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Hepatotoxic effect of Cu (II) in freshwater fish, *Channa punctatus*: A histopathological study

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Abstract: It is known that copper is an essential trace metal for living organisms and plays a crucial role in many biological enzyme systems that catalyze oxidation/reduction reactions and have molecular oxygen as a co-substrate. However, if copper is present at relatively high concentrations in the environment, toxicity to aquatic organisms can occur. Liver is a main storage and metabolizing center of toxicants in the animals including fishes. Disruption of its structural integrity may inhibit physical and biochemical activities in fish. In the sparkle of above fact present study was carried out to asses the hepatotoxic effect of Cu (II) in fish *Channa punctatus*. For this purpose fishes were exposed to sub-lethal concentration (0.36 mg/l) of Cu (II) (taken as $CuSO_4$) for 15, 30 and 45 days of exposure periods. Chronological histopathological damage such as swelling of hepatic cells, hepatocellular necrosis, vacuolization, inflammation and hepatic cell damage were observed in fishes exposed to Cu (II).

Key word: Histopathology, *Channa puntatus*, Liver, Copper sulphate

Introduction

It is known that copper is an essential trace metal for living organisms and it is present in all natural waters and sediments. This metal plays a crucial role in many biological enzyme systems that catalyze oxidation/reduction reactions and have molecular oxygen as a co-substrate. However, if copper is present at relatively high concentrations in the environment, toxicity to aquatic organisms may occur. It forms integral components of proteins involved in all aspects of biological function. However, in excess they are toxic, binding to inappropriate biologically sensitive molecules or forming dangerous free radicals in tissues where copper accumulates. As with mammals , the liver is the major organ involved in copper homeostasis (Grosell *et al*., 1997, 1998a,b; Kamunde *et al*., 2001, 2002a). The liver accumulates a large proportion of the copper absorbed from the diet or water and is the site for synthesis of the most abundant copper-containing protein in the body *viz.,* ceruloplasmin.

Copper sulphate, commonly known as blue stone or blue vitriol, is the best known and the most widely used among all copper salts. Today, there are more than 100 manufacturers of this salt and its world consumption is around 200,000 tons per annum, of which it is estimated that approximately three-quarters are used in agriculture, principally as a fungicide to control bacterial and fungal diseases of fruit, vegetable, nut and field crops. Some of the diseases that are controlled by this fungicide include mildew, leaf spots, blights and apple scab. It is used in combination with lime and water as a protective fungicide, referred to as Bordeaux mixture, for leaf application and seed treatment. It is also used as an algaecide, a herbicide in irrigation and municipal water treatment systems, and as a molluscicide, a material used to repel and kill slugs and snails. It is also used as a mordant in dyeing process. An increased level of copper in aquatic environments also comes from electroplating, mining and metallurgy industries and sewage and agricultural waste (Taylor *et al*., 2000; Di Toro *et al*., 2001).

The physical and chemical characteristics of water play an important role in copper toxicity for aquatic animals. Metal can either increase or decrease hepatic enzyme activities and lead to histopathological hepatic changes, depending on the metal type and concentration, fish species, length of exposure and other factors (Wepener *et al*., 2001; Shastri and Sharma, 1979). The histological changes in fish liver are highly sensitive and accurate way to assess the effects of copper concentration in experimental studies. Essentiality of copper arises from its specific incorporation to variety of enzymes which play important role in physiological processes as well as in to some structural proteins. The specific mechanism of liver toxicity has not been elucidated, but administration of copper to laboratory animal has resulted in hepatocelluar necrosis, degenerative activity, and kupffer cell mobilization (Rabert,1979; Sorenson *et al*., 1980). Keeping all above facts in mind present study have been taken to asses the hepatotoxic effect of copper in fish *C. punctatus.*

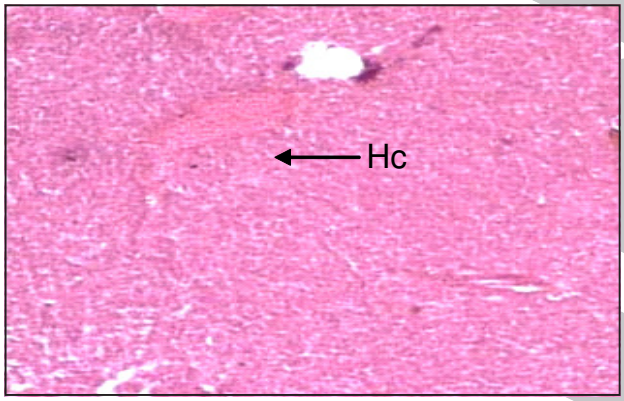
Materials and Methods

The small size freshwater fish, *Channa punctatus,* weighing 15±2 g and measuring11±2 cm, were collected with the help of local fisherman from water bodies located in the sub-region of Lucknow. They were properly washed in tap water and treated with 0.02% $KMNO_4$ and 0.004% formalin solution to remove external infection of fungi, algae *etc*. The fish were acclimatized to laboratory conditions for 15 days prior to the experimentation. The fish were fed with TOKYO made in Japan once in a day. The LC_{50} of test chemical, copper sulphate was estimated by employing Trimmed Spearman Karber method (Hamilton *et al*., 1977). Sub-lethal concentration of $CuSO_4.5H_2O$ (MERCK) test chemical was freshly prepared in distilled water before mixing in aquaria water.The fish were divided into 4 equal groups consisting of 10 each. Each group was transferred separately to glass aquaria of 100 litre volume. Fish of group I were maintained as control, while fish of group II, III and IV were exposed

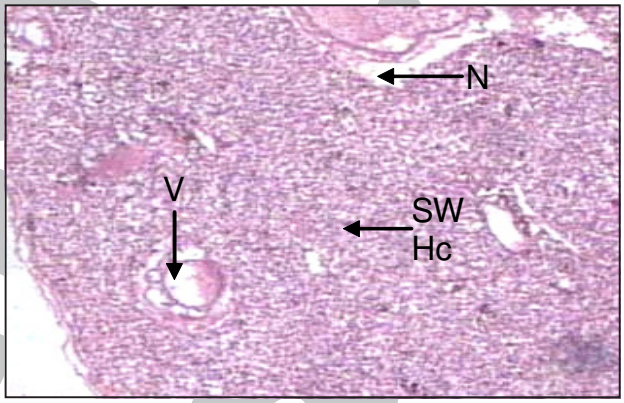
Table - 1: Effect of copper sulphate on physico-chemical profile of water

Parameters	Days of exposure period						
	Zero hr control + treatment	15 day control	15 day treatment	30 day control	30 day treatment	45 day control	45 day treatment
Temperature ^{0}C	22	24	24	25	25	26	26
pH	7.5	7.5	7.2	7.4	6.5	7.3	5.8
TDS (mgL^{-1})	100	105	200	110	230	130	280
DO (mgL^{-1})	7.8	7.5	6.7	7.6	6.8	7.0	6.8
Hardness(mgL^{-1})	110	100	96	95	80	85	50
Alkalinity (mgL^{-1})	350	350	300	340	250	300	200
Chloride (mgL^{-1})	43.50	42.50	36.75	43.00	33.50	40.00	29.70
Iron (mgL^{-1})	0.41	0.35	0.31	0.34	0.29	0.34	0.26

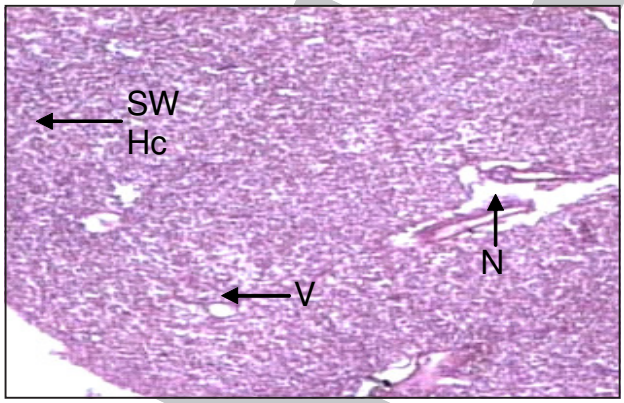
TDS =Total dissolved solids, DO = Dissolved oxygen



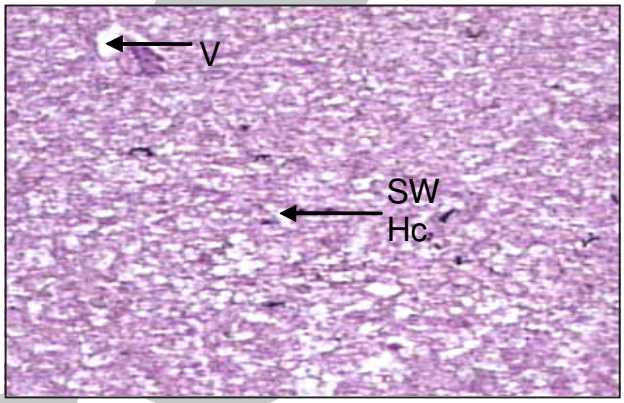
A: Liver of fish from the control group showing (H and E X 10). Hc-Hepatic cells



C: Liver of fish exposed to 1/10 (LC50) copper sulphate for 30th days showing (H and E X 10). SW Hc-Swelling of hepatic cells, N-Necrosis, V-Vacuolation



B: Liver of fish exposed to 1/10 (LC50) copper sulphate for 15th day showing (H and E X 10). SW Hc-Swelling of hepatic cells, N-Necrosis, V-Vacuolation



D: Liver of fish exposed to 1/10(LC50) copper sulphate for 45th days showing (H and E X 10)

Fig. 1: Effect of copper sulphate on liver of freshwater fish *Channa puntatus*

to sublethal concentration (0.36 mgL^{-1}) of copper sulphate for 15, 30 and 45 days of exposure periods. The waste products were removed from aquaria water by using good quality of aquaria water filter. Physico-chemical characteristics of test medium *viz.* temperature, pH, TDS (total dissolved solids), DO (dissolved oxygen), hardness, alkalinity, chloride and iron of each group was analyzed after 15, 30, 45 days of exposure periods following standard methods (APHA, 2005). After the completion of stipulated time of experimentation liver of fish were taken out quickly and fixed in boinins fluid and processed for microtomy. Rotatory microtome was used to cut sections of 3 µm thickness. Section were thin fixed on a slide with the help of Meyer's albumin, heated on hot plate and were double stained by using Ehrlich haematoxylin and the Eosin.

Results and Discussion

Considerable alterations in physico-chemical profile of test medium viz. temperature, pH, TDS, DO, hardness, alkalinity, chloride and iron were observed after each exposure period in comparison to control (Table 1). Temperature and TDS contents were found increased while pH, DO, hardness, alkalinity, chloride and iron contents was found decreased in comparison to control after each exposure periods.

The normal structure of liver of *C. punctatus* comprises of a regular mass of parenchyma cells. The liver parenchyma of control fish shows normal hepatocytes filled with glycogen and kuffer's cells. Normally hepatocytes are arranged in a fashion that they appear as a radiating structure like the spokes of a wheel. The hepatocytes are separated from each other by small blood spaces or sinusoids (Fig. A). The fish exposed to sublethal concentration of copper for 15, 30 and 45 days of exposure periods showed considerable histoanatomical changes in liver. After the initial 15 days of exposure period, the hepatocytes began to swell and were arranged in a disorganized fashion, resulting in an irregular lobular architecture (Fig. B). Mild necrosis and minor vacuolation were also found as is clearly evident from the Fig. B. After 30 days and 45 days of exposure period the outer membrane of the liver was found to be significantly ruptured. The hepatocytes also showed vacuolation, extensive pyknotic nuclei, necrosis and hypertrophy resulting in disappearance of hepatocytes (Fig. C, D).

As liver is the main storage and metabolizing center of toxicants and copper may damage liver of the fish. Necrosis leads to decreased number of hepatocytes which may decrease the function of the hepatocytes and liver as a whole.The specific mechanism of copper toxicity has not been elucidated, but administration of copper compounds to laboratory animals has resulted in hepatocellular necrosis, degenerative activity, cirrhosis, Kupffer cell mobilization and hepatocellular pigment formation (Ram and Sing, 1988). The hepatocellular architecture of fresh water fish *Channa puntatus* found promptly affected due copper exposure (Fig. B, C and D).

Radhaiah and Rao (1992) have also reported similar findings in fish, *T. mossambica* exposed to sublethal concentration of fenvalerate for 10 and 20 days of exposure periods. They observed histopathological lesions such as vacuolated hepatocytes, necrosis, movement of nuclei to the cell periphery, pyknotic nuclei and cytoplasmic degeneration in the liver tissues. According to Risbourg and Bastide(1995), Gingerich(1982), Rahman *et al*. (2002), the exposure of fish to agricultural toxicants increased vacuolization, mild pyknotic and necrotic hepatocytes and rupture of blood vessel causing hemorrhage. Several other workers have also observed hypertrophy of hepatic cells and liver cord disarray, vacuolation of cytoplasm and necrosis, rupture of hepatic cell membrane in fishes *Channa punctatus* and *C. carpio* exposed to mercuric compounds (Kauppasamy, 2000; Masud *et al*. 2003; Nessiem *et al*. 2003).

Fish prefers optimal environmental conditions for their growth and development. Any change in environmental conditions causes stress on fish leading poor fish growth and development. Liver is a great determining factor for hepatotoxicity in living organism including fishes. The process of biotransformation is a critical factor for many xenobiotics, especially since hepatic biotransformation processes are readily modified by exposure to toxic chemicals.

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