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The Histopathological Effects of Copper Sulphate on Rainbow Trout Liver (*Oncorhynchus mykiss*)

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Abstract: Fish were exposed to sublethal dose of copper sulphate ($CuSO_4$) in order to determine the histopathological alterations in the liver of rainbow trout (*Oncorhynchus mykiss*). After 28 days treatment; non-homogenous regions and congestion of central vein, dark-stained hepatocytes, increasing the number of Kupffer cells, vascular degeneration and sinusoidal degenerations were observed.

Key words: Rainbow trout, copper sulfate, liver histopathology

INTRODUCTION

Copper is one of 26 essential trace elements occurring naturally in plant and animal tissue (Anonymous, 1986). For many years, it has been accepted that Cu is necessary and essential element for living organisms from bacteria to human (Ma and Betts, 2000). Copper is a trace element essential to life, but the toxic effect of water contamination to fish is now clearly demonstrated by Roncero *et al*. (1992) and Grosell *et al*. (1998).

$CuSO_4$ is a fungicide used to control bacterial and fungal diseases of fruit, vegetable, nut and field crops. These diseases include mildew, leaf spots, blights and apple scab. It is used as a protective fungicide (Bordeaux mixture) for leaf application and seed treatment. It is also used as an algaecide and herbicide and to kill slugs and snails in irrigation and municipal water treatment systems. It has been used to control dutch - elm disease. It is available as a dust, wettable powder, or liquid concentrate. $CuSO_4$ is highly toxic to fish. Even at recommended rates of application, this material may be poisonous to trout and other fish, especially in soft or acid waters. Its toxicity to fish generally decreases as water hardness increases (Anonymous, 1996).

Sources of aquatic environmental contamination by copper are numerous, e.g., mining, industry and agricultural pesticides. $CuSO_4$ is frequently used in agricultural and viticulture treatments for its antifungus properties. It is present in many fertilizers and pesticides and thus can contaminate aquatic ecosystems (Palacios and Risbourg, 2006).

Since the liver is the center of xenobiotic metabolism, it can be considered as a suitable tissue for micronucleus tests. Use of cells from liver tissue, however, has some limitations mainly due to its low mitotic index (Cavas *et al*., 2005). The main organ responsible for the storage of Cu in the body and its secretion is liver (Geyikoğlu *et al*., 2004).

Although the Cu induced perturbations in fish liver are well documented, the variability of the reported results is large and depends on the degree of contamination, fish species, ages or sexes and water quality. Histocytological reported changes are either adaptative as lysosomal proliferation and reticulum development or degenerative as losses in integrity of mitochondria, plasma or nuclear

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membranes, fragmentation of endoplasmic reticulum and development of autophagic vacuoles. In liver of zebra fish (*Brachydanio rerio*) or roach (*Rutilus rutilus*) exposed to sublethal copper sulphate concentrations these two types of perturbations are observed together (Palacious and Risbourg, 2006).

This study aimed to evaluate the sensitivity of liver cells and determine the histopathological alterations of rainbow trout exposed to $CuSO_4$.

MATERIALS AND METHODS

Fish Maintenance, Water and Experimental Design

Rainbow trout, *Oncorhynchus mykiss* (120±15 g) were purchased from Atatürk University, Faculty of Agriculture, Trout Breeding and Research Center. The research was arranged in the Fish Toxicology Laboratory of Fisheries Department. Fish were acclimatized to this unit for three weeks and maintained in fiberglass tanks. Tanks have 600 L water volume; 0.4 L min^{-1} kg^{-1} fish fresh water input and waste water discharge. Water temperature was 10.5±0.5°C during the experiment. There were three groups (two tanks with copper sulfate and a control tank) and each group included seven fish. During the acclimatization and the experiment, fish were fed with commercial trout feed (Çamlı Feed Co., pellet 4). The treatment extended 28 days and organized during November 2006-January 2007.

Toxicant

$CuSO_4$ is still used as an agrochemical. So it was obtained as commercial package from a pesticide seller. After calculating the tank water volumes and desired concentrations (600 μg L^{-1}) in tank, chemical was put into the tanks once a day as semi-stabile method (Unsal, 1998; Palacios and Risbourg, 2006). No mortality was observed during the adaptation and experiment.

Sample Preparing

The liver of all fish from each treatment and control group were used for histology. After the fish brought out the water waited for losing their livings. The fish were euthanised with a sharp cranial blow at the end of the study and fixed in 10% neutral buffered formalin. Incisions were made in the fish's abdominal wall to allow penetration of the formalin though out the intestinal organs. Following preservation the fish were dissected and their livers were taken for histological examination. The livers were embedded in paraffin, sectioned and stained with haematoxylin and eosin (H and E) using Standard techniques. The sample sections were examined with a compound microscope (200x and 400x magnification) and digital images taken of each of the liver samples (Glencross *et al*., 2004).

RESULTS AND DISCUSSION

Control rainbow trout livers were in normal color of a healthy trout. Central vein and sinusoids were in normal appearance. Regions and congestion of central vein were homogenous (Fig. 1a). This group fish presented a centrally located nucleus, similar in aspect and size. In the ovoid nucleus, the nucleolus was slightly eccentrically located and presented an important electron density. The nucleus and nucleolus structure were a common feature to all cell types and Kupffer cells of healthy fish (Fig. 1b). Our observations on control livers were in agreement with the reports of researchers (Hampton *et al*., 1989; Rocha *et al*. 1997).

Hepatocytes of rainbow trout exposed to copper sulphate were darker than the control group. Severe liver damages in experimental group were established such as: Non-homogenous (light and dark stained) regions and congestion of central vein (Fig. 2), dark-stained hepatocytes (Fig. 3), general degenerations of hepatocytes and sinusoidal dilatations (Fig. 4a, 4b and 5), increasing the number of Kupffer cells (Fig. 6), vascular degeneration and congestion in vessel (Fig. 7).

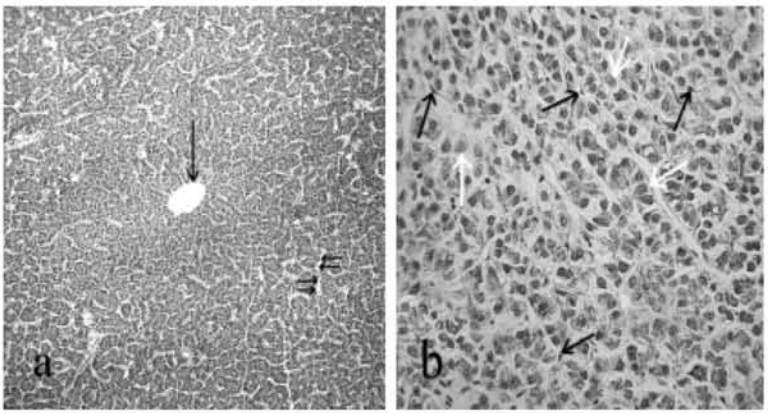

Fig. 1: Liver of the control rainbow trout. (a) Central vein (arrow) and sinusoids (double arrow). Hem-Eo x100, (b) Hepatocytes (white arrows) and Kupffer cells (black arrows). Hem-Eo x400

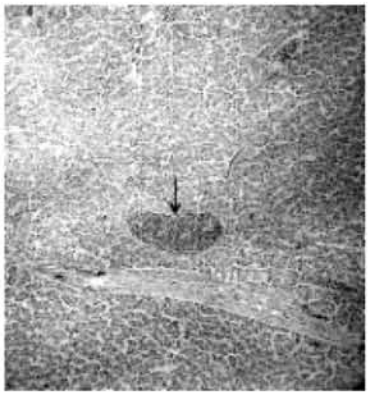

Fig. 2: Non-homogenous (light and dark stained) regions and congestion of central vein. Central vein (arrow). Hem-Eo x100

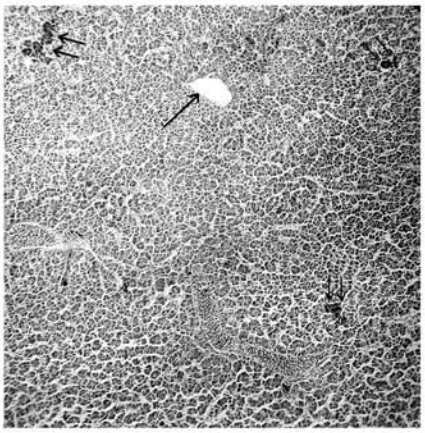

Fig. 3: Dark-stained hepatocytes (double arrow). Central vein (arrow). Hem-Eo x100

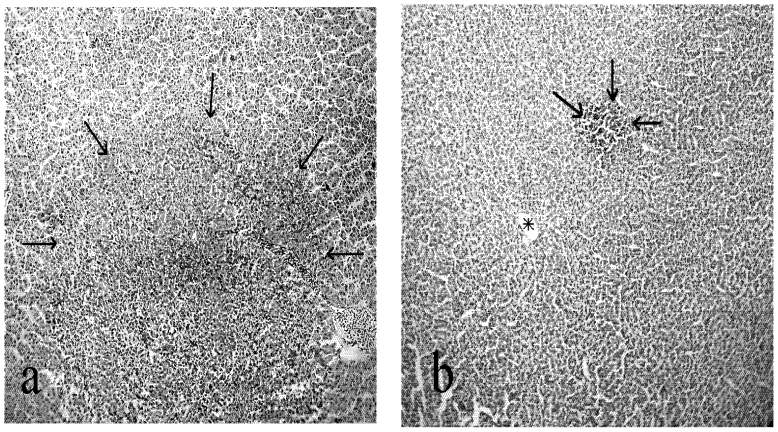

Fig. 4: (a) Degenerations of hepatocytes (arrows), (b) Degenerations of hepatocytes (arrows) and central vein (asterisk). Hem-Eo x100

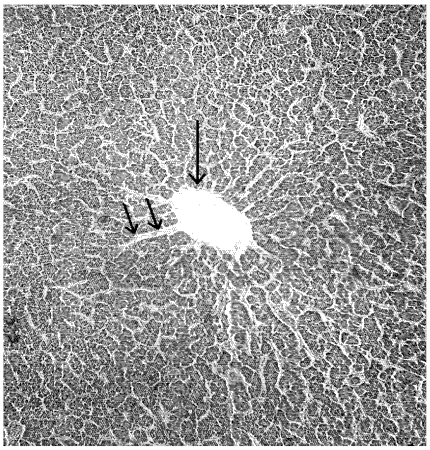

Fig. 5: Sinusoids (double arrow). Central vein (arrow). Hem-Eo x100

This research reveals that $CuSO_4$ has detrimental effects on the liver histopathology of rainbow trout (*O. mykiss*). Copper absorbed across the intestine are carried directly to the liver via the hepatic portal vein and aqueous Cu accumulates in hepatocytes (Handy *et al*., 1999). As a matter of fact, the cytoplasm of hepatocytes in rats fed on 1500 mg Cu kg^{-1} water was darkly stained with eosin (Fuentealba *et al*., 1993). In a previous study was also established that the content of seca became darker and pale, when 120 and 250 mg kg^{-1} Cu was added to chicken fodder (Jensen and Maurice, 1977). In the present study, the same situation was observed in the liver of rainbow trout given 600 μg L^{-1} $CuSO_4$ water. Goldfischer *et al*. (1980) emphasized that Cu was concentrated in cytoplasm of hepatocytes before the formation of necroses. The darkening in the tissues occurred due to Cu. Similarly, degenerations of dark stained hepatocytes were observed after fish treated with 600 μg L^{-1} $CuSO_4$ in this study. It is reported that absorbed Cu especially accumulates in lysosomes of cells. At this point lysosomes play important role in metal homeostatis, storage and in the turnover of cytoplasmic proteins such as metallothioneins. Excessive metal accumulation in lysosomes disrupts the normal process of lysosomal biogenesis causing impairment of this essential cellular system

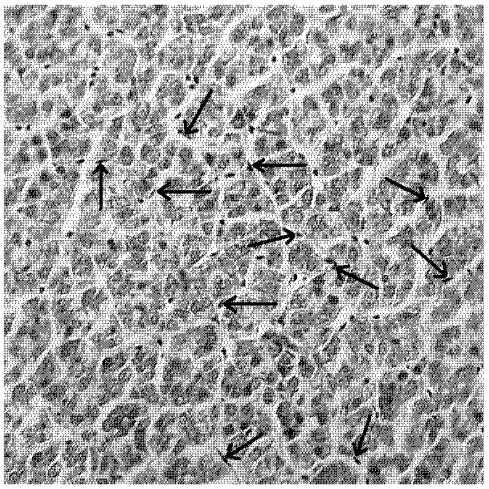

Fig. 6: Increasing the number of Kupffer cells (arrows). Hem-Eo x 400

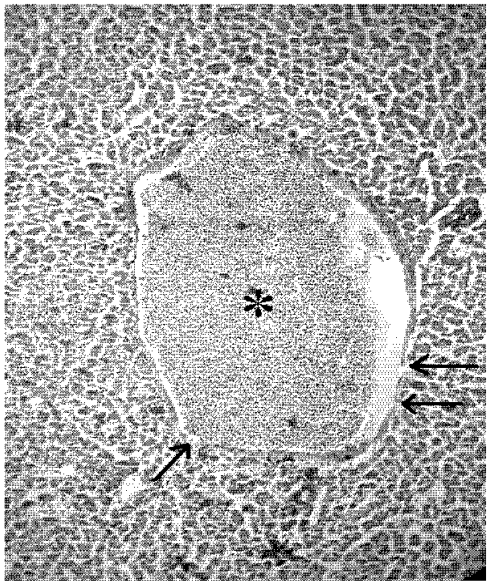

Fig. 7: Vascular degeneration (arrow) and congestion in vessel (asterisk). Hem-Eo x 100

(Fowler *et al*., 1983; Fowler, 1987). Again, degenerative changes occurred when Cu increased in nucleus (Fuentealba *et al*., 1989). In addition accumulation of Cu in nucleolus might suggest a possible inhibition of DNA transcription.

In the current study, vascular disorders revealed by effect of $CuSO_4$. Increased arterial flow also leads to sinusoidal dilatation (Nobuyoshi *et al*., 2005). Sinusoidal dilatations cause to passing of proteins between Cu complexes and hepatocytes (Ettinger, 1984). Thus, liver receive Cu quickly and differently. Irregular sinusoidal structures give signs of congestion, too (Ozturk *et al*., 2005). As regards, the obstruction of hepatic venous outflow leads to intrahepatic venous congestion and portal hypertension (Masaaki *et al*., 2004). Again, Kupffer cells observed in this study, macrophages of the

liver, play an important role in liver damage and regeneration. It is proposed that Kupffer cells are stationary and regenerate after liver trauma by local proliferation (Bair *et al*., 2005). On the other hand, increasing intrahepatic lymphocytes are believed to be directly involved in the immunopathogenesis of chronic liver diseases (Wang *et al*., 2004).

Paris-Palacios *et al*. (2000) demonstrated that the toxic impact of Cu on fish was the induced histo-cytological perturbations. Because the functional hepatocytes have lost typical features of increased metabolism, $CuSO_4$ may cause physiometabolic dysfunction in the fish liver hepatocytes and increase histopathological alterations.

Liver plays a crucial role in metabolism (Yokouchi, 2005). Hepatic metabolism is, first and foremost, a mechanism that converts compounds into products that are easily excreted (Tolman, 1998). For this reason, further studies are needed because the different life stages of this fish and the other species may respond different reactions.

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