

Hepatotoxic Responses in *Heteropneustus fossilis* (Bloch) after Oral Exposure to *Microcystis* under Laboratory Conditions

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Abstract: The effects of cyanobacterial blooms containing *Microcystis sp.* on hematological and blood biochemical changes in a fresh water fish, *Heteropneustus fossilis* were investigated under laboratory conditions. Moreover, a histopathological study of liver, tissues was also performed. Fishes were orally exposed to cyanobacterial blooms containing *Microcystis sp.* at a dosing of 100, 200 and 300ml mixed per liter of water. Results showed a sharp increase in bilirubin concentration in the blood of treated fish. Haemoglobin levels and cholesterol concentration have also been altered due to toxicity of *Microcystis sp.* The microscopic study revealed tissue alteration at various doses. These findings suggest that this fresh water fish can be adversely affected by cyanobacterial blooms in their natural habitat.

Key words: *Microcystis sp.* • Hepatotoxic • Histopathological • Biochemical studies

INTRODUCTION

Dense blooms are regular feature of eutrophic lakes. Using intracellular gas vacuole for buoyancy under thermally stratified condition, phytoplankton can form thick aggregation on Lake Surface [1]. The eyesores and ensuring foul odors associated with biomass decay lead to severe nuisance conditions and challenges to environmental management.

The bloom forming process can be caused by increased levels of nutrients, like phosphorus and nitrogen due to anthropogenic influence. Cyanobacteria have a number of special properties and besides their ability to nitrogen fixation using the enzyme nitrogenase [2] many of them have ability to form several toxic metabolites. The cyanobacterial toxin can be classified in five functional groups. hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (lipopolysaccharides). The hepatotoxins are known to be produced by member of several cyanobacterial genera including *Microcystis*, *Anabaena*, *Planktothrix*, *Oscillatoria*, *Anabaenopsis*, *Nostoc* and *Hapalosiphon* [3,4]. Biosynthesis and extracellular concentration of microcystins have been shown to be enhanced under high light conditions, which might have implication for the toxicity of cyanobacterial blooms in an aquatic ecosystem [5].

Blooms affect terrestrial plants and their reactions to their all toxins attain research interests because of contamination via spray irrigation [6-8]. Aquatic plants have been investigated to a lesser extent but as part of the aquatic ecosystem, they are potentially exposed to higher levels of cyanobacterial toxins.

Microcystin (MC) is a toxin secreted by cyanobacteria, which are the dominant phytoplankton in eutrophic fresh water bodies. A recently identified microcystin (MC) variant, [D-Asp (3) (E)-Dhb (7)] MC-RR, produced by *Planktothrix rubescens* found in lake Zurich, showed higher toxicity to zooplankton when compared to known MC [9].

Mass mortalities of fish have also been associated with toxic algal bloom (Rodger et al., 1994). The main fish organs affected by MC are the liver and kidney with symptoms similar to those described for mammals i.e., total loss of liver architecture causing liver dysfunction and necrosis [10, 11].

Considering the factors cited above, this study aimed to assess the physiological and biological responses in *Heteropneustus fossilis* in order to estimate the maximum dose of cyanobacterial cell containing MCs able to induce hepatotoxic responses and pathological changes in this fresh water fish after acute exposure to a *Microcystis* bloom from Sagar Lake.

MATERIALS AND METHODS

Experimental Set up and Acclimation of Fish:

Male *H. fossilis* sp. average weight 70 ± 2.0 gm were obtained from local fish market and transferred to the laboratory where they were held in aquariums (4 individual /aquarium) with 25 L of fresh water. The fishes were fed with commercial fish food and were acclimatized for 7 days before beginning of the experiment.

Collection of *M. Aeruginosa*: *M. aeruginosa* was collected from Sagar Lake,. Sagar is situated $23^{\circ} 50' N$ latitude; $78^{\circ} 48'$ longitudes lie a few kilometers in north of the tropic of cancer occupying almost a central position in the country. Sagar lake is situated in the heart of the city and is surrounded from sides viz., eastern, western and northern by a number of ghats and houses except from the southern side. The wind plays an important role in circulation of lake water, mainly due to southern open side.

Exposure of *M. Aeruginosa*: The acclimatized fishes were starved for about 48 hours to clear their gut contents. The experiments were then set up in 4 jars containing 5 liter of water with 5 fishes in each jar. *M. aeruginosa* at concentration of 100ml, 200 ml and 300 ml was added in 3 jars. One jar was kept at control without addition of *Microcystis* sp. The water was well aerated and was not changed up to seven days. After seven days, all the fishes were taken out and transferred to jars containing tap water. This was considered as 0 day. Afterwards the

fishes were given normal fish diet and kept in the laboratory for 30 days.

Biochemical Assay: Determination of cholesterol and bilirubin was done by enzymatic method in blood serum of the fishes. Enzymatic analysis was done by AUTOPAK cholesterol and bilirubin kit on Ames SEAC from miles India Ltd. Acid haematin method was followed to determination of haemoglobin levels.

Histopathological Study: For histopathological observations, the fishes were sacrificed and liver was removed. The liver tissues were dehydrated in alcohol series and embedded in paraffin wax ($58^{\circ}C$ to $60^{\circ}C$). $6\mu m$ thick sections were cut and stained in hematology. Lin-eosin and Mallory triple stain.

Statistical Analysis: Values were expressed as mean \pm S.D. The statistical analysis was performed using ANOVA followed by Dunnett's multiple comparison tests in order to compare more than two groups. All the data were processed with instat version 2.1 software.

RESULTS

Effect on Bilirubin Concentration: The concentration of bilirubin was increased in fishes that received *Microcystis* doses as compared to control fishes. A maximum increase was observed in fishes given 300 ml concentration of *Microcystis*. The levels were decreased and returned to normal levels after 30 days (Figure 1).

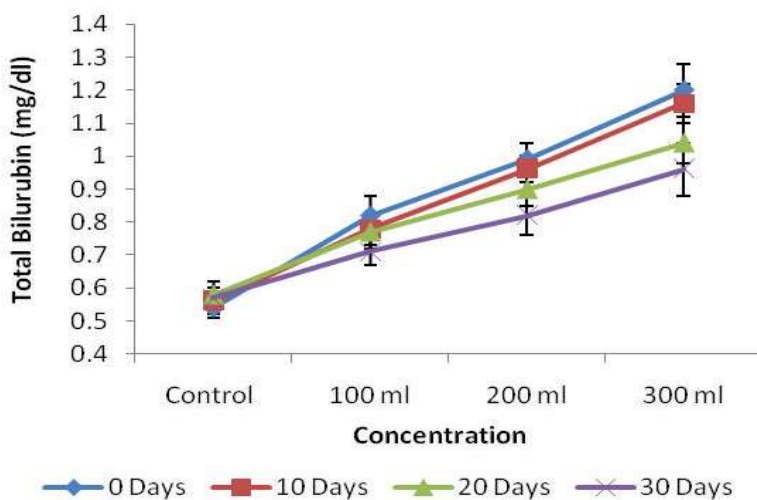


Fig. 1: Effect of bilirubin concentration in control and *Microcystis* fed fishes

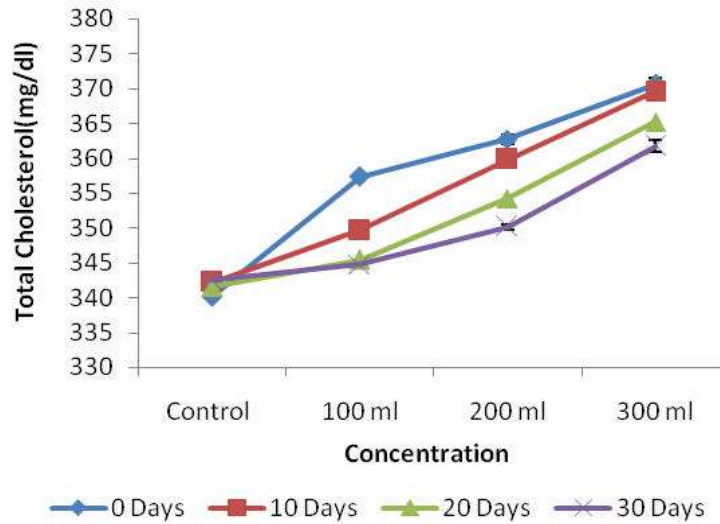


Fig. 2: Effect of cholesterol concentration in control and *Microcystis* feeded fishes

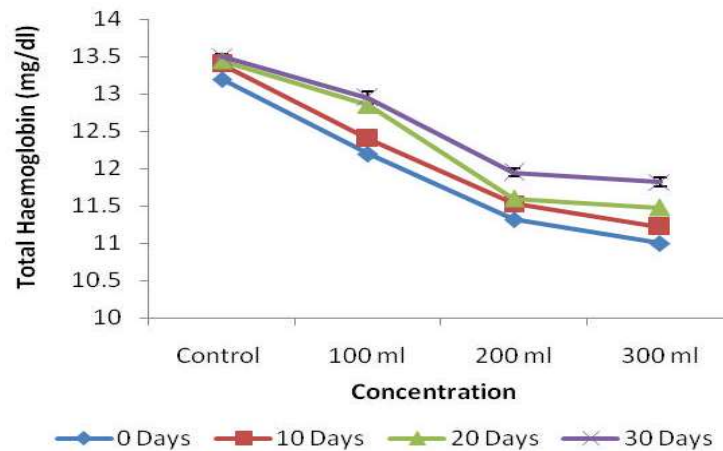


Fig. 3: Effect of haemoglobin concentration in control and *Microcystis* feeded fishes

Effect on Cholesterol Concentration: With increase in concentration of bloom, the blood cholesterol levels significantly increased and reached from 340.20 mg/dl in control fishes to 370.60 mg/dl of 300 ml dosed fishes on 0 day. A further decrease in cholesterol levels was obtained on 10th, 20th and 30th day of experimentation (Figure 2).

Effect on Haemoglobin Levels: The haemoglobin levels that were decreased initially due to *Microcystis* toxicity were recovered at the end of the experiment. The levels were improved after decreasing upto a maximum level of 11.00 mg/dl in fishes kept at 300 ml concentration (Figure 3).

Effect on Liver Tissues: From the histopathological studies, damages in the liver of fishes were evident the histopathological damages like necrosis, hypertrophy, dystrophy and degeneration were noticed in the hepatic cells. The severity of damage was observed maximum on 0 day in 300 ml concentration. Cytoplasmic degeneration, cytoplasmic clumping and cellular vacuolation were the main features of the affected hepatic tissues. Central blood vessels of the capsule are ruptured and with few blood corpuscles in extreme toxic condition in 300 ml concentration. However the ruptured hepatic cells were slightly recovered after 30 days. Fishes kept at 100 and 200 ml concentrations were almost fully recovered but 300 ml concentration was nearer to lethal dose that couldn't be recovered fully.

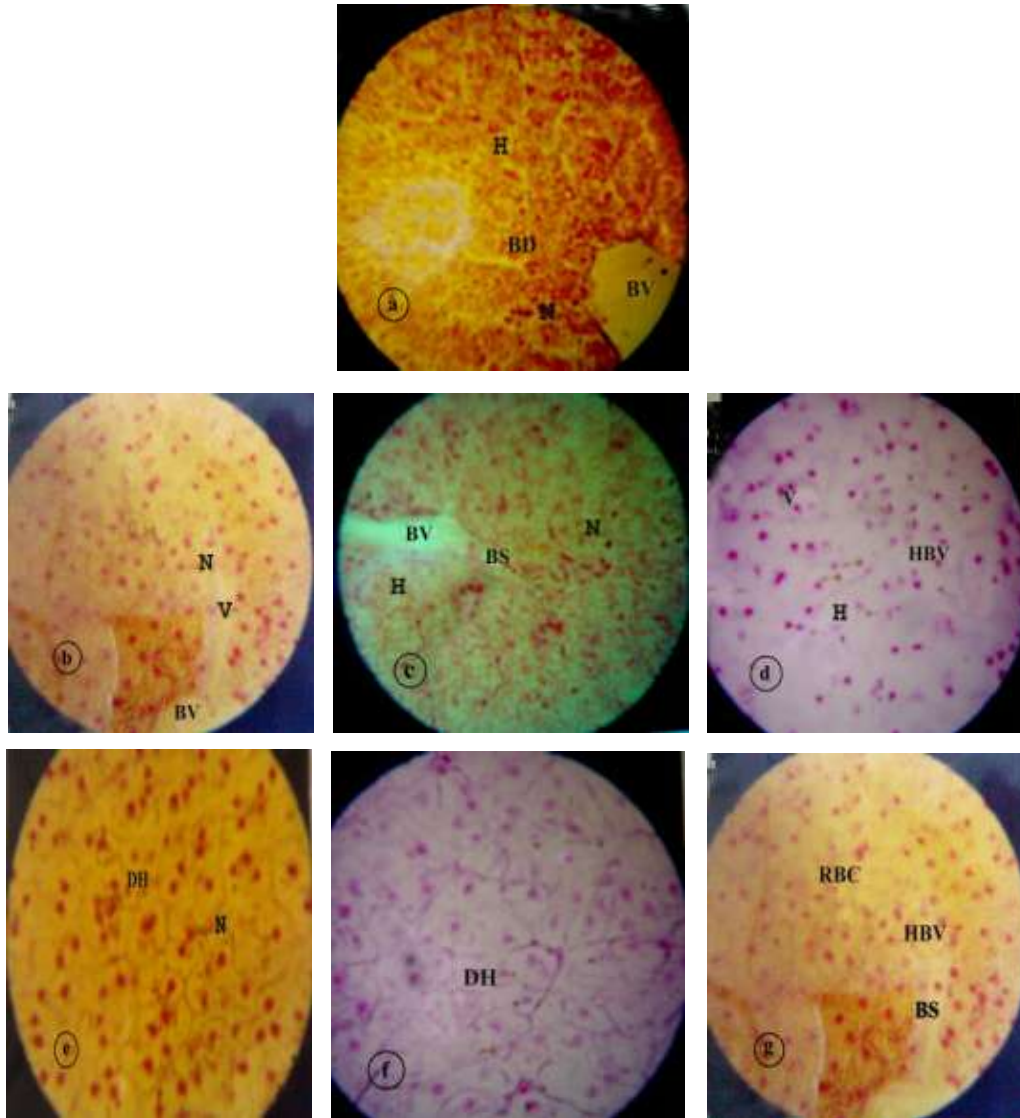


Fig. 4: Liver section of *Heteropneustes fossilis*. a, Control group showing normal nucleus and hepatocytes. H, Hepatocyte; BD, Bile duct; N, Nucleus; BV, Blood vessel; 10×45 (H&E stain). b, Experimental group (0 days in 100 ml concentration) showing vacuole in cytoplasm and small nucleus in hepatocyte. V, Vacuoles; 10×45 (H&E stain). c, Experimental group (0 days in 200 ml concentration) showing dissociated hepatocytes and BS, Blood seeping;. d, Experimental group (0 days in 300 ml concentration) showing HBV, Haemorrhagic blood vessel. e, Experimental (10 days in 100 ml concentration) showing DH, Dissociated hepatocytes; 10×45 (H&E stain). f Experimental group (20 days in 2000 ml concentration) showing showing DH, Dissociated hepatocytes and cytoplasm of hepatocyte; 10× 45 (H&E stain). g, Experimental group (30 days in 300 ml concentration) HBV, Haemorrhagic blood vessel and BS, Blood seeping; HBV, Haemorrhagic blood vessel; RBC, Red blood cell.

DISCUSSION

There have been several laboratory and field studies that document fish impairment associated with cyanobacterial blooms [12, 13] and some of them have

been reported as time or dose dependent on microcystin exposure [14, 15]. Microcystins have been reported to cause toxic effects when the toxins are applied by oral route [16, 17], by immersion [18] or intraperitoneally [19]. Our work showed for the first time in *H.fossilis* exposed by

the oral route to single doses of cyanobacterial cells, dose dependent and tissue specific changes as well as metabolic alterations.

There are considerable differences in the susceptibility to MC-LR among fish species [20]. Histopathological components in liver and blood biochemical components of fishes from different trophic levels to toxic cyanobacterial blooms also varied and might be responsible for their resistance to microcystins.

Studies focusing on acute oral effects in the first day and subsequent days after exposure are still very scarce, although [21] studied the oral toxicity of *Planktothrix rubescens* in European white fish, showing toxic effects from 24 hours after exposure.

The present study revealed that liver was highly affected after microcystins toxicity. Blood biochemical parameters of *H. fossilis* showed alteration after exposure to microcystins.

Bilirubin is the main bile pigment that is formed from the breakdown of heme in red blood cells. Serum bilirubin is considered a true test of liver function, as it reflects the liver ability to take up, process and secrete bilirubin into the bile. Elevated levels of bilirubin indicated the hepatic damage. [22] reported increase in bilirubin concentration due to microcystin intoxication.

The increased levels of cholesterol in the blood were also observed in the present study. Increase in cholesterol and triglycerides in the blood may be due to structural damage of the liver and kidney. The concentration was increased greatly at 0 day and subsequently decreased up to 30 days. There are also several studies on accumulation and depuration of microcystins content in several organs (liver and muscles) in a time dependent manner [23]. These findings also suggest that microcystins affect liver tissue greatly as they get accumulated in them and that liver can detoxify the accumulated microcystins after a certain time period. Findings of [24] also supported this view.

Variation in haemoglobin content in blood of normal and treated fishes have also been depicted from our study. Initial decrease and further increase in haemoglobin shows toxic effects of microcystins and synthesis of new haemoglobin molecules after 20 days of exposure. [25] reported significant changes in haemoglobin and RBC's after application of the biomass of blue green algae.

Histopathological examination of the liver of *H. fossilis* after exposure to *Microcystis sp.* Clearly shows that the parenchymal architecture of the liver is disturbed and hepatocytes show dissociation after 7 days

of exposure. The hepatocytes appear swollen and cytoplasm appear granular. [26] have reported the effect of *Microcystis* in rainbow trout *Oncorhynchus mykiss*. The typical chord structure of trout liver disappeared and cytoplasm of hepatocytes was condensed. *M. aeruginosa* can cause severe damage in liver and also to other organs like intestine, kidney, heart, spleen and gills [27, 28]. Route of exposure has been shown to affect the damage in fishes more significantly [29].

Besides physiological and toxicological harm, cyanobacterial toxins cause fish behavioral changes. Environmentally relevant concentrations of MC-LR (0.5-50 μ g/L) influenced the diurnal rhythm of zebra fish leading to impairment of food uptake and spawning success.

In conclusion, our study reveals that *Microcystis sp.* cause significant damage to the fishes that are feeded upon them. The engulfment of *Microcystis* cause hepatodegeneration and their increased concentration may even lead to fish mortality. Consumption of such toxicated fishes by humans and other animals may lead to several health complications. So the present study recommends a strict monitoring for microcystins presence in case of bloom formation in local water bodies to avoid risk of public health and aquatic resources.

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