

Research Article**Effects due to sub-lethal exposure of zinc sulphate on liver of *Mystus cavasius* fish in fresh water: A histological study**

Shampa Jain, Deepmala Batham*, Zubair Azad

Department of Zoology and Biotechnology, Govt. M.H. College of Home Science and Science for Women, Jabalpur (M.P.) India

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Abstract

Objective: In our present investigation the histopathological study was conducted in liver of *Mystus cavasius*, exposed to three sub-lethal concentration of a zinc sulphate after determination of LC₅₀. **Materials and methods:** The healthy fish *Mystus cavasius* were exposed to sub-lethal concentrations of 2mg/L, 2.5mg/L and 3mg/L of zinc sulphate for 10 and 20 days. Liver from those fishes were taken and fixed in bouin's fluid for 24 hours for fixation. This fixed tissue was then processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Sections of the paraffin wax were processed to obtain six micron thick paraffin sections and then stained with Hematoxylin and Eosin and examined under light microscope. **Results:** Histopathological changes revealed that the results observed in the liver includes degeneration of hepatic cells, vacuolar lesions, haemorrhages and ruptured blood vessels. Liver observed microscopically showed increasing degrees of damage in the tissues in correlation with the concentration of zinc sulphate. **Conclusion:** It can therefore be concluded that concentration of zinc sulphate caused histological alteration in the liver of *Mystus cavasius*.

Keywords: Histology, zinc sulphate, haemorrhages, liver, *Mystus cavasius*

Introduction

Heavy metals are found in fluctuating levels in the aquatic bodies and can occur naturally in the environment. Sometimes, these heavy metals can be vulnerable to aquatic organisms at peculiar high levels. Therefore, due to insistent nature of heavy metals and their vergency to accumulate in tissues, they have been known as strong biological poisons (Nahed, 2006; Berntessen et al., 2003; Ek et al., 2005). Zinc (Zn) is among one of the most crucial trace elements in the body, and can take part in several natural function of proteins and enzymes (Maity et al., 2008). Most of the heavy metal ions are toxic in nature (Damien et al., 2004; Farombi et al., 2007) and may pose a threat to human health and the environment.

Despite being a fundamental trace element, Zinc above definite concentrations can be toxic to most organisms (Ho, 2004). Oxidative stress can be achieved due to a large part of these elements which can employ their toxic effect by generating reactive oxygen species (ROS).

Material and methods

Fresh water fish *Mystus cavasius* measuring 8 to 10 cm in length and weighing 6.5 to 7.5 gm were collected from local fish pond and were allowed to acclimatize for 10 and 20 days in the laboratory after which, the healthy fish were exposed to sub-lethal concentrations 2mg/L, 2.5mg/L and 3mg/L of zinc sulphate. Tissues like liver were isolated from untreated and treated fish. Experimental saline solution (NaCl) was used to clean the tissues. This tissue was fixed in aqueous Bouin's solution for 24 hrs. This fixed tissue was then processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Sections of the paraffin wax which contains tissue were cut at 6 µm thickness with the help of microtome, stained with hematoxylin-eosin and were mounted. The obtained slides were studied at 400X magnification.

Results**Histology of control liver**

The control liver is a solid glandular organ bearing rounded hepatic lobules in the form of branched columns which were separated from one another by the connective tissue. Each hepatic lobules contains hepatic cells. Hepatic cells were polyhedral, covered by very thin membrane. In the liver

***Address for Corresponding Author:**

Deepmala Batham

Govt. Model Science College, Jabalpur (M.P.), India

Email: deepmalabatham4@gmail.com

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mass, blood spaces of various sizes are also present. Around a central vein in inter connected lamina, the hepatocytes are arranged in radial manner. Each hepatocytes contains a centrally located spherical nucleus, the cytoplasm is granular which takes deep hematoxylin stain (Figure 1(A)).

Toxic Effect of zinc sulphate on treated liver

After exposure to 2.0 mg L⁻¹ zinc sulphate for 10 days, space between hepatocytes was not much broad. Cells were clearly distinguishable and only small degenerations of some hepatic cells occurred, vacuolization in cytoplasm was conspicuous (B). After 20 days exposure degeneration of hepatic cells was seen. Wider blood spaces appeared. Vacuoles were more frequent, numerous and larger in size (C).

After exposure to 2.5 mg L⁻¹ zinc sulphate for 10 days, (D) the liver showed large size of blood space, though with less quantity of haemorrhages and disturbed blood vessels were occurred. Vacuoles were occurred in great size and the nucleus was shifted to the margins of the hepatic cells. After 20 days exposure the nucleus was absent in some of the hepatic cells. Larger blood spaces were observed (E).

When the fishes were exposed to 3.0 mg L⁻¹ concentration of zinc sulphate for 10 days, intrahepatic spaces were broad with frequent haemorrhage and rupture blood vessels. Nucleus could not be identified in the hepatic cells (F). After 20 days of exposure liver showed broad intrahepatic spaces with frequent haemorrhages and occurred damages of blood vessels. Cells were contract and no nucleus was observed. Small vacuolar lesions were also observed (G).

Discussion

Histological alterations related to heavy metal toxicity in the liver of fish have been showing that the substances cause severe damage to the liver cells. Liver is an important organ for detoxification and due to this reasons the hepatic cells are severely damaged. Our present study focuses on histopathological investigations on Liver which have been proved to be a sensitive tool to detect toxic effects of chemical compounds.

After the exposure of zinc sulphate concentration degeneration of hepatic cells (was seen and wider blood spaces appeared) and the nucleus was either absent or shifted to the margins of the hepatic cells. Liver showed broad intrahepatic spaces with frequent haemorrhages. Our results were also supported by the results of (Bakshi, 1999) who reported the rupture in the hepatic cells of liver of a teleost *Heteropneustes fossilis* due to toxic effect of copper. Shifting of nucleus and clear atrophy in the nucleus of hepatic cells in urea exposure was observed by (Tiwari, 1988). Resembled results were also observed by findings of (Abdel-Warith et al., 2011) they reported that Zn

concentrations damages cell structure with increase of hypertrophied hepatocytes in liver of fish. Moreover, (Kaoud, and El-Dahshan, 2010) revealed that liver showed degeneration of the hepatocytes in *O. niloticus* during the exposure of heavy metals Cd, Pb, Hg and Cu.

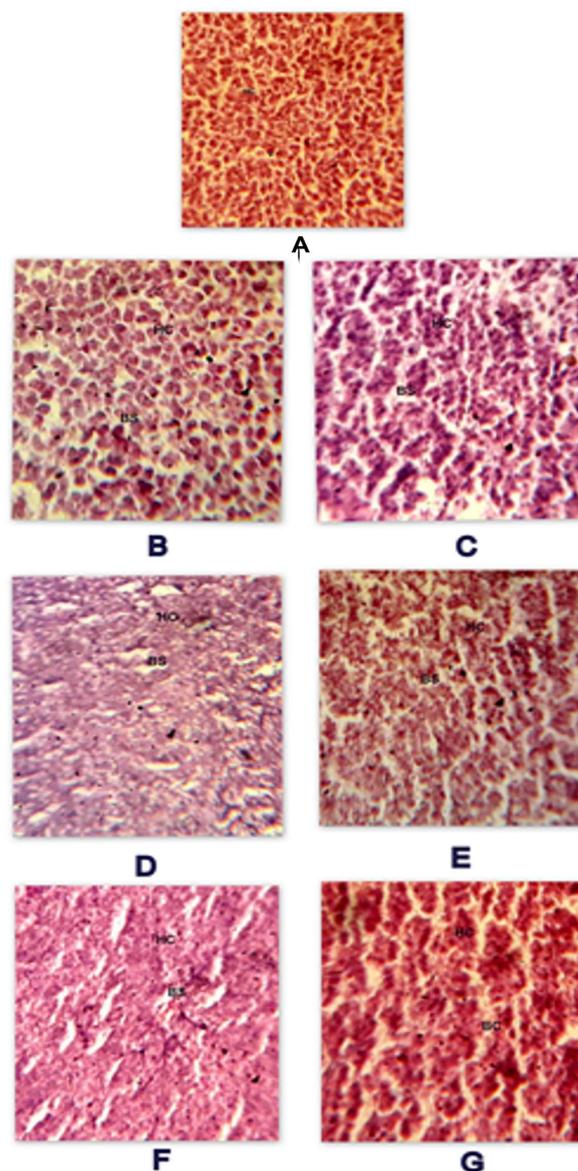


Figure 1. Histopathology of: (A) untreated liver of *Mystus cavasius*. (B) Treated liver of *Mystus cavasius* after 10 days exposure to 2mg/l zinc sulphate. (C) Treated liver of *Mystus cavasius* after 20 days exposure to 2mg/l zinc sulphate. (D) Treated liver of *Mystus cavasius* after 10 days exposure to 2.5mg/l zinc sulphate. (E) Treated liver of *Mystus cavasius* after 20 days exposure to 2.5mg/l zinc sulphate. (F) Treated liver of *Mystus cavasius* after 10 days exposure to 3mg/l zinc sulphate. (G) Treated liver of *Mystus cavasius* after 20 days exposure to 3mg/l zinc sulphate. Slides were stained with Hematoxylin and eosin staining, 400X (HC=hepatic cell, HR =Haemorrhage, BS= Blood spaces.)

(Selvanathan et al., 2013) reported the exposure of mercury in liver of *Clarias batrachus*. They showed that the liver tissue was disrupted, the hepatocytes appeared swollen and liver showed acute inflammation. (Mobarak and sharaf, 2011) revealed histopathological changes in the liver of *poecilia latipinna* treated by lead acetate their observed liver showed disarrangement of hepatic cord, shrinkage of hepatocytes also the presence of a hemorrhagic spot was detected in the hepatic parenchyma. Due to the effect of copper, the changes in the liver hepatocytes such as vacuolization, necrosis was also reported by (Figueiredo-Fernandes et al., 2007).

Olojo et al. (2005) found that when *Clarias gariepinus* treated to lead, hepatic tissue showed varied degree of hepatic cirrhosis as evident in the density of fibrous connective tissue within and around the hepatic parenchyma and hypertrophy of hepatocytes. The liver showed large size of blood space after the treatment of zinc sulphate though with less quantity of haemorrhage, disturbed and ultimately damaged blood vessels were occurred. Similar findings were observed by (Abdel-Warith et al., 2011) who showed the intra hepatic blood vessels were dilated and signs of blood vessels fibrosis were noted. The part of liver namely vacuoles were occurred in great size and small vacuolar lesions were also observed after the exposure of zinc sulphate. Similar observation was found by (Senarat, 2015), he found that histopathological finding of liver of the yellow *Mystus Hemibagrus filamentus* showed vacuolar degeneration in the hepatic cells. (Sultan and Khan, 1986) reported destruction of cytoplasmic vacuolation of hepatocytes in *Carassius auratus*. (Mishra and Mohanty, 2008) observed in the liver of *Channa punctatus* (Bloch) that hepatocytes showed cytoplasmic vacuolization when exposed to chromium toxicity. (Selvanathan et al., 2013) recorded vacuolization and hypertrophy of tissue when exposed to mercury as well as they were also reported toxic effect of cadmium in which the liver was highly damaged, sub capsular vacuolization, vacuolation space formation was evident, throughout the tissue in liver of fish *Clarias batrachus*.

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Conflict of interest

All the authors are not having any conflict of interest in this article.

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