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Copper and Cadmium induced histopathological alterations in liver of *Heteropneustes fossilis* (Bloch) at varying water pH

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ABSTRACT

Histology of liver exposed to 36 hour LC₅₀ concentration of copper sulfate (CuSO₄.5H₂O) and cadmium chloride (CdCl₂.H₂O) with varying water pH (4±0.5, 7±0.5 and 8±0.5) was studied in an air-breathing catfish (*Heteropneustes fossilis*). The histopathological changes observed in the liver tissue post exposure included necrosis, degradation of hepatocytes, degeneration of blood vessels, distended sinusoids with pyknotic nuclei and vacuolation of cells. The degree of damage to the liver tissue was proportional to the nominal concentrations of the metals used. Further the pH of diluent water affected the alteration more acutely signifying a synergistic effect.

Keywords: *H. fossilis*, pH, Copper sulfate, Cadmium chloride, Histopathological changes.

1. Introduction

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades [1]. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals such as copper (Cu) and cadmium (Cd) have gained wide interest in the scientific community in recent years due to its potential human health hazards. Cu is an essential trace nutrient which is discharged into freshwater environments in large concentrations as an industrial effluent and severely affects the freshwater fauna, especially fishes [2]. Elevated levels of Cu may become acutely or chronically toxic to aquatic lives. Although hepatic Cu levels vary greatly between teleost species, the liver is the main area of Cu storage in almost all species studied [3]. Cd is a non-essential toxic heavy metal and its bio-accumulating property causes toxicity to aquatic organisms even in minute concentrations. In fish, Cd has been shown to alter the structure and to cause morphological changes of varying severity in various organs [4].

Aquatic organisms, including fishes, accumulate pollutants directly from contaminated water and indirectly via the food chain. The organ most associated with the detoxification and biotransformation process of toxicants including metals is the liver, because the liver of fish can be considered a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health and exhibit the effects of a variety of environmental pollutants [5, 6]. Histopathological changes in different tissues of fish assess the extent of damage caused by pollutants and is recognized to be a reliable biomarkers of stress in fish [7]. Histological changes appear as a medium-term response to sub-lethal stressors, and histology provides a rapid method to detect effects of irritants in various tissues and organs [8]. Another important parameter influencing the toxicity of metals is pH, which shifts speciation to or away from toxic effect depending upon the ions formed. In general a lower pH will increase free metal ions, while more alkaline pH will result in more carbonate complexes and fewer toxic free metal ions [9]. In general, metals such Cd and Cu are more bio available and toxic to aquatic organisms in acidic water [10]. Therefore, the present study is aimed to analyze the histopathological changes in the liver tissue of the fish *H. fossilis* exposed to sub lethal concentration of copper sulfate (CuSO₄) and cadmium chloride (CdCl₂) at varying water pH.

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2. Materials and Methods

The experimental fish *H. fossilis* was purchased from the local landing sites and care was taken to minimize stress incurred by the fish during transportation and were maintained in glass aquaria containing tap water and acclimatized to laboratory conditions. All the necessary precautions for maintaining the fish were laid down as per the recommendations of APHA [11]. Different water quality parameters viz., Dissolved Oxygen (DO), Specific Conductivity, alkalinity, hardness, pH and temperature were analyzed and recorded (Table 1). Healthy fishes were selected for experimentation. The experiment was carried out using 3 ppm (48 hour LC₅₀ value at pH 7) of CuSO₄.5H₂O and 24 ppm (48 hour LC₅₀ value at pH 7) of CdCl₂.H₂O in 8 l of tap water. The pH was adjusted using ultra-pure HCl and/or NaOH and was continuously monitored

during the toxicity test, with adjustments made as required. A control was run without the addition of metals for each of the three pH treatments. 36 hour post treatment fishes from both control and experimental groups were sacrificed and the liver tissue was excised out. The tissues were then cleared in 9.5% saline solution and immediately fixed in Bouin's fluid for 24 hour processed through a graded series of alcohol, cleared in xylene and embedded in paraffin wax. The sections were cut at 6 µm thickness with the help of MT-1090A Weswox rotary microtome. Sections were then spread on grease free albuminized slides, stained with haematoxylin eosin and were mounted in DPX [12]. The photographs at 40X magnification were taken with a computer aided microscope (Leica DM 1000).

Table 1: Water quality parameters at different pH

pH	Water Temperature (°C)	Specific Conductivity (S/m)	Dissolved Oxygen (mg l ⁻¹)	Total Hardness (mg l ⁻¹)	Total Alkalinity (mg l ⁻¹)
4±0.5	17±1	0.13±0.01	6.14±1.00	25±1.00	4±1.00
7±0.5	17±1	0.12±0.01	6.14±1.00	32±1.00	35.5±1.00
8±0.5	17±1	0.13±0.01	6.14±1.00	39±1.00	48.2±1.00

3. Results and Discussion

The morphological section of liver of control fish (acclimatized under laboratory condition) showed the normal hepatocytes and exhibits a homogenous cytoplasm around the spherical nucleus. There was no clear division of hepatic cells into lobules (Fig 1). Normal hepatocytes and sinusoids with prominent nuclei were observed in control liver tissue of *H. fossilis* at pH 7±0.5 while at pH 4±0.5 there was moderate degradation of cellular hepatocytes in comparison to the control liver tissue at pH 8±0.5 which showed slight degradation of the same (Fig 2a, 3a, 4a).

Liver tissue treated with Cu at pH 4±0.5, showed severe necrosis, hemorrhage and degeneration of blood vessels. Pyknotic nuclei were more prominent with indistinct cells. The histological alterations in the liver of *H. fossilis* on treatment with Cd revealed vacuolated hepatocytes in diffused manner, probably due to glycogen infiltration, and the necrosis was a common feature. Sinusoids with pyknotic nuclei was another prominent feature noticed in Cd treated liver tissue at acidic

pH (Fig 2 b, c). At pH 7±0.5 degradation of cellular hepatocytes was observed on treatment with Cu in addition to distended sinusoids with pyknotic nuclei. There were extensive dilation of sinusoids with blood congestion and the cell death (necrosis) was observed increasingly on treatment with Cd (Fig 3 b, c). On treatment with Cu at pH 8±0.5, hepatocytes exhibited an appearance of some small vascular structures, probably due to the presence of lipids. Major structural changes in the hepatocytes such as focal necrosis, severe congestion in sinusoids were also observed. Hypertrophy of hepatocytes with pyknotic nuclei was quite evident in liver tissue exposed to Cu. On treatment with Cd, it was seen that the liver cells degenerated; the normal architecture of the liver was markedly disorganized. In addition, dilated sinusoids with congestion were noticed. First signs of pathological processes such as necrosis and lipid infiltration around the blood vessels, regarded as a sign for toxic liver injury, were noticed at this stage (Fig 4 b, c)

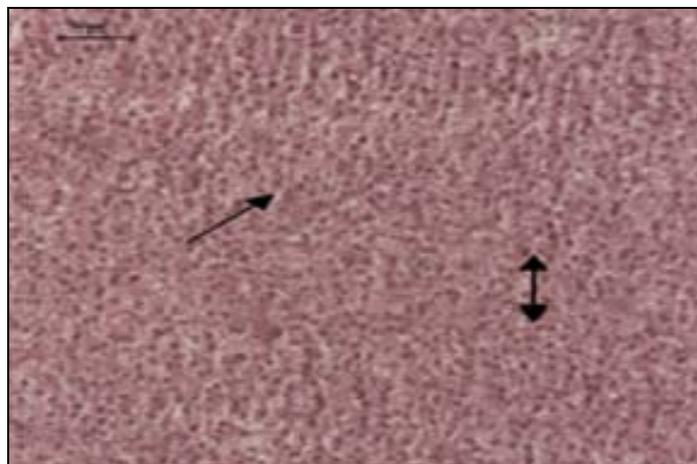


Fig 1: Normal structure of liver of *H. fossilis*-H&E-40X, Hepatocytes (Single headed arrow); Nucleus (Double headed arrow).

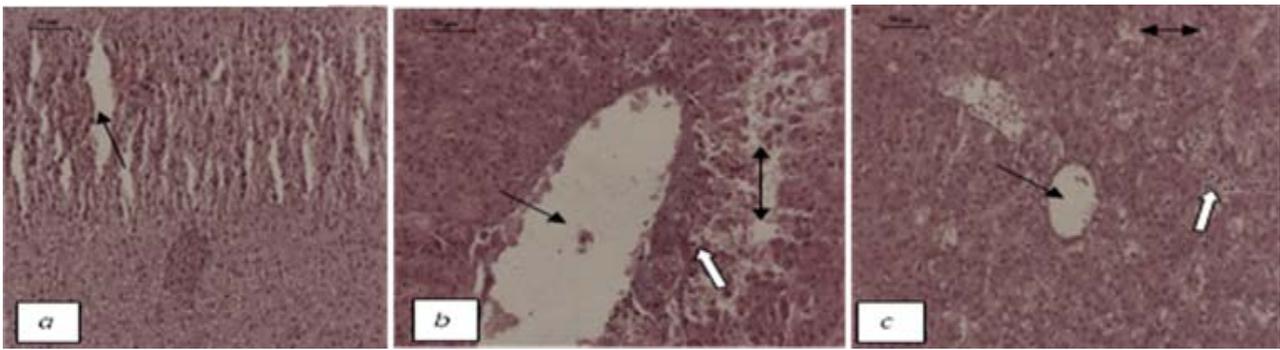


Fig 2: Transverse sections of *H. fossilis* liver at pH 4±0.5 after 36 hour-H&E-40X (a) Control: Moderate degradation of cellular hepatocytes (single headed arrow) (b) Copper treated: Vacuolization (single headed arrow); severe Necrosis (double headed arrow); Pyknotic nuclei (white arrow) (c) Cadmium treated: Vacuolization (single headed arrow); Necrosis (double headed arrow); sinusoids with pyknotic nuclei (white arrow).

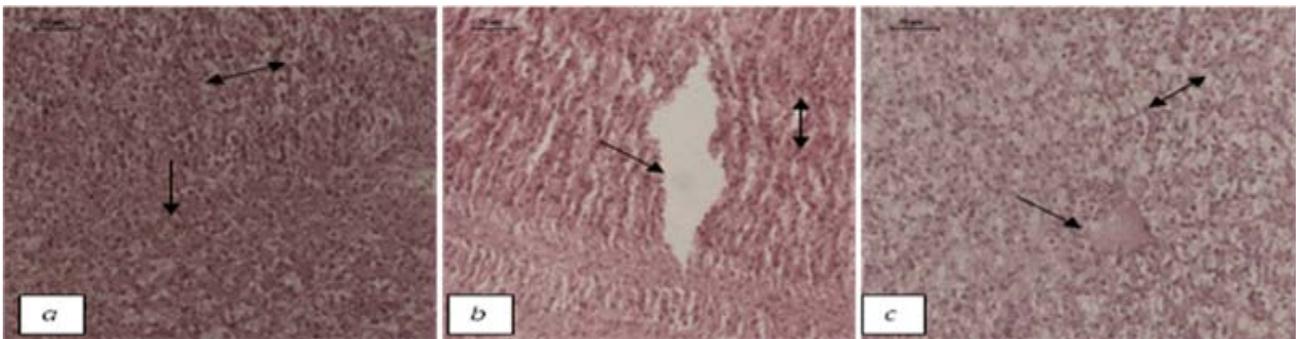


Fig 3: Transverse sections of *H. fossilis* liver at pH 7±0.5 after 36 hour-H&E-40X (a) Control: Normal hepatocytes (single headed arrow); Sinusoids (double headed arrow) (b) Copper treated: Degradation of cellular hepatocytes (single headed arrow); sinusoids with pyknotic nuclei (double headed arrow) (c) Cadmium treated: Necrosis (single headed arrow); Sinusoids (double headed arrow).

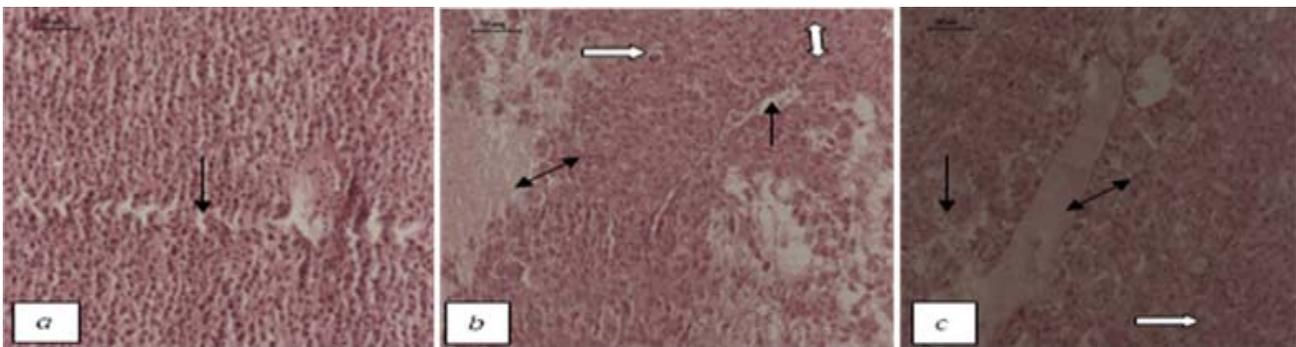


Fig 4: Transverse sections of *H. fossilis* liver at pH 8±0.5 after 36 hour-H&E-40X (a) Control: Little degradation of cellular hepatocytes (single headed arrow) (b) Copper treated: Formation of vacuoles (single headed arrow); Necrosis (double headed arrow); Sinusoids (white arrow); Pyknotic nuclei (white arrow, double headed) (c) Cadmium treated: Bile canaliculus (double headed arrow); Distended Sinusoids (white arrow); Necrosis (single headed arrow).

Histopathological alterations result depending upon the metal type and concentrations, length of exposure, fish species, and other physico-chemical factors. It is brought about due to either increase or decrease in hepatic enzyme activities, [13]. Hepatocytes may thus be expected to be the primary targets of toxic substances, providing an excellent biomarker of aquatic pollution [14]. The acidic pH of water magnifies the toxic effect of certain environmental pollutants including heavy metals [15]. The alterations of the liver parenchyma, such as vacuolation and necrosis are often associated with acid water [16]. The present findings are in accordance with above studies.

Vacuoles in the cytoplasm of the hepatocytes can contain lipids and glycogen, which are related to the normal metabolic function of the liver [17]. Pacheco and Santos [18] described vacuolation of the hepatocytes as a signal of degenerative process that bring about metabolic damage on exposure to contaminated water. Similar histopathological alterations with both Cu and Cd were observed in liver of many teleostean fishes [19, 20]. In the present study, it was observed that the severity of the lesion was metal specific and pH dependent. The liver of fish exposed to 3 ppm and 24 ppm of copper sulfate and cadmium chloride respectively for 36 hr at

different pH exhibited several histological alterations like degradation of hepatocytes, distended sinusoids with pyknotic nuclei, development of vacuoles in cell cytoplasm and necrosis of hepatic tissue. The magnitude of changes differed in proportion to the concentrations of two different metals and variation in water pH, probably due to the synergistic effect of metal and pH. Similar results have been reported in *Clarias batrachus*, *Cyprinus carpio*, *Oreochromis mossambicus* and *Oreochromis niloticus* exposed to copper sulfate and cadmium chloride [21, 22, 23, 24]. Many organic compounds induce toxicopathic lesions in the liver of fish species. The acute toxic injury usually includes cloudy swelling or hydropic degenerations and pyknosis, karyorrhexis and karyolysis of nuclei [6, 25, 26, 27, 28]. Alteration in the liver of Nile tilapia, *Oreochromis niloticus* exposed to 38.19 (acute), 35 $\mu\text{g L}^{-1}$ subchronic of alachlor for 24, 48, 72 and 96 h and 90 days, respectively, found hydropic swelling of hepatocytes, lipid vacuoles were observed in hepatocytes in the 2nd and 3rd month of sub chronic exposure [29]. According to Rodrigues and Fanta [30] the disarray and vacuolization of hepatocytes were observed in liver of *Brachydanio rerio* exposed to organophosphate.

4. Conclusion

Histopathological alterations in air-breathing catfish, *H. fossilis* under the influence of heavy metals such as Cu and Cd at different pH can be used as a sensitive model to monitor the aquatic pollution. The severity of damage is more in liver tissue exposed to Cu as compared to Cd and acidic pH of the medium showed the highest toxicity.

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