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Sub-lethal effect of copper toxicity on liver lesions of Roach (Rutilus rutilus caspicus) juveniles

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ABSTRACT: Copper is one of the Heavy metals that it is so toxic for both humans and animals. This study was carried out to find the effects of sub lethal concentrations of Copper sulfate on Roach (*Rutilus rutilus caspicus*) juveniles. Fish were exposed to concentrations of LC_{50} 24h, 48h, 72h and 96h of Copper sulfate for four days. Then behavioral changes and histopathological alternations of liver were studied. Hyperactivity, swimming sideways, lack of balance and increase in rate of swimming and opercula activity in treatment groups were seen. Liver showed increase in sinusoidal space, hepatocytes necrosis and degeneration, hypertrophy and intercellular oedema**,** hepatocytes atrophy. Also hemorrhage dilation, dilation of sinusoid, hydropic swelling and lipidosis were seen in liver. The results showed that sub lethal toxicity of Copper sulfate severely affects the vital organs and normal behavior. No behavioral complications were observed in the control group and liver tissue was normal. The severities of liver symptoms were different between treatment groups andwere dose-related lesions in the liver of Roach exposed to $Cuso_4$. Therefore the Usage of the Copper sulfate might be dangerous and mortal for fish healthy.

***Keywords*:** Histopathological changes, Heavy metals, copper sulfate.

INTRODUCTION

Some of heavy metals are essential to living organisms and they are commonly found in natural waters but high concentrations and accumulation of them may become so toxic. Copper is a necessary metal with a recognized biological role and like other heavy metals, it is potentially toxic at high concentrations. Copper salts are commonly used algaecides and agents for removal of ecto-parasites in the aquaculture industry (Noga, 2000). Copper sulfate ($CuSO_4$) is used in freshwater aquaculture to control external parasites, bacterial diseases and also as fungicide. It is also used as herbicide (Reddy et al., 2006).

Most of heavy metals accumulate mainly in liver, kidney and gills of fish. Histological changes in animal tissues provide a rapid method to detect effects of irritants, especially chronic ones, in varioustissues and organs (Bernet et al., 1999).

Heavy metals accumulation in fish organs may lead to structural wounds, functional faults and toxic impact on alteration in behavioral patterns. When examining the toxicity of substances, assessing the lethal concentration at which 50% mortality occurs over a 96-h time interval (96 h LC50) is a standard procedure, which allows comparing different fish species without requiring a prior understanding of the involved mechanisms.

The liver is a critical organ that plays an important role for metabolism and excretion of waste chemicals by detoxification (K. Mishra and Mohanty, 2008) and the concentration of these toxic materials on liver is more than other body organs. The alterations in liver due to toxicity impact are often associated with a degenerative necrotic

condition (Arellano et al., 1999; Olojo et al., 2005; Figueiredo-Fernandes et al., 2007). Therefore hepatocytes are the first target of toxins and they are the best biomarkers for examination of aquatic pollutant.

MATERIALS AND METHODS

Fish and acclimation conditions

In this investigation Roach juveniles were collected from Sijwal Fish Reproduction Center (Golestan province, Northeastern Iran). They weighed 2.06g ± 0.05 g (mean±S.D.) and their length was in the range 5-7 cm in the experiment time. The fish were acclimated to the laboratory conditions for two weeks prior to experiment in a glass aquarium (20L) filled with clean water. During the acclimatization, which lasted for two weeks, the fish were fed twice a day with commercial fingerling size fish food (Sari, Iran). Then Fish were randomly transferred into tanks at a density of 7 fish per each tank.

Experimental set-up

Fish were maintained in dechlorinated tap water for 2 weeks in 20 glass aquaria with proper aeration. The parameters of used water such as temperature, pH, salinity and DO were measured and were stable during the experiment period (Table 1). Juveniles were divided to 5 groups (one control and four treatments).The control group had no dosage of copper sulfate. The remaining groups were exposed to sublethal concentrations of copper sulfate. the dosages were LC_{50} 24h, 48h, 72h and 96h of copper sulfate which was determined 0.15, 0.31, 0.46, 0.62 mg/L authors during pre-experiment. In experiment duration no water exchange was occurred. Juveniles weren't fed during the study.

Table1. Chemical parameters of water used during the experiment

chemical Parameters of water	Measurement
Temperature	27.3 °C
pH	7.3
Salinity	1.2 ppt
DO	7.07 mg/L

Preparation of tissue samples

At the end of fourth day,both the experimental and control fishes removed from tanks and anesthetized using clove oil (150ppm).Liver tissues were removed and dropped in aqueous Bouin's fluid. After fixation for 24–30 h, tissues were dehydrated through a graded series of ethanol, cleared in xylene, and infiltrated in the paraffin. Sections of 4–6µm were prepared from paraffin blocks by using a rotary microtome. These sections were then stained with haematoxylin–eosin (Velmurugan et al., 2007). Photos were taken with using Nikon E 200 eclipse microscope. Histopathological changes in the tissues were examined in the randomly selected fish from each group. Tissue changes are shown in the figures and also in tables as mild (+), moderate (++), severe (+++) and none (-).

RESULTS AND DISCUSSION

Behavioral alternations

The fish exposed to $Cuso_4$ exhibited abnormal behavior like lack of equilibrium. They become lethargic and scales were secreted from the most sections of body and swam to surface more often than the control fish. But no visible change observed in in the control group. Summary of the different behavioral changes observed due to $Cuso_4$ is presented in Table 2.

Table 2.Summarized behavioral changes of Roach during sub-lethal exposure to $CuSo_4$ for four days

Behavioral alternations	sub-lethal exposure (ppm)				
	0	0.15	0.31	0.46	0.62
Lack of balance	-	+	++	++	++
Rate of swimming	-	-	-	-	+
Rate of opercular activity	-	+	-	+	+++
hyperactivity	-	-	++	+	++
swimming sideways	-	-	+	+	++

None (−), mild (+), moderate (++) and severe (+++).

Liver

No histopathological changes were observed in the liver of the control fish. The structure of the healthy liver of control *R.rutilus*is shown in (Fig2. G). In the liver tissues of fish exposed to concentrations of 0.15, 0.31,0.46, 0.62 mg/L $Cuso_4$increase in sinusoidal space, hepatocytes necrosis and degeneration**,** hypertrophy and intercellular Oedema**,** hepatocytes atrophy, hemorrhagedilation, dilation of sinusoid, hydropic swelling and lipidosis were seen. The histological changes seen in the $Cuso_4$ exposed are shown in (Fig2. A B C D E F) and Table 3.

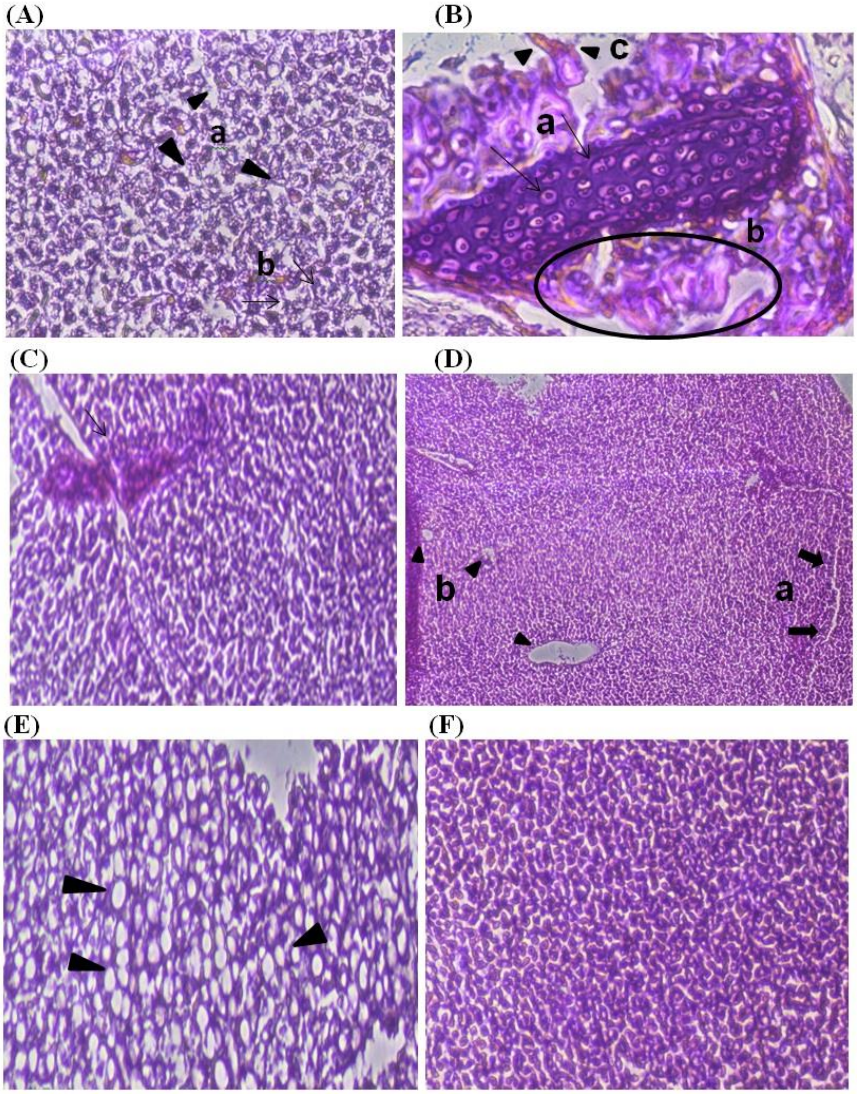

Figure 2. (A) liver tissue of *R.rutilus* exposed to $Cuso_4$ for 4 days. Increase in sinusoidal space (a), Hepatocytes Necrosis and Degeneration (b). (B) Hypertrophy and intercellular Oedema (a), Hepatocytes atrophy (b), Hemorrhagedilation(c). (C) Hemorrhagedilation. (D) Dilation of sinusoid (a), Hydropic swelling (b). (E) Lipidosis. (F) Liver tissue of *R.rutilus* in control group(G).H&E, (100* & 400*)

Table 3. Summarized liver wounds of Roach during sub-lethal exposure to $CuSo_4$ for four days

Wounds	sub-lethal exposure (ppm)				
	0	0.15	0.31	0.46	0.62
Cytolysis	-	-	+	-	+
Hemorrhagedilation	-	+	+	++	+++
Hepatocytes atrophy	-	+	+	++	+++
Necrosis &degeneration	-	+	+	+	++
increase in sinusoidal space	-	+	++	++	+++
Hypertrophy and intercellar oedema	-	+	++	+++	+++

None (−), mild (+), moderate (++) and severe (+++).

Discussion

Many Studies described that the effects of metals like Hg, Cd, Pb, Zn, Al and Cu on survival and histopathology of various organs in fish are available (Skidmore and Tovell, 1972; Kendall, 1975;Karlsson-Norggren et al., 1986; Kirubagaran and Joy, 1988; Arellano et al., 1999; Thophon et al., 2003; Van Heerden et al., 2004; Olojo et al., 2005; Figueiredo-Fernandes et al., 2007). In this study we described acute toxicity effects of $Cuso_4$ on behavior and histopathology of liver of Roach (*Rutilus rutilus caspicus*) juveniles. The LC_{50} 24h, 48h, 72h and 96h value of $Cuso_4$ was determined Roachby authors.

Copper is an important additive in the aquaculture industry for control of algal growth and ecto-parasites (Tom-Petersen et al., 2007). Effects of Cu on living organisms are traditionally related to total dissolved Cu concentrations as determined by chemical procedures (Niyogi and Wood, 2004). However, the bioavailability and toxicity of Cu depends on water parameters such as pH, alkalinity and concentrations of dissolved and particulate organic matter (Boyd et al., 2005; Brooks et al., 2008).

The fish, as an indicator animal, plays an important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden mortal of fish indicates heavy pollution and the effects of exposure to sublethal levels of pollutants can be measured in terms of biochemical, physiological or histological responses of the fish organism (Mondon et al., 2001). Hyperactivity, loss of balance, increase in rate of swimming and opercular activity andConvulsions sowed in *Channapunctatus*(Bloch) exposed to hexavalent chromium by K. Mishra et al. (2008).

The histopathological alterations in liver tissues of Roachexposed to0.15, 0.31, 0.46, 0.62 mg/L of copper sulfate were obvious. Thus acute $Cuso_4$ exposure may cause destructive physiological problems that finally leading to the death of fish. Osman et al. (2009) showed the histopathological changes under study of inducing change in different fish organs (gills, liver, kidneys and spleen) after 2 and 6 weeks of treatment and three different concentrations. These histopathological changes were positively correlated in its effects with the increase of pollutants concentration and time of exposure.

Liver being the main organ of many key metabolic pathways and toxic effects of chemicals usually appear firstly in the liver. Many heavy metals induce toxicopathic lesions in the liver of fish species. Acute toxic injury usually includes cloudy swelling and hydropic degenerations (Hawkes, 1980; Hinton and Lauren, 1990; Visoottiviseth et al., 1999). In the liver of fish exposed to $Cuso_4$, hydropic degenerations were observed in this present study. The intensity of liver cell damage increased with increasing concentration and exposure to the Formalin treatment fish in study of Cruz et al (1989).Treatment groups showed cloudy swelling, hemorrhage, deposition of pigments, and necrosis in liver parenchyma.

All the histopathological observation showed that exposure to sub-lethal concentrations of $Cuso_4$ caused destructive impact in the liver tissues and therefore may have dangerous result in severe physiological problems and ultimately cause the death of fish.

CONCULSION

The discharge of industrial wastes containing toxic heavy metals into water bodies may have significant effects on fish and other aquatic organisms, which may endanger public health through consumption of contaminated seafood and irrigated food crops. As a conclusion, histological investigations demonstrate a direct correlation between toxicants exposure and histopathological symptoms observed in several tissues**.**

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