cadmium, such as corrosion resistance, thereby leading to an increased metal production. The contamination of natural water resources by cadmium can occur due to direct entry of runoffs from industrial discharges or by means of mining and smelting operations.

Pollution of fresh water reservoirs by cadmium and other heavy metals have been reported by (Srivastav and Srivastav 1998); (U.S.ATSDR 1999);(Senthil *et al.,* 2008); (Tripathi and Dubey 2008); (Ebrahimi *et al., 2009*). Fish may accumulate concentration of heavy metals directly from contaminated water or indirectly by feeding on living organisms in the contaminated water and have been recognized as major vectors for transferring contaminants to humans (Suedel *et al., 1997*); (Javed Md., 2005). Cadmium has been considered as a contaminant which adversely affects water quality, feeding and swimming behaviour of fish (Atif *et al.,* 2005); (Laovitthyanggoon S., 2006). It was also reported that Cadmium delays the hatching and maturation period in *Channa punctatus* (Srivastav and Srivastav 1998). The main objective of this study was to determine the lethal concentration (LC_{50}), behavioural alterations and histopathological changes in liver of *Oreochromis mossambicus* (Cichlidae).

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MATERIAL AND METHODS

Fish and acclimation conditions

In the experiment, *Tilapia mossambicus,* weight ranging from 50-100 grams were obtained from local pond namely Supatal, Jabalpur. These fishes were first treated with 0.1% potassium permanganate solution for 15 minutes to get rid of dermal infections. The fishes were acclimatized for 2 weeks in de-chlorinated water of 30-50L glass aquariums under laboratory conditions. The water temperature, PH and dissolved oxygen of the experimental water and cadmium sulphate concentration in the experiments are given in table 1.

Test chemical and determination of LC50

The Cadmium Sulphate ($3CdSO_4.8H_2O$) from Central Drug House (Batch no. 13059) was used as the test compound for determination of median lethal dose (LC_{50}) of Cadmium Sulphate. A total of eight fishes were treated in eight different aquaria with continuous aeration and the fishes were fed once a day with wheat flour during this conditioning period. The fishes were accepted as well adapted to laboratory conditions when mortality less than 1% was recorded during acclimatization period of 14 days. Fish of both sexes were used without discrimination. The temperature, pH and dissolved oxygen of the aquaria were monitored on daily basis throughout the experiment. The acute toxicity test was performed for 4days with a replicate of seven different concentrations (350, 400, 450, 500, 600, 650 and 700 mg/l) at 24, 48, 72, and 96 hours. Dead fishes were counted along with the control group and dead individuals were removed immediately, and behavioural changes were observed closely. In this study the data of toxicity for Cadmium sulphate upon the *Oreochromis mossambicus* were determined using probit analysis technique (Finney, 1952) as given in (Table 2 and Table 3). Also the logarithm of Cadmium sulphate concentrations against the empirical probit values of the mortality of *Oreochromis mossambicus* individuals is given (Fig. 1).

Experimental exposure

After determining LC_{50} value for Cadmium, fishes were divided into nine groups with six fishes in each group. The fishes were exposed to the sub lethal concentrations of Cadmium (50mg/l, 75mg/l and 100mg/l) each for 10, 20 and 30 days of exposure for histopathological studies.

Tissue preparations

At the end of exposure time, fish of all the groups were sacrificed. Liver of control and alive treated fish were dissected out, and the tissue were fixed in Bouin's solution for 24 h and then were washed with water to remove the fixative. After washing, tissues were treated with ascending series of alcohol for dehydration and then were processed for paraffin embedding.

Histology

Paraffin blocks of liver of all the groups were cut at 6 micron thickness and stretched on sterilized glass slides. After deparaffinization and dehydration, sections were stained with Haematoxylin–Eosine and observed under light microscopy. The histopathological changes in the tissues were examined from each fish. Histopathological changes induced by treatments in the tissues were photographed using Censico photomicroscope.

Table 1 The water temperature, pH and Dissolved oxygen values of each aquarium used in the toxicity experiments of Cadmium sulphate upon *Oreochromis mossambicus.*

Conc. µl/L	Tempe-rature °c	PH	Dissolved oxygen mg/l
350	22.3	8.22	8.1
400	22.6	8.43	8.3
450	22.7	8.54	8.3
500	24.0	8.23	8.2
600	23.7	8.22	7.8
650	24.4	8.35	7.7
700	24.9	8.67	7.9

Table 2 The mortality rate of *Oreochromis mossambicus* individuals in 96 h at different concentrations of cadmium sulphate.

Con. mg/l	log dose	24 h	48 h	72 h	96 h	Total
Control	0	0	0	0	0	0
350	2.544068044	0	0	0	0	0
400	2.602059991	0	0	0	1	1
450	2.653212514	0	0	1	2	3
500	2.698970004	0	1	1	1	3
600	2.77815125	0	1	1	2	4
650	2.812913357	1	1	2	3	7
700	2.84509804	2	2	1	3	8

Table 3 Mortality and empirical Probit values for the concentration of cadmium sulphate after 96 h for calculation of LC_{50}.

Con. µl/l	log dose	Mortality	Percentage	Probit
400	2.602059991	1	12.5	3.85
450	2.653212514	3	37.5	4.68
500	2.698970004	3	37.5	4.68
600	2.77815125	4	50	5.0
650	2.812913357	7	87.5	6.15

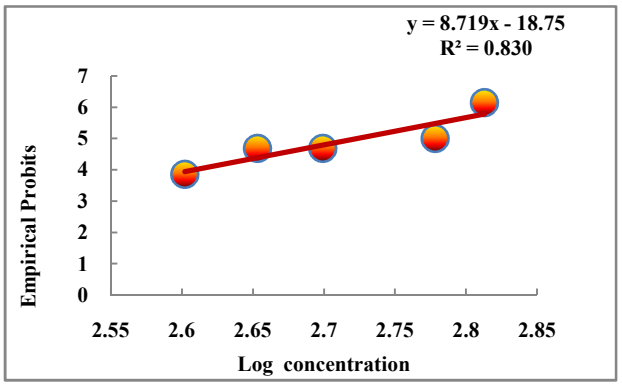

Fig 1 The logarithm of Cadmium concentration against the empirical probit values of the mortality of *Oreochromis mossambicus* individuals.

RESULT

The histology of *O. mossambicus* liver in the control group showed normal structure of hepatic cells, the connective tissue of liver expressed normal condition, normal hepatic mass granulation were observed. Hepatocytes are seen as polygonal cells with a spherical central nucleus. Each sinusoid consists of an outer peripheral connective tissue and an inner lining of endothelial cells. A large number of blood sinusoids were found in the hepatic mass of these cords. Thin bile canaliculi are observed between the hepatic cells (Fig 2.1).

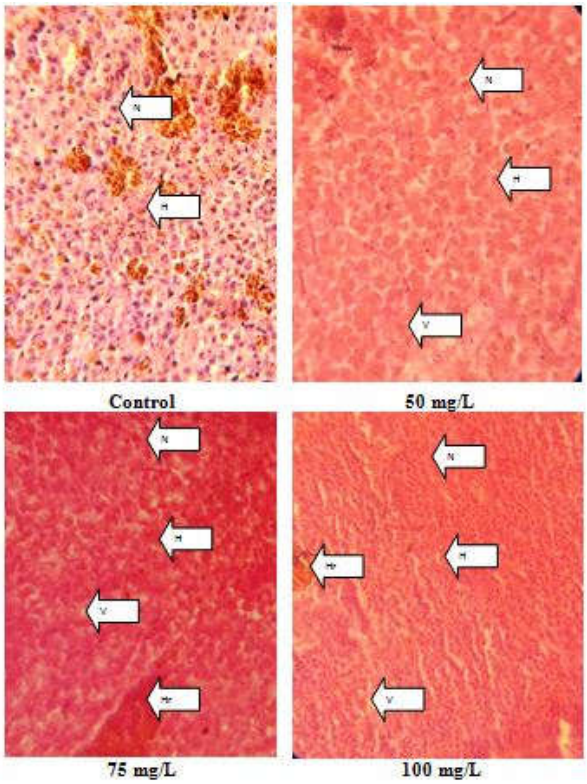

Fig 2.1: Effect of cadmium sulphate of different concentrations on histopathology of liver of *Oreochromis mossambicus* after 10 days of exposure as is shown by Haematoxylin and Eosin (H&E) staining (400x). H-Hepatocyte, N-Nucleus, V-vacuolation, Hr-Hemorrhage

When the fishes were exposed to the different concentrations of cadmium sulphate for 10 days, lower concentrations revealed the hepatic cells with the characteristic round or polygonal shape with the shrunken nucleus. On increasing the concentration to 75 and 100 mg/L, the histological sections of the liver of the fish was observed the change in shape of hepatocytes, increased vacuolation as well as some haemorrhage (Fig 2.1).

The changes in liver histology became more significant with the increase in time of exposure. After 20 days of exposure, the hepatic cells started changing the shape because of shrunken nucleus, resulting in disintegration of bile ducts. The vacuoles were larger and haemorrhage was seen with all tested concentrations (Fig 2.2).

As the exposure time increased, more deleterious effects were seen in the liver of *O. mossambicus.* The histopathological changes were the complete disintegration of hepatocytes. The pyconotic nucleus was observed in the liver sections, as well as extensive vacuolation of all the hepatocytes and finally lost the polygonal shape. Haemorrhage was frequently seen with all tested concentrations after 30 days of exposure (Fig 2.3).

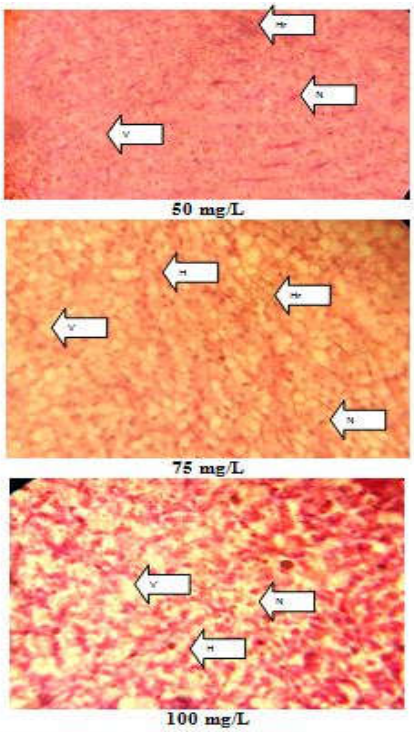

Fig 2.2: Effect of cadmium sulphate of different concentrations on histopathology of liver of *Oreochromis mossambicus* after 20 days of exposure as is shown by Haematoxylin and Eosin (H&E) staining (400x). H-Hepatocyte, N-Nucleus, V-vacuolation, Hr-Hemorrhage

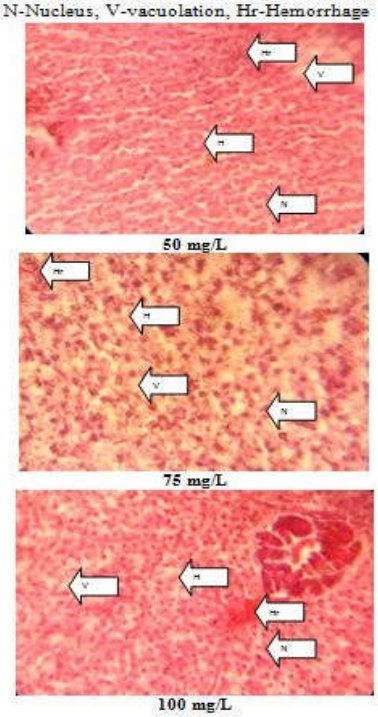

Fig 2.3: Effect of cadmium sulphate of different concentrations on histopathology of liver of *Oreochromis mossambicus* after 30 days of exposure as is shown by Haematoxylin and Eosin (H&E) staining (400x). H-Hepatocyte, N-Nucleus, V-vacuolation, Hr-Hemorrhage

DISCUSSION

Liver is an important detoxifying organ which breaks down toxic substances and metabolites of administered substances. This process is carried out by hepatocytes. Due to this reason the hepatic cells are severely damaged on exposure to toxicants.

In the present study Cadmium exposed to liver showed vacuolation, degeneration of hepatocytes, swollen and pyconotic nucleus, necrosis, disintegration of bile duct, Haemorrhages and lost the polygonal shape of hepatocytes. Similar changes in the liver of fishes had been reported in different species treated with various toxicants. Focal coagulative necrosis and early cirrhosis had been reported in the liver of *Heteropneustes fossilis* exposed to sunset yellow and brilliant blue (Rajiv *et al*., 1994). Ahmed *et al.* (2013) observed that the liver of *Oreochromis mossambicus* exposed to arsenic showed intense degenerative changes, and vacuolization of hepatocytes. Gaber *et al.* (2014) examined that degeneration of hepatocytes, focal necrosis, edema in liver of seabream and seabass. The liver of tilapia treated with cadmium showed degeneration of the hepatocytes with nuclear pyknosis, sinusoids with necrosed hepatocytes (Kaoud *et al.,* 2011).

Studies describing the effects of metals like Hg, Cd, Pb, Zn, Al and Cu on survival and histopathology of various organs in teleost fish are available (Van Heerden *et al*., 2004; Figueiredo-Fernandes *et al*., 2007, Mishra and Mohanty, 2008). The alterations in liver due to toxicity impact are often associated with a degenerative necrotic condition (Figueiredo-Fernandes *et al*., 2007).

The present study suggests a strong link between heavy metals and lesions in the liver. Sorensen *et al.* (1991) cited that heavy metals in Elbe were might cause liver damage. Aly *et al*. (2003) obtained similar results after exposure of *Clarias gariepinus* to lead pollution. They found that the vacuolar degeneration and necrosis of hepatocytes. Olojo *et al.,* (2005) found degeneration of the hepatocytes and focal necrosis in the liver of *Clarias gariepinus* exposed to lead. Similar and supportive findings of Gupta (2008), Vinodhini and Narayanan (2009) and Pathan *et al.,* (2010), who too have reported severe necrosis of hepatocytes and enlargement of blood spaces to hamper the haematopoiesis, of the fish that offers a strong support for the present point of view. Deka and Mahanta, (2012) reported at 10 days treatment, the liver of *Heteropneustes fossilis* revealed the hepatocytes dissociated, with swollen shape and granular cytoplasm. The nuclei turned pycnotic in addition, patchy degeneration, with increased fibrosis in tissue causing congestion in sinusoids.

Degenerative changes in the liver of *Oreochromis mossambicus* demonstrated that the liver happen to be the organ affected most severely in response to chromium intoxication.

CONCLUSION

In the present study the toxicity tests showed that heavy metals, i.e, cadmium is toxic to fresh water fish species which constitute the non-target organism. The sensitivity of *O. mossambicus* to this toxicant is evaluated for short and long term duration in this study. The sub-lethal concentrations caused considerable deterioration to fish health effecting the structure and function of liver. Thus the present study suggests that because of its toxicity the cadmium release must be well monitored in aquaculture farms and agricultural fields.

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