Effect of copper chloride on the histopathology of liver of the freshwater fish, "*Clarias batrachus*" (Lin.)

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Abstract
The experimental fish exposed to different graded concentration of toxicant (copper chloride), exhibited abnormal behavioral response. During the exposure time $CuCl_2$ treated fish initially showed rapid movement faster opercula activity, surfacing and gulping of air. The study was assess the effect of copper chloride on histopathology of liver in the fresh water fish *Clarias batrachus*. The fish were dividing in 5 groups. After exposure to 1/5 of sub lethal concentration of copper sulphate, the tissue like liver of the fish *Clarias batrachus* showed various degenerative changes after 7, 14, 21 and 28 days. The degenerative histopathological change observed in the liver are remandable in the copper chloride treated fish.

Keywords: Copper chloride, *Clarias batrachus*, histopathology

1. Introduction

Pollution of the aquatic environment generally cause in the physiological and structure of the inhabitant organisms heavy metals have become major environmental pollutant today. Heavy metal compound are the main source of water pollution and are through sewage and industrial effluents in the aquatic environment. Some heavy metal compounds are also used in fish pond by the aqua culturists to control algal blooms. They acts as an algaecides and are known to contaminate natural water bodies. Fish are in direct contact with environment and are susceptible to any change that may occur. In the growth and development of terrestrial and aquatic animal natural condition play one of the important role in the natural environment various chemical occur, most of them however do not penetrate in significant amount in organisms, despite being in direct contact with them. During evolution countless relationship have been develop between organism and their environment when those relationship are distrupted by change environmental condition disease or even death of organism may occur [1]. Heavy metals have been recognized as strong biological poison because of their persistent nature toxicity tendency to accumulate in organisms and undergo food chain amplification. They also damage the aquatic fauna including fish [2]. Measuring heavy metals in aquatic organism may be a bio indicator of their impact on organism and ecosystem health [3-4]. But a true evolution of the damage inflicted by heavy metals should come from comprehensive biomarker studies biomarkers are more telling them bio indicator as a measurement of heavy metal contamination because they deal with chemical and physiological changes on the organism level and assess contamination based on direct measure of change in the organism [5]. The main source of fresh water pollution can be attributed to discharge of untreated waste, dumping of industrial effluent and run off from agricultural field. Stress response in characterized by physiological changes and human destructive influence on the aquatic environment is in the form of sub-lethal pollution which result in chronic stress condition effect of pollution on fish is assessed by acute and chronic toxicity tests. Copper is essential part of variety of enzyme (free radical defense) and liver proteins homocuprien and hepatocuprien. It is also used as fungicide, algaecide and herbicide and in municipal water treatment system [6].The accumulation of contaminants in fish occurs through biological uptake and retention of chemical contaminants derived from various exposure pathways such as the uptake of dissolved chemical constituents from the water and ingestion and assimilation of pollutants in tissues of fish are often related to those found in the environment [7]. Various metals like mercury, cadmium and lead are injurious to the fish even al low concentration, whereas other zinc, copper and cobalt are biologically more critical that can only become toxic at elevated concentrations [8]. Although toxicants impair the metabolic and the physiological activities of the organism, physiological studies alone do not satisfy the complete understanding of pathological condition of tissues under toxic stress. Hence, it is useful to have an insight in histological analysis, as they acts as biological marker's to access the toxicity condition [9]. Fish liver histology could therefore serve as the model for studying the interaction between environmental factor and hepatic structure and function [10]. The liver histology is used as biomarker for the environmental pollution and there have been numerous reports of histopathological changes in liver of fish exposed to a wide range of organic compound and heavy metal [8]. The liver is an important organ performing vital functions including biotransformation, migration of lipids, glycogen storage and release of glucose into the blood. Liver showed highest accumulation rate, the metal from the gill and other organ will be transported to liver for their detoxification more over live is the sight of various protein other molecule which has high affinity with metal forming complexes. Bioactive metal plays important role in metabolism metal like Zn, Cu or Mn function as cofactor in several enzyme system. Bioactive metal like Fe, Mn, Cu and Zn accumulation can actively controlled by the fish through different metabolic processes and usually ambient concentration. Copper is an essential trace metal for life present in natural water and sediments and virtually in other media including air, water and soil. It is abundante in factory effluents related to manufacturing of electric goods, fertilizer, fungicides and metal plating byproducts. This trace metal is vital part of many biological enzyme system to catalyse oxidation or reduction reaction having molecular oxygen as a co substrate. However, higher

concentration of Cu in an aquatic ecosystem would become toxic to organism due to increased product ion of free radicals in the body, tetragenecity and chromosomal aberration.

2. Material and Method

Live specimens of the fresh water fish, *Clarias batrachus* were selected for the present piece of research work. The fish were obtained for the experimental purpose from the Adan project Taluka–Karanja (Lad) Dist. Washim, M.S. This fish were brought to the laboratory in well oxygenated bags without any injury. They were washed with 1% $KMnO_4$ solution for 5 minutes for dermal disinfection. The fish were allowed to acclimatize to the laboratory condition before conducting the experiment particularly in the morning hour's fish fed on small pieces of boiled eggs ones in a day. The fishes including males and females weighting between 37 to 83.4 gms and length between 19 to 24 cms were selected for experimental work. They were maintained in separate aquaria containing free aged tap water.

2.1 Preparation of experimental aquarium

96 h Lc_{50} and sub-lethal concentration of calcium chloride for the fish *Clarias batrachus* was taken from literature. This was 0.005 mg/lit from 96 h Lc_{50} value, approximately 5 times less concentration (0.001 mg/L) Used to exposed the fishes for 28 days to study the histological structure of liver. The physiochemical characters of water used were also analyzed by using standard method [11]. (APHA – 1998) as the heavy metal toxicant was of unknown toxicity. First literature survey was made and then probable concentration was selected. Aqueous solution of calcium chloride ranging from 10-100 ppm was added to glass aquaria containing 40 liter of water to toxicant solution was added drop by drop with constant steering and then acclimatized fishes were transfer into glass aquaria (60 x 30 x 30 cm) containing 40 liter of toxicant treated water. The fish were feed pieces of boiled eggs ones in a day especially in morning hour's observation made for 24 hours from which the different concentration were selected for the full scale experiments, Behavioral changes in the fishes were observed and recorded.

For the above studies acclimatize fishes were divided into 5 groups as below.

Group 1: Containing fish in aged tap water which serve as control.

Group 2: Fish kept in toxicant water containing 0.001 mg/L $CuCl_2$ for 7 days

Group 3: Fish kept in toxicant water containing 0.001 mg/L $CuCl_2$ for 14 days.

Group 4: Fish kept in toxicant water containing 0.001 mg/L $CuCl_2$ for 21 days.

Group 5: Fish kept in toxicant water containing 0.001 mg/L $CuCl_2$ for 28 days.

The toxicant solution and aged tap water were renewed every day to maintain uniform test concentration throughout the experimental work.

2.2 Histopathological studies

Fish in each group were tested for histopathological studies of fish liver. The sections were cut at 5µ thickness and were stained with haematoxylene Eosin (H.E.) stain.

3. Result and Discussion

Histological studies revealed that liver section from control fish show normal histoarchitectrue, characterized by polygonal shaped hepatocyte with granular cytoplasm and centrally placed round nuclei. Hepatocyte arranged in well-organized hepatic cords and separated by narrow blood sinusoids. After exposure to Sub-lethal concentration Calcium chloride, the tissue like liver of *Clarias batrachus* showed varied degenerative changes observe in the liver remarkable in copper chloride treated fish. (Fig.1)

3.1 Effect of sub-lethal concentration of toxicant copper chloride on liver

In copper chloride toxicant exposed fish, the histopathological changes observed in liver were duration dependent after 7 days copper chloride exposure the hepatocyte showed disruption of regular cordal arrangement and prominent shrinkage hepatic cells (Fig. 2) The nuclei of hepatocyte observed become prominent along with disarray of hepatic cords was noticed after 14 days of exposure to toxicant copper chloride (fig. 3)After 21 days of explosure the hepatocyte become vacuolated and blood coagulation was also observed shrinkage of blood vessels, clumped erythrocytes wildly separate bile canalliculi were noticed several other degenerative changes also occurred due to acute toxic effect of copper chloride, include picnotic nuclei and damaged connective tissue, disorganization of hepatic cells and hepatic cords. Focal necrosis and acute hemorrhage was also prominent (fig. 4)

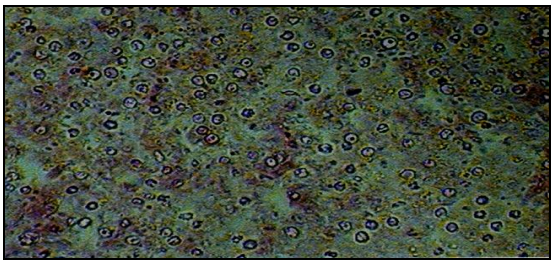

Fig 1: T.S. of Liver of *clarias batrachus* illustraling normal structure.

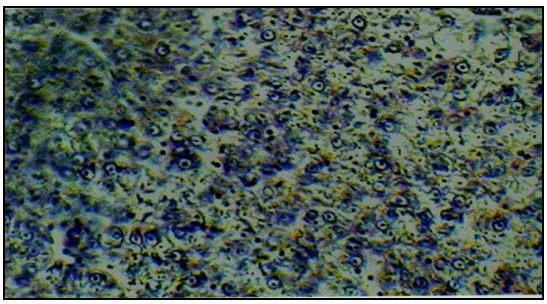

Fig 2: T.S. of Liver illustraling histomorphological changes after exposure of the fish to toxicant Copper chloride for 7 days.

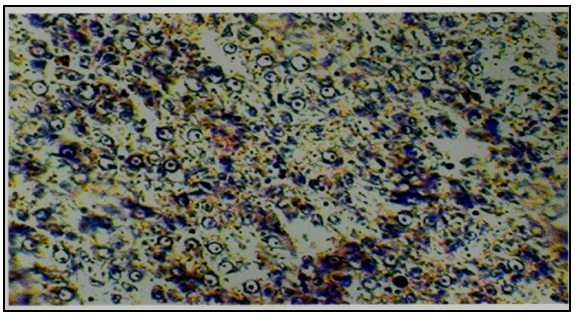

Fig 3: T.S. of Liver illustraling histomorphological changes after exposure of the fish to toxicant Copper chloride for 14 days

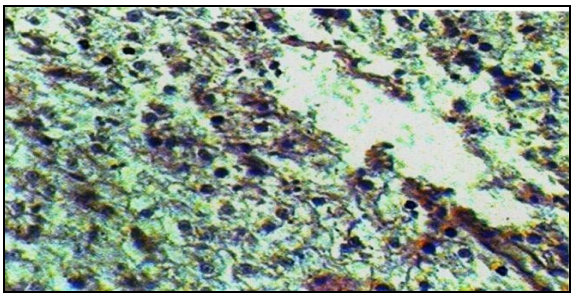

Fig 4: T.S. of Liver illustraling histomorphological changes after exposure of the fish to toxicant Copper chloride for 21 days.

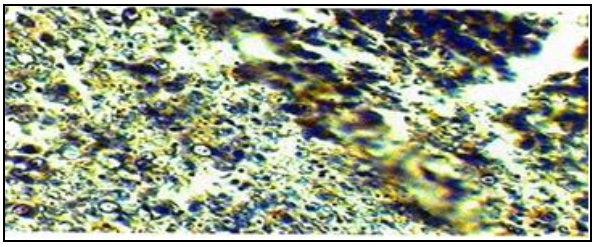

Fig 5: T.S. of Liver illustraling histomorphological changes after exposure of the fish to toxicant Copper chloride for 28 days.

Toxic effect of copper chloride after 28 days of exposure the high degree of atrophy was notice in the center of the liver. There was a typical change in the size of hepatocyte and their nuclei were displacing to the periphery. The cell membrane of some cells were ruptured resulting in to fusion between two or more cells and exhibiting binucleate or multinucleate appearance of the cell at many places. The vacuolization and clumping of hepatocytes was prominent hemorrhage in hepatic blood vessels and widening of blood cyanocytes was also noticed. The entire necrotic spongy mass and the liver of whole showed cirrhosis. Other degenerative changes like increase in connective tissue damage that occur in hepatic celss of fish liver which is exposed to Copper chloride toxicant (Fig. 5)In the present study the histopathological lesions were concentration of experimental toxicant up to 28 day's. Histopathological lesion began with hypertrophy of hepatocyte and disorientation of liver cords. After 7 days of exposure the hepatocyte showed disruption of regular hepatic cordal arrangement and shrinkage of hepatocytes. After 14 days of exposure the nuclei of hepatocyte become prominent along with disarray of hepatic cords. After 21 days of exposure the liver of *Clarias batrachus* show hepatocytes becomes vacuolated and coagulation was also observed several other degenerative changes occurred due to acute toxicity of Copper chloride include picnotic nuclei and damaged connective tissue disorganization of hepatic cell and hepatic cords. After 21 days of exposure the hepatocyte become vacuolated and blood coagulation was also observed shrinkage of blood vessels, clumped erythrocyctes wildly separated bile canalliculi are found. After 28 days of exposure was observed typical changes in the size of hepatocytes and their nuclei were displaced to the periphery. The result of present investigation is in confirmative to the result reported by singh and Bhati by 1994, also reported [12] that alacholar technical and lasso 50% EC has induced discrete pathological changes in the liver tissues of fish *Channa punctatus*. These changes include degeneration of cytoplasm in hepatocytes, atrophy, formation vacuole repture in blood vessels, necrosis and disappearance of hepatocyte wall and disposition of hepatic cords [8] have reported the exposure to sub-lethal concentration of cadmium chloride dose and duration dependant histopathological alterations in the liver of *Clarias batrachus* the lesions in these vital organ might have resulted in physiological and metabolic disregulations, which further led to behavioral alterations and growth impairment [13].also observed necrosis in liver of copper exposed fish in aspirate amino transferase and analine amino transferase activities were increased due to damaged of liver in the section. These results of present investigation with Copper chloride as toxicant are almost similar to those of above investigator.

4. Conclusion

After exposure to sub-lethal concentration of copper chloride the tissue of liver of fish *Clarias batrachus* shows degenerative changes. The degenerative histopathological changes in the liver were severe and duration dependent. The necrosis observed in the liver after exposure to copper chloride might be due to the liver which bursts open the lysosome, liberating autolytic enzyme which digest the cell producing necrosis. The degenerative changes observed after sub-lethal exposure might result of biomagnifications or degraded forms of heavy metal compounds. The fish survived after 28 days which could be due to the compensatory mechanism played by the endocrine glands their secretion, involving pituitary – internal axis and pituitary liver axis.

5. References

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