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EFFECT OF ALUMINIUM CHLORIDE TOXICITY AGAINST HISTOPATHOLOGY OF GILL AND LIVER TISSUE OF INDIAN MAJOR CARP, *CATLA CATLA* (HAMILTON)

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ABSTRACT

Histopathological studies in organs like gill and liver of *Catla catla* (Hamilton) were made to assess tissue damage due to Sublethal concentration of aluminium chloride. Histopathological study provides a real picture of the detrimental effects and the involvement of the heavy metal toxicants in the major vital functions such as respiration metabolism and reproduction in aquatic animals. It is generally evident that changes in microscopic abnormalities. The gill of aluminium exposed fish exhibited vacuole formation in epithelial cells, swollen of lamellar tips, rupturing of epithelial wall, dilation of blood vessels and fusioning and shortening of secondary lamellae and loss of broken lamellar structure. The liver of *Catla catla* exposed to aluminium showed distinct hepatic lesions. The histopathological changes included necrosis, psychosis, disintegration of cells and vacuolization. In addition internal haemorrhage, necrosis and empty blood vessels were also seen. Such pathological changes were observed in all the tissues were more pronounced in sublethal concentration for 28 days of exposure at 10 % and 20% of the 96hrs LC_{50} value. The observation of the present study is to suggest that the damage of these tissues is caused by cumulative accumulation of aluminium in tissues.

KEY WORDS: Aluminium chloride, *Catla catla*, Histopathology, Gill, Liver

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INTRODUCTION

Freshwater ecosystem has been polluted by continuous discharge of waste water from industries, human dwelling and agricultural practices. The wastewater contains various amounts of chemical substances, such as pesticides, fertilizers and industrial pollutants. These chemical substances are accumulate in large quantities of water. Often accumulate in large quantities. Such poisonous substances on reaching aquatic ecosystem have a fatal effect on biota including fishes. These chemical substances are accumulate in large quatities of water it will accumulate in organisms through food chain and harmful to human beings. Industrial pollutants occupy prime position than the others, as they release heavy metals in to the aquatic environment [1]. Heavy metals pose a serious threat to the aquatic environment because of their greater toxicity and persistence to accumulate in organisms through food chain amplification[2]. Aluminium metal and its compounds have a wide variety of uses including structural materials in construction auto mobiles, air crafts and the production of metal alloy, glass, ceramic, rubber pharmaceuticals and water proofing textiles. The presence of aluminium in water found to be in different forms[3] among fish species considerable differences in sensitivity to metals have been reported. *Catla Catla* have the ability to accumulate and concentrate iron to the levels, several order of magnitude above those found in their environment . The aqueous aluminium is recognized as the principal toxicant to fishes [4,5]. Sastry and Shukla [6] have shown that exposure to heavy metal pollution results in decreased oxygen consumption in fish Indian major carp *C.Catla* is an important cultured fish species in fish ponds due to its fast growth and economic value. However the studies on the histopathological effect of aluminium on the fresh water fishes are very limited. Therefore, the present study is proposed to assess the aluminium toxicity on the histopathological changes of vital organs like gill and liver of fish *C. catla*.

MATERIALS AND METHODS

COLLECTION OF EXPERIMENTAL ANIMAL

The fresh water fish *Catla catla* average length 3 ± 0.50 cm, weight 2.5 to 3gm were procured from the B.S.P. aquarium, Puthur, near Chidambaram, Tamil Nadu. They were brought to the laboratory in polythene bags filled with aerated water and stocked in a tank of 60litre capacity filled with tap water and fishes were acclimatized in the laboratory conditions for about two weeks. Significant sign of stress or unusual behavioural criteria were not observed in the control fishes throughout the acclimation and test period. During the acclimatization the fishes were fed with pellet feed daily in the evening uneaten feed was removed next day morning followed by 100% water exchange.

PREPARATION OF STOCK SOLUTION FOR ALUMINIUM CHLORIDE TOXICITY TEST

One gram of Aluminium chloride (Merck, Germany) was dissolved in 1 l of double-distilled water and used as the stock solution for preparing different concentrations of aluminium chloride in rearing water. It was stored in a clean standard flask at room temperature in the laboratory

SUBLETHAL TOXICITY TESTS

For sublethal toxicity tests, the fishes were grouped into three batches. Each batch had ten fishes and three replicates were maintained. Fishes maintained in normal fresh water served as control (group I). Fishes were exposed to concentration of 5.31ppm (10% of 96 hr LC_{50}) of Aluminium chloride in fresh water (group II). Fishes were exposed to the sublethal concentration of 10.62ppm (20% of 96 hr LC_{50}) of Aluminium chloride in fresh water (group III). The media were renewed every alternate day. Fishes were fed daily with artificial feed. Two specimens each from the groups I, II and III were sacrificed after 28 days of the experiment.

HISTOPATHOLOGY

Fishes were exposed to Aluminium chloride at 5.31 and 10.62 ppm for 28 days. Sampling was done on the 28th day of exposure; two fishes in each group were sacrificed. The gills and liver of representative fishes from each test and control group were dissected out and fixed in Davidson's fixative for 24hrs. The preserved tissues were processed by a routine histological method [7], dehydrated in alcohol series and embedded in paraffin wax. They were cut into sections of 6 mm thickness by a rotary microtome (Weswox, MT1090:1090A, India). The thin sections of the tissues were stained by haematoxylin and eosin for observation by the Nikon Bright field transmission microscope with Koechler illumination, and automatic exposure unit was used.

RESULTS

GILL

In the gill of control fish *C catla* the primary gill lamellae are laterally compressed leaf like structures, attached alternately on either side of the interbranchial septum. Each primary gill lamellae consist of secondary gill lamellae on both the sides, which are perpendicular to its long axis. Primary gill lamellae comprised of a central core of cartilaginous rod and linings of epithelial cells are closely applied to gill ray. The tip of the primary lamellae contains a large dense mass of red blood cells. The secondary lamellae consisted a layer of flattened epithelial cells arranged to the basement, membrane. Further, the secondary lamellae consist of pillar cells situated in between blood capillaries and chloride cells located at the base of the two adjacent lamellae (Fig. 1). During the present study, *C.catla* subjected to sublethal concentration of aluminium chloride has initially exhibited a film of coagulated mucous over gill surface and marked histopathological changes of gill in both exposure for a period of 28 days at 10% and 20% of 96hrs LC_{50} value of the gill rays have become thicker, cartilaginous cells of the gill rays undergo hyperplasia, vacuole formation in epithelial layer, swollen lamellar tips and rupturing of epithelial wall of secondary lamellae. Moreover, the degeneration of pillar and chloride cells, dilation of blood vessels, fusion and shortening of secondary lamellae and loss of broken lamellar structure are the important histopathological changes in both treatment. In addition, owing to hyperplasia, inter lamellar epithelium exhibits a characteristic aggregation in fish exposed to sublethal concentration of aluminium. The lesions observed in the 20% of the 96hrs LC_{50} vaule of sublethal exposure were more pronounced than those in the sublethal exposure of aluminium chloride (Fig. 2&3).

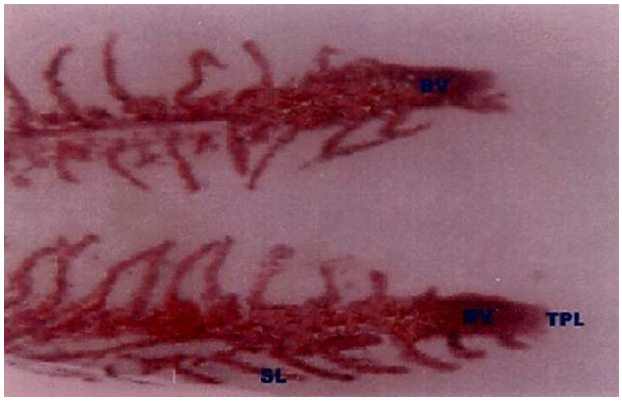

Plate 1

Histology of control gill in C.catla photomicrographs of the paraffin section stained with haematoxylin and eosin (×40). BV- Blood Vessels, SL - Secondary Lamellae, TPL -Tip of Primary Lamellae.

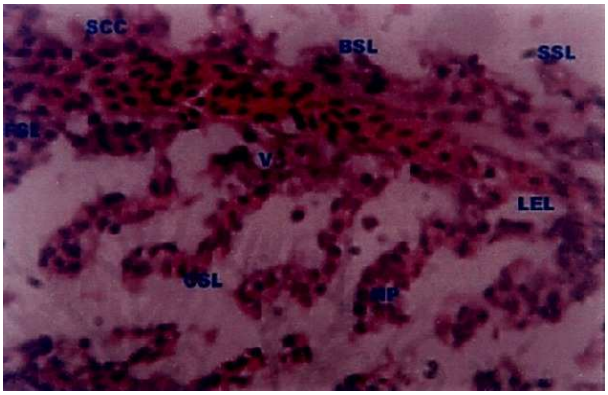

Plate 2

Histopathological changes of gill in C.catla photomicrographs of the paraffin section stained with haematoxylin and eosin (×40) after 28 days of exposure to 5.31ppm concentration of Aluminium chloride. BSL- Broken of Secondary Lamellae, CSL- Curling of Secondary Lamellae, FSL - Fusion of Secondary Lamellae, HP - Hyperplasia, LEl - Lifting on Epithelial Layer, SCC- Swelling of Chloride Cell, SSL - Shortening of Secondary Lamellae, V - Vacuole.

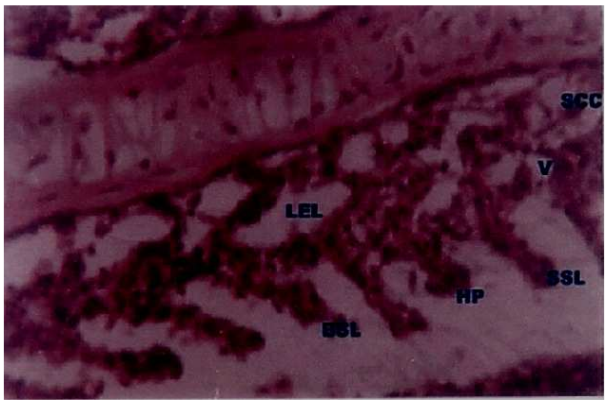

Plate 3

Histopathological changes of gill in C.catla photomicrographs of the paraffin section stained with haematoxylin and eosin (×40) after 28 days of exposure to 10.62ppm concentration of Aluminium chloride. BSL - Broken of Secondary Lamellae, HP – Hyperplasia, LEL- Lifting on Epithelial Layer, SCC - Swelling of Chloride Cell, SSL- Shortening of Secondary Lamellae, V - Vacuole.

LIVER

Microscopical observation shows that the normal liver of *C. catla* consists of large number of hepatocytes arranged regularly in cords. The hepatic cells are large, polygonal in shape with prominent nucleus. Blood sinusoids are also seen among the hepatocytes. Liver cells are similar in size. The nuclei of the liver cells are spherical and show uniform size, shape and orientation (Fig.4). In the present study the following histopathological changes have been observed in the liver of *C. catla e*xposed to sublethal concentrations of aluminium chloride for 28 days at 10% and 20% of 96hrs LC_{50} value. In both exposures the liver parenchymal cells arc found to shrunken and appear smaller in size, Variations in the shape of hepatocyte is evident in different regions of liver, Intrahepatic spaces have been dilated. The extension of nucleus called the pyknosis and cytoplasmolysis are observed in different region. The cytoplasm becomes granulated and vacuolated. The blood vessels are severely damaged leaving large empty spaces. The liver

cord has become disarrayed in both treated groups.These above observed histopathological changes in the median lethal exposures are more pronounced than those in the sublethal exposure (Fig.5 &6).

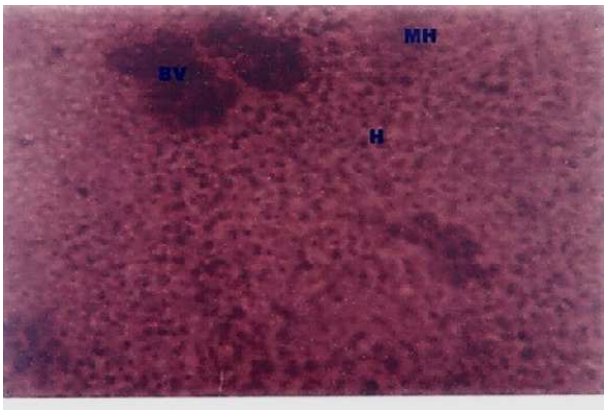

Plate 4

Histology of control liver in C.catla photomicrographs of the paraffin section stained with haematoxylin and eosin (×40). BV - Blood Vessels, H - Hepatocyte, MH- Mass of Hepatocyte

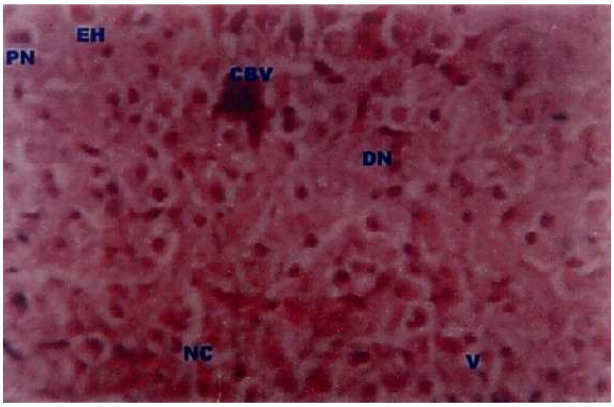

Plate 5

Histopathological changes of liver in C.catla photomicrographs of the paraffin section stained with haematoxylin and eosin (×40) after 28 days of exposure to 5.31ppm concentration of Aluminium chloride. CBV- Congested Blood Vessel, DN - Disintegration of Nucleus, EH - Enlargement of Hepatocyte, NC - Necrosis in Cytoplasm , PN - Pyknotic Nuclei, V - Vacuolation.

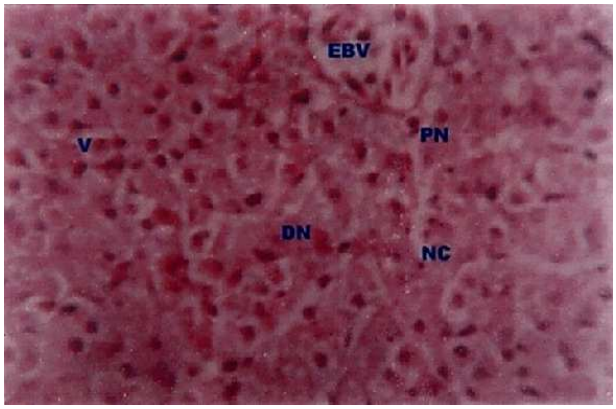

Plate 6

Histopathological changes of liver in C.catla photomicrographs of the paraffin section stained with haematoxylin and eosin (×40) after 28 days of exposure to 10.62ppm concentration of Aluminium chloride. DN - Disintegration of Nucleus, EBV -Empty Blood Vessels, NC - Necrosis in cytoplasm, PN - Pyknotic Nuclei, V - Vacuolation

DISCUSSION

The liver of the fish can be considered as a target organ to pollutants. Alteration in its structure can be significant in the evaluation of fish health and exhibit the effects of variety of environmental pollutants. The gills of fish are the most important organ for respiration and osmoregulation and its external location renders it most vulnerable target organ for the pollutants [8]. The study on morphometry and histopathology of gills may be used to monitor the quality of the environment. During the present study, several histopathological changes were observed in the gills of *C. catla,* when they exposed to sublethal concentrations of aluminium chloride after 28 days at 10% and 20% of the 96hrs LC_{50} value. The common histopathological changes caused by aluminium were curling of lamellae, desquamation of the lamellar epithelium, hyperplasia and degeneration of the primary lamellae and secondary lamellae. Similar histopathological changes were also observed in *C. punctatus* exposed to methyl ethyl mercuric chloride [9].

In the present study, epithelial cells of the secondary gill lamellae of aluminium treated C. *catla* depicted an initial hypertrophy and vacuolization. These appear to be the general responses of the gill to heavy metal pollution [10,11].The observed progressive hyperplasia, necrosis, desquamation of the epithelial cells and multiple telangiectasia in *C. catla* exposed to aluminium was quite comparable to the fish *Sulmo gairdneri* [12]. The Filling up of inter-lamellar spaces by hyperplastic epithelium under aluminium treatment tends to explain the high mortality of the fish, probably due to asphyxia [8]. Mallatt [13] suggested that hyperplasia of squamous epithelial and chloride cells serves as a physiological defense mechanism against the toxicants. In addition to hyperplasia of inter lamellar cells, necrosis and pycnotic epithelial cells are also observed in the treated fish. These changes might he resulting a decrease in energy metabolism due to degeneration of respiratory epithelium and the damage of the gill tissues lead to tissue hypoxia. Gardner and Yovich [14], Mathivanan[15],Jagadeesan[16] and Karuppasamy [17] have also been observed the impairment of respiration and external functioning in the gills of estuarine teleost fish, *Fundulus heterocliitus* ,fresh water fish. *Anabas testudineus* when exposed to cadmium, *Labeo rohita* fingerlings when exposed to mercury and *Channa punctatus* when exposed to PMA (Phenyl I Mercuric Acetate) respectively. The survival of *C. catla* at 10% of 96hrs LC_{50} value of sublethal concentration of aluminium chloride

after 28 days might be due to the lesser damage whereas in 20% of 96h LC_{50} value sublethal concentration the intensity of the metal effect is more pronounced and rapid. leading to the mortality of fish.

The liver is an important vital organ through which most of the important metabolic functions are occurring and the entry of toxicants primarily affects the liver. Toxic induced changes in the liver of the fishes can be regarded as an index for the identification of pollution present in the environment [18]. During the present study marked histopathological lesions were observed in the liver of C. *catla* exposed to sublethal concentrations of aluminium chloride. At sublethal concentration histopathological lesions were mild and observed as degeneration of hepatocytes, cell wall rupture, necrosis, picnosis and cytopalsmolysis. parenchymal cells leading to appear smaller in size, cytoplasm become granulated and vacuolated, damaged and empty blood vessels, and swelling of liver cord. These severe effects in sublethal concentration may be due to the possibility of more accumulation of aluminium in liver. Similar histopathological changes were observed in the liver of fishes exposed to different metal toxicants [19,20,21&17] noticed disintegrated hepatic cells and nuclei in the liver of mercury treated *C. carpio.* Akkilender Naidu [22] showed granular degeneration and vacuolization of hepatic cells, liver cord disarray, damage of blood vessels leaving large empty space and enlargement of nuclei when S. m*ossambicus* was exposed to mercury. Sastry and Rao [20] observed vacuolization of hepatocytes, necrosis, rupture of cell membrane and enlargement of intercellular spaces in the liver when *C. punctatus* was exposed to methoxy ethyl mercuric chloride. Rajamanickam [21] exposed *M. vittatus* to copper and observed necrosis of hepatocytes, loss of adhesion between cells, enlargement of nucleus. Vacuolization, karyolysis and swelling of liver cord. Karuppasamy [17] observed severe hepatic lesions of ruptured outer membrane, necrosis, pycnosis, vacuolat ion, damaged blood cells and accumulation of cytoplasmic granules in *C. punctatus* when exposed to PMA. Shastry and Sharma [23] exposed *C.punctatus* to sublethal concentration (0.01mg/l) of endrin were observed hypertrophy of hepatic cells and liver cord disarray, vacuolation of cytoplasm and necrosis, rupture of hepatic cell membrane and necrotic centrolobular area.

CONCLUSIONS

The histopathological changes observed in different fishes exposed to various concentrations of metals and other toxicants have caused deleterious effects. Since liver is one of the major sites of metabolic activities, the toxic effects of heavy metals including aluminium are more pronounced and cause the malfunction resulting in the altered metabolism in fish. Although it is too early to conclude all of the unknown effects of aluminium, our findings strongly suggest that aluminium is very toxic for *Catla catla*. Surely more information is needed to decide either aluminium affects the gills and a liver directly or indirectly. By this context, the aluminium has to be taken into more consideration as an environmental contaminant. Further details should be obtained from advanced investigations.

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