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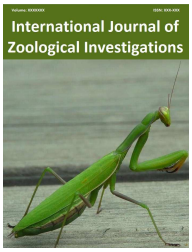

Biochemical Changes in Blood of Freshwater Catfish *Heteropneustes fossilis* Exposed to Microcystin-LR

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Abstract: Present study reveals the effects of microcystin-LR (2.5 µg/25g bw) toxins on certain biochemical parameters in blood of *H. fossilis.* In treated fish serum bilirubin, serum cholesterol, serum creatinine and serum urea levels are increased. Serum protein content of treated fish is decreased. Present study suggests that MCLR is toxic to fresh water catfish *Heteropneustes fossilis.*

Keywords: Blood, microcystin-LR, *Heteropneustes fossilis*

Introduction

The toxic cyanobacterial blooms in eutrophic lakes, reservoirs and recreational waters has been a worldwide problem (Paerl et al., 2001). Many cyanobacterial species most commonly associated with microcystin production is *Microcystis aeruginosa* (WHO, 1999). There are more than eighty microcystins possessing a ring structure of seven amino acids, which compose one unique phenyl deca- dienoic acid, four invariable D- amino acid, and two variable L-amino acids. MC-LR (MC Lysine and Arginine) is the most widely investigated cyanobacterial peptide toxin because it is frequently present in cyanobacterial blooms in river and lakes (Sivonen and Jones, 1999).

Various experimental studies have documented the impact of microcystin on fish given either by intraperitoneal (i.p.) injections (Rabergh et al., 1991; Kotak et al., 1996; Malbrouck et al., 2004), oral gavaging via the diet (Tencalla and Dietrich, 1997; Fischer and Dietrich, 2000; Li et al., 2004) or emersion is water containing purified microcystin MC or lysates or whole cells of cyanobacteria (Carbis et al., 1996). In acute toxic experiment, when fish were treated with high doses of MC, liver pathology was characterized by condensed cytoplasm, the appearance of massive pycnotic/apoptotic nuclei (Rabergh et al., 1991; Kotal et al., 1996; Fischer and Dietrich, 2000; Li et al., 2007). Rabergh et al. (1991) have reported degenerative changes in epithelial cells, tubules and glomeruli of kidney of common carp (*Cyprinus carpio* L) exposed to a sublethal dose of MC-LR (150µg/MC-LR kg^{-1}). In the present study we have

investigated the effects of microcystin LR on certain biochemical parameters of blood of a freshwater catfish *H. fossilis*.

Materials and Methods:

Animals:

Adult fresh water fish *Heteropneustes fossilis* (both sexes; body weight 23-37 g) were collected locally and acclimatized to laboratory conditions for 15 days in plastic pool. During experiment fish were kept in aquarium (8 fish/aquarium) with 30 L of fresh water (water temperature 23± 2^{o}C, dissolve oxygen between 6-8 mg/l, pH- 7.8 ± 0.2). Fish were not fed 24 h before and during the experiment.

Chemical:

In this study microcystin-LR (purchased from Enzo Life Science, USA Product No. ALX-350-012-C500; isolated from *Microcystis aeruginosa*) was used. Mcrocystin was dissolved in ethanol (1ml) and diluted with 0.6% NaCl (saline) to prepare the stock solution (100µg/50ml). 150 fish were taken for the experiment and divided into two groups, each containing 75 fish and employed as follows:

Group-A: This group served as control and intraperitoneally injected with saline (0.6% NaCl) at day 1, day 10 and day 20 of initiation of the experiment.

Group-B: The fish from this group were intraperitoneally injected with microcystin-LR (2.5 µg /25g) at day 1, day 10 and day 20 of initiation of the experiment,.

15 fish were sacrificed (under slight anesthesia with MS 222) from each group (A and B) on day 1, 5, 10, 15 and 30 after initiation of experiment. On day 1 i.e. the day of initiation of experiment, the fish were sacrificed 2 h after the injection. Blood samples were collected after sectioning of the caudal peduncle. The sera were separated by centrifugation at 3,500 rpm and analyzed for protein (Lowry et al., 1951), bilirubin (Erba kit, Transasia Bio–medicals Ltd), creatinine (Erba kit), urea (Erba kit) and cholesterol (Erba kit). All samples were analyzed in duplicate and mean value was taken for statistical significance.

Statistical analysis:

All data were presented as mean ± SE and studentes t-test was used for the determination of statistical significance. In all studies, the experimental group was compared with its specific time control group.

Results:

Serum protein:

There is no perceivable change in serum protein levels in group A (control) throughout the experiment. The serum protein levels in microcystin-LR injected *H. fossilis* (group B) remain unchanged up to day 5. The level indicates a progressive decrease from day 10 till the end of the experiment (day 30) (Fig. 1).

Serum cholesterol:

No significant change in serum cholesterol level of the fish of group A (control) is noticed throughout the experiment. In fish of group B the serum cholesterol level is unchanged up to day 5. The cholesterol level increases from day 10 to day 30 (Fig. 2).

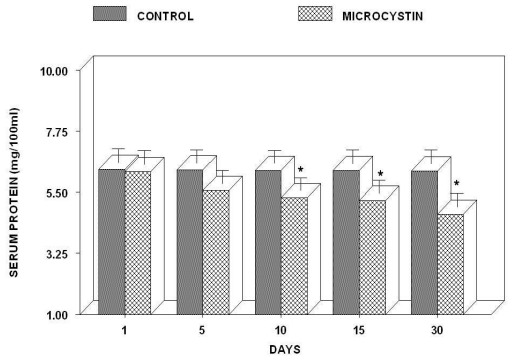

Fig. 1: Serum protein levels (mg/100 ml) of microcystin LR treated fish. Values are mean ± SE of six specimens. Asterisk indicates significant differences ($P < 0.05$) from control.

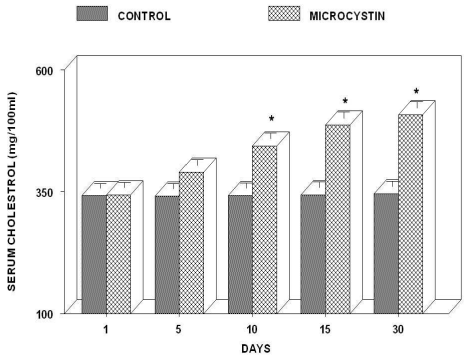

Fig. 2: Serum cholesterol levels (mg/100 ml) of microcystin LR treated fish. Values are mean ± SE of six specimens. Asterisk indicates significant differences (P < 0.05) from control.

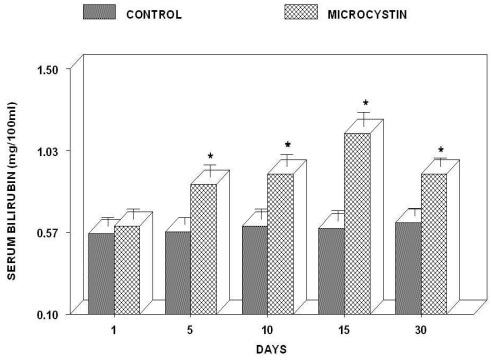

Fig. 3: Serum bilirubin levels (mg/100 ml) of microcystin LR treated fish. Values are mean ± SE of six specimens. Asterisk indicates significant differences (P < 0.05) from control.

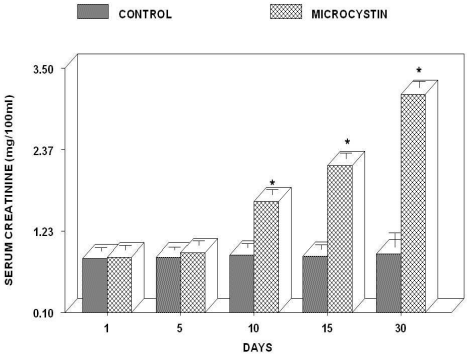

Fig.4: Serum creatinine levels (mg/100 ml) of microcystin LR treated fish. Values are mean ± SE of six specimens. Asterisk indicates significant differences (P < 0.05) from control.

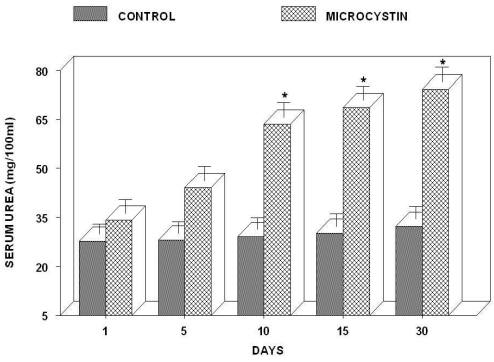

Fig. 5: Serum urea levels (mg/100 ml) of microcystin LR treated fish. Values are mean ± SE of six specimens. Asterisk indicates significant differences (P < 0.05) from control.

Serum bilirubin:

In group A the serum bilirubin levels remain unaffected throughout the experiment. In group B the serum bilirubin level increases from day 5 to day 15. Thereafter the level exhibits slight decrease on day 30 (Fig. 3).

Serum creatinine:

The serum creatinine level of fish of group A (control) is unchanged throughout the experiment. In group B the serum creatinine level remains unaffected up to day 5. The ceartinine level progressively increases from day 10 to day 30 (Fig. 4).

Serum urea:

The serum urea level in fish of group A (control) is unaffected throughout the experiment. In fish of group B the serum urea level remains unchanged up to day 5. Thereafter the level increases from day 10 to day 30 (Fig. 5).

Discussion:

In the present study the microcystin LR treatment induced a significant decrease in serum protein level. A decrease in blood protein content has also been reported in a veriety of stressed fish *Ictalurus punctatus* exposed to zinc and copper (Lewis and

Lewis, 1971); *Salmo gairdneri* exposed to DDT (Mehrle et al., 1971) and dialdrin (Grant and Mehrele, 1973); *Clarias batrachus* exposed to mercury (Bilgrami and Qayyum, 1978) and *H. fossilis* exposed to dimethoate and fenvalerate (Srivastava, 2000).

In the present study concentration of serum cholesterol of fish exposed to MC-LR significantly increased from day 10 to day 30. The increase in cholesterol in blood may be due to the structural damage of liver and kidney (our unpublished work). The present observation derives support from Gupta and Guha (2006) who have also reported increase in cholesterol content in blood of *H. fossilis* after MC-LR treatment and suggested that increase in cholesterol was due to liver and kidney damage.

In this study serum bilirubin content of fish injected with microcystin exhibited an increase from day 5 to day 30. Increase in bilirubin content was reported earlier by Young et al. (1994) and Gupta and Guha (2006), in fishes exposed to microcystin. The increase in bilirubin content may be attributed to damage caused by toxicants to liver.

In the present study serum creatinine and serum urea content exhibited an increase from day 10 to day 30. Rani (2015) noticed significant increase is urea and creatinine level in *Channa punctatus* after intoxication with Nuvan (an organophosphate compound). The higher level of urea might be possibly due to kidney impairment and possible enhancement of protein catabolism together with accelerated amino acid deamination for gluconeogenesis. This interpretation has been extended by Adamu and Siakpere (2008) for rise in urea level in *Channa punctatus* after Nuvan stress. Elevation in cratinine level may be due to glomerular insufficiency and renal injury causing tubular cell necrosis and increased muscle tissue catabolism by the direct action of Nuvan on fish kidney (Hasan et al., 2007).

Conclusion:

We conclude that microcystin- LR alters the blood biochemical parameter of the fish particularly serum urea, serum cholesterol, serum bilirubin, serum creatinine and serum protein which is important for several physiological processes. Any alteration in these contents may cause physiological disturbances which would seriously affect the normal vital functions, growth rate and their survival in nature.

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