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Experimental Study Of Catfish, H. Fossilis Liver Protection With Chlorella Against Mercuric Chloride Induced Histological Changes

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***Abstract:** The effect of mercuric chloride on the liver in Catfish Heteropneustes fossilis was analyzed histologically. Fish, when treated with 0.01 ppm of mercuric chloride for 21 days exhibited degeneration, vacuolization, swelling in hepatocytes and necrosis. Fish of recovery groups showed reduced vacuolization and reduced necrosis. The marked degeneration, fragment cytoplasm was lost and evenly distributed in the cell and large nuclei, arranged correctly and displayed a high affinity toward Haematoxylin- Eosin and Mallory's triple stain.*

***Keywords:** Chlorella, Heteropneustes fossilis, Mercuric chloride, Histology.*

I. INTRODUCTION

Heavy metals are present in all phases of the environment- air, water, and land. These heavy metals are released in the water phase of the environment, the fluid motion transports them, are further transferred to the atmosphere and land, subjected to various physiochemical and biochemical reactions and are being assimilated by all levels of the aquatic food chains. They are also transmitted by direct interaction through the food chain to higher organisms and ultimately to human beings (Kumar and Kakrani 1995). Among them, mercury is one of the most toxic metals in our environment, including the lithosphere, hydrosphere, and atmosphere (Barbosa et al. 2001).

The liver is an important organ performing vital functions including biotransformation, migration of lipids, glycogen storage, a release of glucose into the blood, etc. Moreover, liver contains many enzymes and proteins; heavy metals chelation may disrupt the liver tissue by disintegrating the functional and structural properties of the cells. The liver is responsible for detoxifying the chemical substances in the blood. When, it is exposed to the high concentrations of toxicants and toxic metabolites, making it susceptible to injury (Glaister, 1986).

Several aquatic macrophytes have been used in the removal of heavy metals from the waste water. The use of these plants in biomonitoring of metals (Cardwell et al., 2002) or as biofilters for polluted water (Dunbabin and Bowmer, 1992) and the aspects of removal (Miretzky et al., 2004; Hassan et al., 2007) besides the toxicity of these metals through the plants (Drost et al., 2007).

Marine algae have the potential to impure the biochemical imbalances induced by various toxins associated with free radicals. The unicellular green algae Chlorella contain many bioactive substances with medicinal properties. Chlorella helps in detoxification of metals while reduces UV-B degradation of the skin, decreasing dioxin and increasing immunoglobulin concentration in breast milk. It has powerful chelating properties, potent cancer fighter, and balances the body pH.

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Some studies have determined the antioxidant properties of chlorella against various diseases, heavy metals and β-exposure (Vijayavel et al., 2007; Blass- Valdivia et al., 2010).

It is worthwhile to explore the effects of mercury on the liver in H. fossilis to (or "intending to") observing the recovery of damages after giving chlorella with the diet.

II. MATERIALS AND METHODS

EXPERIMENTAL ANIMAL: Heteropneustes fossilis (Bloch)

The metal used: Mercury was used for present study in the form of mercuric chloride (HgCl2). Fisher Scientific mercuric chloride, molecular weight -271.50, (CAS No: 7487-94-7), Product no: 15564.LC 50 value decided the dose of mercuric chloride. It was found to be 0.75 mg/l.

RECOVERY AGENT: Chlorella vulgaris (powdered form) was used as a recovery agent for the present investigation.

Living and healthy specimens of Catfish Heteropneustes fossilis (20±3 gm body weight, length 13±3 cm) were collected from local fisherman of Ujjain and were kept in glass aquaria. Fishes were acclimatized into dechlorinated water for 7 days. Fishes were treated with 0.01% of Kmno4 solution to avoid dermal infection. They were fed chopped dried prawn every day and water was renewed an alternate day, leaving no fecal matter, unconsumed food or dead fish in aquaria. Water in all aquaria was daily aerated by an aquarium pump for 30 minutes.

Fish were divided into 3 groups of 12 fishes each and were kept in glass aquaria; each containing 20 liters stored tap water. No mortality was recorded during the experimental period.

The experiment was conducted in the following manner:

- ✓ *CONTROL GROUP:* Fishes of this group were fed with everyday food and keep only in stored tap water without HgCl2 solution.
- ✓ *TREATED GROUP:* Fishes of this group have been addressed with HgCl2 (0.01ppm) solution for 21 days and fed with everyday food.
- ✓ *RECOVERY I GROUP:* Mercuric chloride pretreated fish were used. Fish of this group were treated with HgCl2 (0.01ppm) solution for 21 days another and then chlorella powder (5% of the diet) with food was given to these treated fishes.

The fish was depicted on the day 21 and their liver was fixed in Bouin's solution and 10% formalin and sectioned following the routine method. Chrom alum heamatoxylin-phloxine (CAHP) staining method was applied. The microphotographs of sections were taken using Leica ATC 2000 light microscope with MINTRON auto iris capture unit and PCTV Vision software.

III. RESULTS AND DISCUSSION

A. CONTROL GROUP

Results indicate the parenchyma cell analyzed to form a lattice network. The pentagonal hepatocytes were seen in the section containing well defined centrally located, a spherical nucleus with a prominent dark nucleolus, distinct bile passages, and blood capillaries. Hepatocytes were found among blood capillaries, called sinusoids. The sinusoids make continuous communication as they are seen covering into a central vein. Hepatocytes were uniformly distributed throughout the section of liver. The basophilic cytoplasm was evenly distributed in each hepatic cell. (Fig.1,2).

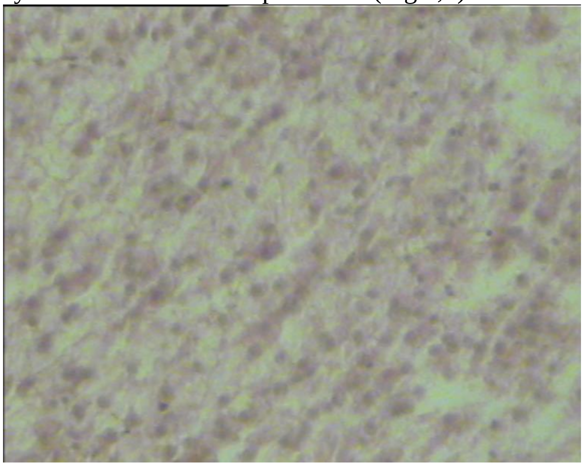

Figure 1: control group HE, 400x

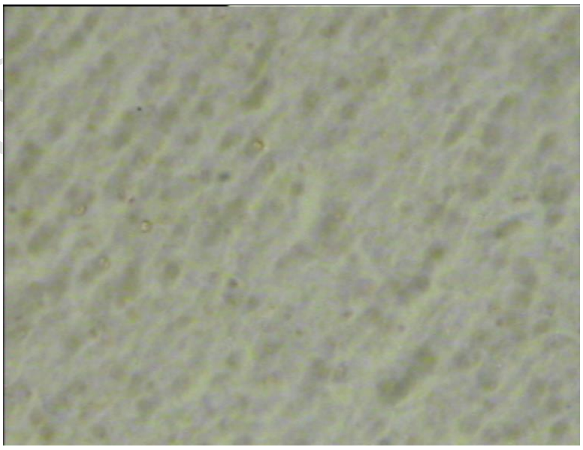

Figure 2: control group Mallory's, 400

EXPERIMENTAL GROUP

21 DAYS TREATED GROUP

In this duration, the liver showed severe degeneration of the hepatic cords, damaged cells and extensive necrosis of hepatocytes were observed. Hepatocytes cytoplasmic vacuolization was the most evident pathological alteration observed. The cellular structure was totally denatured. The nuclei of the hepatocytes showed pyknosis and few cells were on hypertrophied stage. The notable determinate changes were marked swelling in the hepatocytes and sinusoid cells. The necrotic cells were quite prominent; almost all cells become necrotic due to this liver displayed a spongy appearance.(Fig.3,4).

Shrivastava and Shrivastava (1994) reported high accumulation of lead in the liver of fish. Fanta et al., (2003) reported that due to bioaccumulation of the trace element in the liver tissue, impeded the liver function thus resulting in gradual degeneration of the liver cell arrangement. The intracellular spaces are seen with total degeneration of hepatic cells.

Hg-induced changes as observed during this study in the liver, such as swelling and degeneration of hepatocytes, infiltration of blood cells among hepatocytes are similar to those reported in C. batrachus (Bhoraskar and Kothari, 1993). Bano et al., (1990) also observed degeneration of liver cells along with disorganization of hepatic cells in H. fossilis. (Fig.3,4).

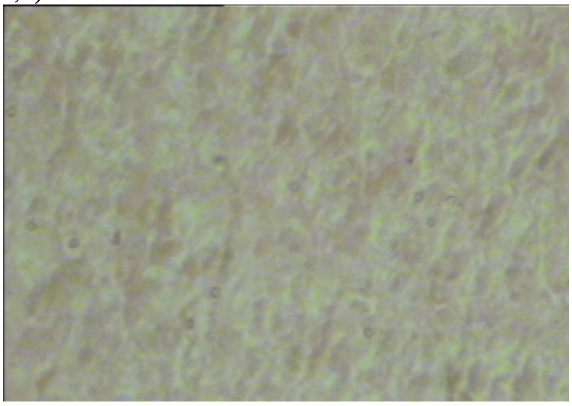

Figure 3: 21days treated HE 400x

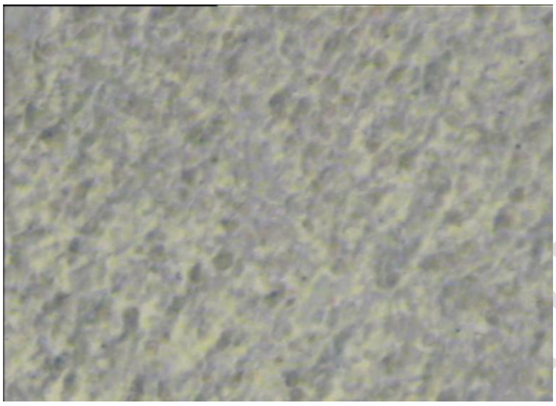

Figure 4: 21days treated Mallory's 400 x

21 DAYS RECOVERY GROUP

While in 21 days, the hepatic cells lost their hypertrophied nature and gaps among cells were filled by the connective tissues. The marked degeneration, fragment cytoplasm was lost and evenly distributed in the cells and large nuclei, arranged correctly and displayed a strong affinity toward Haematoxylin- Eosin and Mallory's triple stain. (Fig.5,6).

Chlorella vulgaris – unicellular freshwater green alga – is a useful test object to study the toxicity of water-soluble compounds, metals, radionuclides and other pollutants (Shehata et al., 1999; Franklin et al., 2002; Hogan et al., 2005).

Many studies have been performed on metal detoxification of various chlorella species. Many algae growing in metal-polluted environments display an ability to tolerate high concentrations of toxic metals (De Filippis and Pallaghy, 1994). Different groups of algae exhibit varying levels of tolerance to heavy metals.

Inside the cell, metals are chelated, and excess metal is sequestered by transport into the vacuole showing intracellular detoxification mechanisms. Clemens et al., 2002; Mehta and Gaur,1999 noticed that the greater the toxicity of metal, the greater is the intracellular concentration of proline in Chlorella Vulgaris, which is induced to protect the algae from metal toxicity.

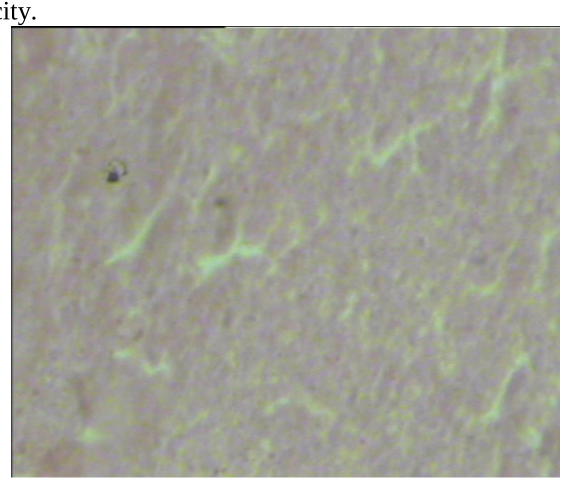

Figure 5: 21 days recovery HE 400x

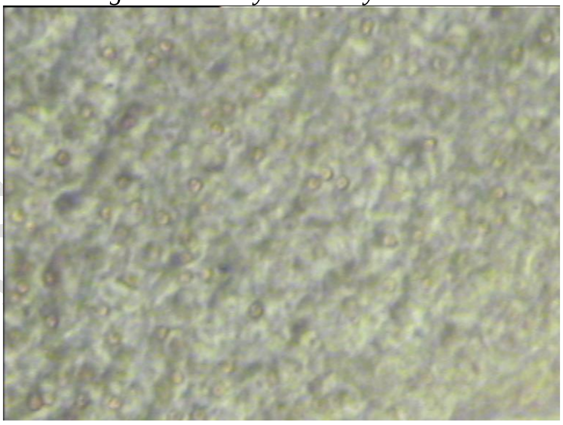

Figure 6: 21 day's recovery Mallory's 400x

ABBREVIATIONS
C – Cytoplasm
HC -Hepatic cells
VC -Vacuolized cell
N – Nuclei

IV. CONCLUSION

By these studies, it is concluded that Mercuric chloride brings histological changes in the liver of H. fossilis; and chlorella played a role against effects of mercuric chloride in the liver, suggesting that simultaneous exposure of fish to chlorella along with Hg helps in maintaining almost normal histology of liver of the catfish.

Further detailed investigation is needed to understand the preventive mechanism of chlorella against the hazardous effects of mercury.

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