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Effect of Cu heavy metals on fish liver and kidney of *Channa punctatus* fish

Pranjuli Tomar, Umesh Shukla
Department of Zoology, Agra College, Agra, Affiliated to Dr. Bhim Rao Ambedkar University, Agra, Uttar Pradesh, India

Abstract
The present study was under taken to assess safety and efficacy of Cu on fingerlings of *Channa Punctatus*. Fingerlings of an average weight (10-15 gm) and average length 6-8 cm. were selected from the stock and kept for laboratory condition in aquarium of size 60x30x30 cm with 30 L. capacity for one month. After acclimatization *Channa Punctatus* fish (n=10) were exposed to Cu concentration for 10,20 and 30 days. Fingerlings in each group contains six were given graded doses of Cu (0.2,0.4 and 0.6 ppm). First group received normal water (Tap water) as a control. The fingerlings were then maintained and observed daily for 10,20 and 30days for further any toxicity. The numbers of survival were noted. Complete postmortem was done on all survivor or if any fingerlings found dead or moribund condition during the study period. Histopathological examination was performed on collected tissues liver and kidney of individual fingerlings.

Keywords: Toxicity, Sublethal, Heavy metal, and *Catla catla* fish

Introduction

In small amounts, heavy metals is a necessary trace metal for a number of fish metabolic processes. The unique incorporation of heavy metals into a range of enzymes—such as those involved in cellular respiration, free radical defense, neurotransmitter function, connective tissue biosynthesis, and other processes—as well as into certain structural proteins, gives rise to the element's essential status. sDespite heavy metals's important function in a number of enzymatic activities, when large amounts of this heavy metal are found in water, they can have harmful toxicological consequences. In actuality, it may be hazardous when the inside Physiological alterations and aquatic ecosystem health are indicators of environmental contamination. An analysis of the liver's histology may yield significant and practical information on the impacts these compounds may have on fish species and, in turn, human populations. Some heavy metals are required for particular Heavy metal contamination has been reported in aquatic organisms. Kock *et al*. (1996) [5]. Previous studies indicated that these substances are harmful to fish, even in low concentrations. Meyer and Hendricks (1982). Fish are relatively sensitive to changes in their surrounding available concentration exceeds the capacity of physiological detoxification processes. An analysis of the liver's histology may yield significant and practical information on the impacts these compounds may have on fish species and, in turn, human populations. Because certain heavy metals have nutritional value, they are required for certain bodily activities. The goal of the current study is to record the heavy metals's toxicity in *Channa punctatus* fish.

Materials and Methods

1. Study area

Channa punctatus was chosen for the fish under research in this study. The fish were collected from fish market and were given time to acclimatized in laboratory conditions.

2. Test for bioassay

The hazardous and sublethal concentrations of heavy metals were discovered during 48-hour exposures in an initial investigation. In a bioassay test, the response of a living creature to a heavy metal is used to measure the metal's concentration. The purpose of the bioassay testing was to determine the LC_{50} values for varying exposure times (24, 48, 72, and 96 hours). The collected fishes kept in heavy metals at different concentrations in a glass tank that was cylindrical in shape. The amount of water present in the 30 liters of water were kept in each of the glass aquaria. Ten fishes were placed into each heavy metal concentration, and fish death was monitored.

After being exposed to each of the four-test media for 24, 48, 72, and 96 hours, the fish were examined for behavioural changes, and the mortality was noted. The concentration that resulted in 100% mortality during a 96-hour period was identified as the fatal concentration water (96 h LC_{100}, 1 ppm), whereas the concentration that resulted in 100% survival after 96 hours was identified as the sublethal concentration 0.2 ppm (96 h LC_0). Profit analysis was used to determine the LC_{50}, or lethal concentration, which kills 50% of fish after 96 hours of exposure. Thus, using Finney's (1978) method of profit analysis, the sublethal LC_{50} and lethal amounts of heavy metals were ascertained.

3. Heavy metals

Heavy metals were produced at different concentration of parts per million for the heavy metals under investigation. Every day, for an hour, between 10:00 and 11:00 a.m., fish meals were fed to the fish any leftovers were then collected with pipettes. Thirty days were dedicated to the conduct of the experiments. In experimental tanks, water was replaced every other day with heavy metals. Simultaneous control and experimental runs were done in triplicate.

Table 1: Effect of different concentration of copper on mortality percentage of *Channa punctatus* fish as a function of different exposure time.

Concentration of Cu (ppm)	% Mortality / Duration of Exposure			
	24 hrs	48 hrs	72 hrs	96 hrs
1.0	6/10=60	7/10=70	9/10=90	10/10=100
0.8	5/10=50	7/10=70	8/10=80	9/10=90
0.6	3/10=30	5/10=50	7/10=70	8/10=80
0.4	2/10=20	3/10=30	4/10=40	5/10=50
0.2	-	-	1/10=10	3/10=30

4. Fish sample preparation

Following a 30-day trial, ten fish from both the control and experimental groups were removed and their muscle tissue was removed using a saline 0.9% solution (Lockwood, 1971). Two of the fish were retained in this condition. After being placed in a watch glass, they were left to dehydrate at 500°C in an oven. Dehydrated thereafter, they were ground into a powder using a mortar and pestle, and then stored for later use in little plastic bags.

Heavy metals (Cu) Reagent Estimation

a. Specific gravity of ammonium hydroxide is 0.90.
b. AR grade for chloroform.
c. Concentrated hydrochloric acid (C).
d. The solution of hydroxylamine hydrochloride is made by dissolving 40 g of the compound in 200 ml of distilled water.
e. Alcohol isopropyl.
f. Neocuproine solution: Made by mixing 50 ml of isopropyl alcohol with 0.1 g of neocuproine, then diluting the mixture to a volume of 100 ml using double-distilled water.
g. Concentrated nitric acid.
h. Concentrated sulfuric acid.
i. Sodium citrate solution: created up to 1000 ml, this solution is created by dissolving 250 g of hydrated sodium citrate in water. Neocuproine solution (10 ml) and hydroxylamine hydrochloride solution (10 ml) were added to this. Heavy metals impurities were extracted using chloroform, and the chloroform layer was discarded.
j. Stock solution for heavy metals II: prepared by heating six milliliters of 1:1 nitric acid to dissolve 0.2 grams of pure heavy metals metal. One milliliter of concentrated H2SO4 was added to this, and the mixture was dried by evaporating it. The leftover was then mixed with 1 liter of distilled water (1 ml = 200 g 1-1).
k. Distilled water was used to dilute 100 milliliters of stock solution to one liter (1 milliliter = 20 µg 1-1). This created the intermediate heavy metals II solution.
l. Standard heavy metals: Made by adding distilled water to 1 ml of the intermediated stock solution to make 50 ml (1 ml = 0.4 µg 1-1).

Procedure On a hot plate, 1 ml of reagent (H) and 5 ml of reagent (G) were added and evaporated to produce dense fumes of white sulfur trioxide in order to eliminate the interfering substances. After adding 5 ml of reagent (G) and hydrogen peroxide each time, the treatment was repeated and the mixture was evaporated until it was completely dry. Then the residue was dissolved in 80 cc of pure water, heated, cooled and filtered. Reagent (A) was added to the content dropwise until the pH was between 4 and 6. Reagent (C) was added in 0.2 ml increments and diluted with 100 ml of distilled water.

5. Extraction

In a separating funnel, 50 ml of acidified sample was taken. To this 5 ml of reagent (D) and 10 ml of reagent (F) were added and shaken well. To the contents 20 ml of reagent (B) was added and shaken for 1 minute in order separate aqueous and chloroform layer. Chloroform layer was collected in a dry flask. Olojo *et al*. (2005) [8]. This procedure was repeated with another 20 ml aliquot of chloroform. Finally, the extracts were pooled and made up to 50 ml with reagent (E). Reagent blank was prepared by treating 50 ml of double distilled water in the same way as described above. The optical density of the sample solution was measured at 457 nm against the reagent blank. The quantity of heavy metals was determined by utilizing a calibration graph made from pure heavy metal in the concentration range 0.2, 0.4, 0.6, 0.8, 1 ppm (µg/g in fish tissue) Quabius Li *et al*. (1998).

Calculation

mg 1-1 = M/V x 100 for heavy metals where M is the sample's mass in milligrams of heavy metals and V is the sample's volume in milliliters.
Paraffin techniques in histology (Humason, 1979)
Agents

a. Physiological saline (0.9%)
b. Bouin-Hollande fixatives
c. Ehrlich`s haematoxylin Eosin

Process

After the livers of the control and parallel experimental groups were dissected, they were wiped clean of membranes, cut into the appropriate size pieces, and fixed for a full day in Bouin's-Hollande fixative. Following fixation, tissues were dehydrated in a series of progressively stronger alcohols and rinsed three days in 70% alcohol to eliminate surplus picric acid. After three changes (20–30 min each), the tissues were infiltrated with molten paraffin at 58–600C and then embedded in paraffin El moselhy (2001) [2]. Using a rotary microtome (Leica, Germany), slices of all the tissue were cut to a thickness of 3–5 mm, and Ehrlich's haematoxylin was used to stain it, with eosin serving as a counterstain. The DPX mountant was used to mount the slides. A Nikon microscope was used to examine the sections in field lighting, and specific regions were captured on camera at magnifications of x40, x100, x200.

Result

Heavy metal's Cu acute toxicity tests on *Channa punctatus* are displayed in (Table 1). The current investigation of Cu heavy metals 96-hour LC_{50} value was 0.8 ppm. The percentage of death from *Channa punctatus* exposure varied with exposure duration and heavy metals content.

Fish histology

The liver and kidney underwent a thorough examination, was weighed, kept in formalin 10%, and then processed using the conventional micro method for paraffin

embedding. For histological investigations, a 5 µm segment of the organs stained with alum hemotoxylin and eosin was examined under a microscope. From a histopathological perspective, the control group I animals had significant centrilobular necrosis, vacuolization, and macro vesicular fatty alterations in addition to normal liver architecture. Live show 45% damage necrosis by the treatment of heavy metals. In liver heavy metal caused central vein congestion, inflammation, edema degeneration and necrosis of hepatocytes.

Catla catla fish liver tissue of 3 group exposed to different ppm (0.2, 0.4, 0.6) of heavy metal (group I,II, III) 10 days, 20 days, 30 days respectively. Hemolysis (H), dilated sinusoids (DS), Raptured hepatocytes (RH), Repture central vein (RCV), Necrosis, Edema of hepatocytes (E), Inflammation (I) H&E stain 100X.

Kidney of control fish showed normal structure and consisted of anterior head, middle and posterior trunk kidney. The anterior kidney is mainly composed of hematopoetic interregnal and chromaffin tissue and is deficient in tubules and nephrons. Hamed (1992) [3]. In the middle kidney the hematopoitic tissue decreases and nephrons and tubules increase in numbers. the posterior trunk kidney is composed of numerous nephrons that consist of malpighian corpuscles composed of well vascularized glomeruli enclosed by Bowmean's capsule, proximal and distal tubules and the collecting duct system. Brown *et al.* (1984) [1]. The control fish did not exhibit morphological alterations expect of slight nuclear and cellular hypertronic changes in the tubules. Kidney anormalies included obliteration of Bowman's space shrinkage and degeneration of tubules and glomerulus.

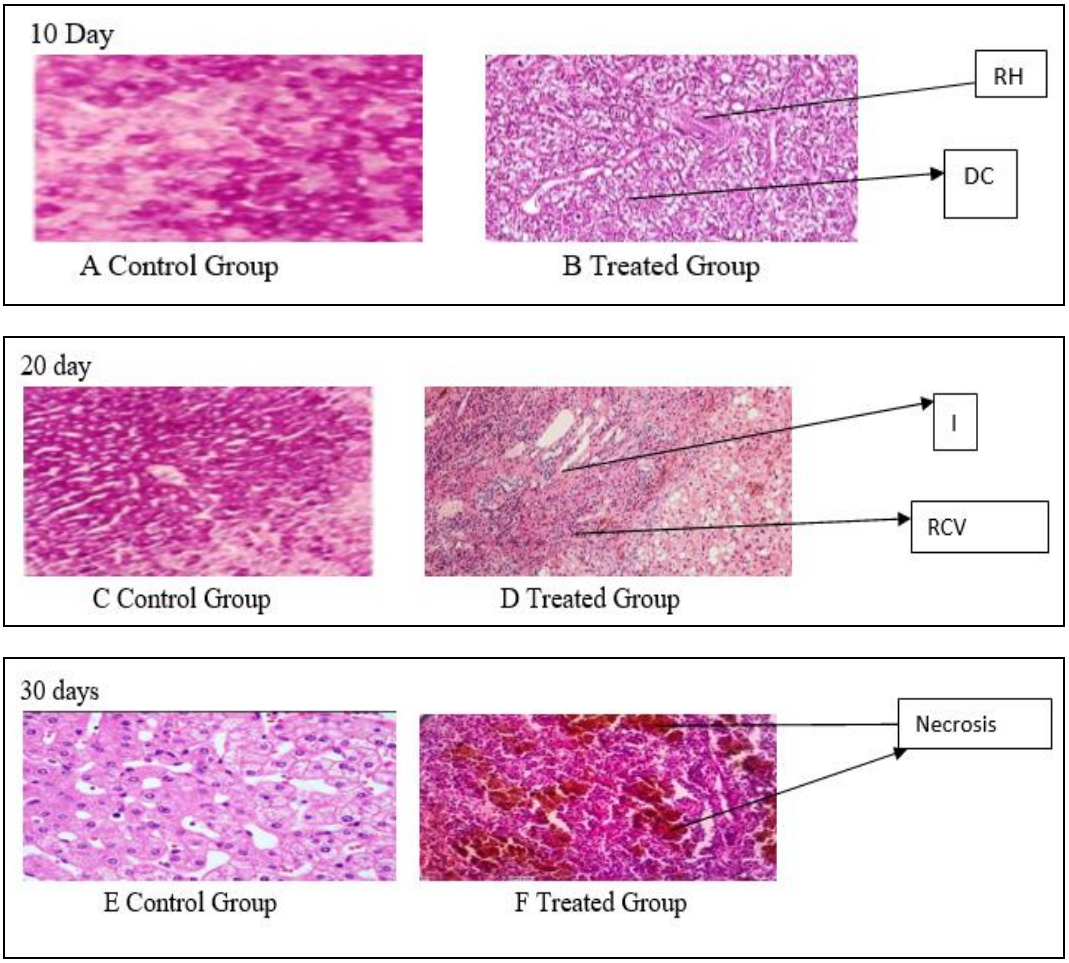
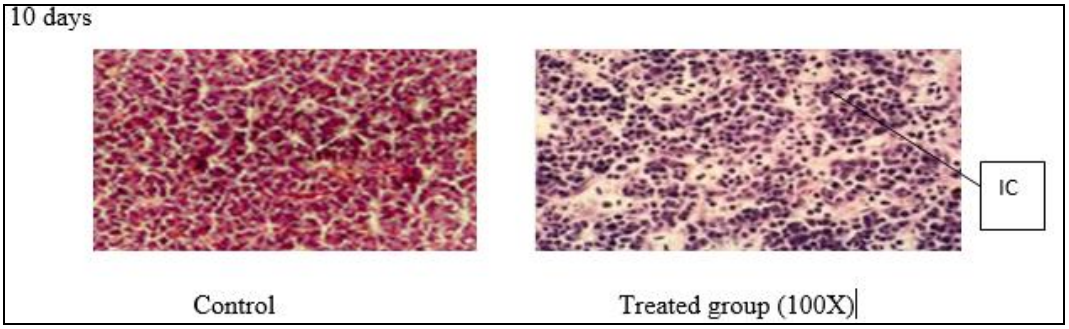


Fig 3.1: A, B, C, D, E &F Showing liver Necrosis in treated group of *Channa Punctatus* (100X)



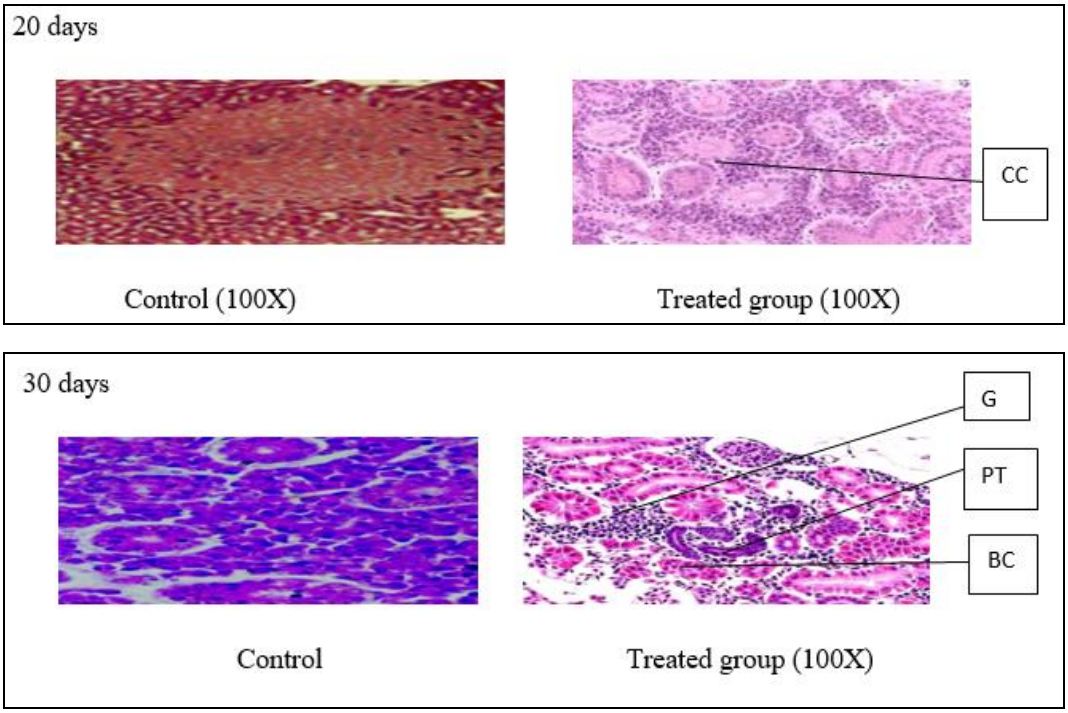


Fig 3.2: Shows histological section of kidney of fish *CHANNA PUNCTATUS* control and treated group (100X)
Kidney tissue from treated and control group- GBowman capsule (BC), Glomerulus (G), proximal tubules (PT). Cromaffin cells (CC), Interstitial cell (IC). hemotoxiline and eosin stain (100X).

Discussion

Channa Punctatus is more vulnerable to heavy metals than other fish, according to a review of the acute toxicity of heavy metals to *Channa Punctatus* and other fish (Table 1). It's possible to explain the variations in the 96-hour LC_{50} values between *Channa Punctatus* and other fish by the fact that metals alter the physiology and ability of aquatic creatures to survive under because these variations occur across metals, between species, and between experimental conditions, metallic stress is complexs. Sharma and Agrawal (1996). The death of *Channa Punctatus* may be caused by the cumulative effects of heavy metals and dolerite at several metabolic sites. During our experiments, we discovered that the toxicant's concentration and time of exposure affected the behavior and death rate of *Channa Punctatus*. Pagenkopf (1986) [9]. The 96-hour lower limit of detection (LC_{50}) values for *Channa Punctatus* fingerling (30.4 mg/L in a static bioassay test method) are clear indications of this. When exposed to a deadly quantity of heavy metals, fingerlings displayed strange behaviors such jumping out of the water, producing a lot of mucus, and moving quickly. These behavioral changes may be caused by an osmatic imbalance, which can have an impact on the neurological system.

Conclusion

The fish *Channa Punctatus* released too much mucus at a deadly dose (10 ppm), lost its balance, and eventually perished. These investigations clearly showed that as heavy metal concentrations rose, so did the death rate. At a concentration of 10 ppm heavy metals, there was 100% death. For more than a heavy metal, the fishes easily lived at concentrations of 0.2 ppm and lower. Hence, the sublethal or approximately sublethal concentration is defined as 0.2 ppm and lower. Nevertheless, further research is required to fully comprehend its harmful consequence.

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