

Microcystin-LR: Effects on Freshwater Catfish *Heteropneustes fossilis* Prolactin Cells

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Article Info

Article Type:

Research

Article History:

Received: 01.08.2019

Accepted: 13.10.2019

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ABSTRACT

Background:

Previous studies have been reported on the toxicity of Microcystin-LR, which is produced by cyanobacterial growth in fish, such as *Heteropneustes fossilis* (*H. fossilis*). However, no studies have been conducted on the effects of Microcystin-LR on the prolactin cells of *H. fossilis*.

Methods:

H. fossilis fish were intraperitoneally injected with Microcystin-LR (2.5µg/25g) and sacrificed after 1, 3, 5, 10 and 15 days. The blood samples were analyzed for the calcium levels. Histological slides of the pituitary prolactin cells were stained with Herlant's tetrachrome and Heidenhan's azan techniques, and examined under light microscopy.

Results:

The prolactin cells exposed to Microcystin-LR exhibited no structural changes on day 1. However, hyperactive prolactin cells exhibited cellular degranulation from day 3 to day 5. On days 10 and 15, degenerated and vacuolated prolactin cells were also observed. The nuclear volume of prolactin cells exposed to MC-LR increased progressively from day 3 to day 10 but on day 15 the nuclear volume returned to normal. The serum calcium level of MC-LR injected specimens showed hypocalcemia from day 3 to day 10. On day 15, the level became normal.

Conclusions:

Our results indicated that the serum calcium levels and prolactin cells were altered after exposure to microcystin-LR.

Keywords:

Blood Calcium Level; *Heteropneustes Fossilis*; Microcystin-LR; Prolactin Cells; Prolactinemia

How to cite this paper:

Prakash C, Srivastav SK. Microcystin-LR: Effects on Freshwater Catfish *Heteropneustes fossilis* Prolactin Cells. Iran J Toxicol. 2019;13(4): 17-20

INTRODUCTION

Many cyanobacteria species (a.k.a. blue-green algae) produce a group of toxins, known as microcystins. In freshwater, including drinking water reservoirs, there is increased occurrence and intensity of cyanobacterial growth (1, 2). The species most commonly associated with microcystins production is *Microcystis aeruginosa* (3). Microcystin-LR is named for the leucine (L) and argentine (R) amino acids, and is the first identified and the most commonly studied. Microcystins are actively absorbed by fish, birds and mammals primarily via drinking water. Microcystins affect the liver, depending on the amount of the toxins absorbed. Microcystins persist even after boiling, indicating that boiling is not sufficient to destroy these toxins (3). Cyanobacterial toxins are divided into three groups, based on their toxicological target. The first group consists of hepatotoxins including microcystins, nodularins and cylindrospermopsins. The second group is neurotoxins, including anatoxin-A, homoanatoxin-A and saxitoxins. The third group

involves irritants including lyngbyatoxin A, aplysiatoxin and lipopolysaccharides (4).

Injected intraperitoneally into carp at (50µg/kg), microcystins kill the fish but an oral dose of 250µg/kg in similar fish results in no lethality or may cause minimal liver damage (5). Microcystins accumulate in the liver and there is evidence that these toxins can pass to other organs, including muscle, kidney and brain in significant amounts (6-8). Histopathological changes in fish tissues, such as liver, kidney, gonads and gills after exposure to microcystins have been reported (9). The purpose of this study was to investigate the toxic effects of microcystin-LR on the prolactin cells in the pituitary gland of a freshwater catfish, known as *Heteropneustes fossilis* (*H. fossilis*). Such a study has not been conducted previously.

MATERIALS AND METHODS

Collection and Acclimatization of Test Animal:

Freshwater catfish *H. fossilis* (both sexes, average body weight 25-35g) were collected and acclimatized for

two weeks in a 250 liter plastic pool. Small mesh, dip net of soft material was used for gentle handling of fish throughout the study. Care was taken to minimize stress to the fish and dead fish were removed immediately.

Experimental Design: Microcystin-LR was dissolved in 1ml ethanol and diluted in 0.6% saline to prepare the stock solution (100µg/50 ml). One hundred *H. fossilis* fish were used in the experiment, divided into two equal groups and were utilized as follow:

Group A served as controls and were injected intraperitoneally with 0.6% saline (placebo).

Group B was injected intraperitoneally with Microcystin-LR (2.5µg/25 g) at the initiation of the experiments.

Biochemical Estimations: Fish from both groups were sacrificed under slight anesthesia, using MS222, after 1, 3, 5, 10 and 15 days into the study. Blood samples were collected after sectioning of the caudal peduncle, sera was separated by centrifugation at 3,500 rpm, analyzed for calcium (calcium kit, RFCL Limited, India) and inorganic phosphate levels (inorganic phosphorous reagent kit, RFCL Limited, India). The biochemical data were consistently expressed in mg/100 ml.

Ethical Approval: The Ethics Committee of Department of Zoology, DDU Gorakhpur University, India approved all aspects of the experimental protocols used in this study.

Histological Assessments: Pituitary glands and the brain were fixed in aqueous Bouin's fluid and Bouin's-Hollande fixatives. Tissues were routinely processed in graded series of alcohol, cleared in xylene and embedded in paraffin. Serial sections were made at 6µm. The pituitaries were stained with Herlant's tetrachrome and Heidenhan's azan techniques.

Nuclear Volume: Nuclear indices (maximum length and width) of the prolactin cells were measured, using an ocular micrometer to calculate the nuclear volume as: $\text{volume} = \frac{4}{3} \pi ab^2$, where 'a' was the major semi-axis and 'b' the minor semi-axis. The nuclear volume of prolactin cells with degenerating nuclei were not measured.

Statistical Analysis: All data were presented as the mean \pm SE of six specimens and Student's *t*-test was used for the determination of statistical significance. In all studies, the experimental group was compared with the controls for the specific time of each experiment.

RESULTS

There was no detectable change in serum calcium level in group A throughout the experiment. The serum calcium level in group B that had been injected with microcystin-LR, remained unchanged on day 1. From day 3 to day 5, the calcium levels decreased progressively, but tend to recover between day 10 and day 15, when the experiments ended (Fig. 1).

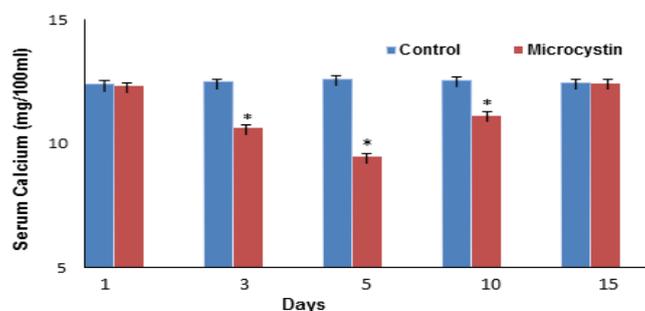


Figure 1. Serum calcium levels of microcystin treated *H. fossilis*. Values are mean \pm S.E. of six specimens. Asterisk indicates significant differences compared with the controls ($P < 0.05$).

The pituitary gland of *H. fossilis* is attached to the brain by a distinct stalk, i.e., leptobasic type. The gland is oval shaped and is divided into two major parts, neurohypophysis and adenohypophysis. The glandular part (adenohypophysis) is further divided into rostral pars distalis (RPD); proximal pars distalis (PPD) and pars intermedia (PI), which are dorsoventrally arranged one after the other. The rostral pars distalis consists mainly of prolactin cells. In our study, the prolactin cells had distinct nuclei with dense chromatin granules whereas the cell boundaries were indistinct. The cytoplasm of prolactin cells exhibited affinity to azocarmine and erythrosine (Fig. 2).

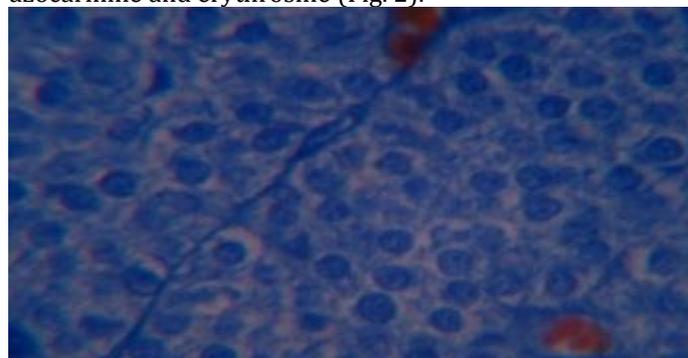


Figure 2. Prolactin cells of *H. fossilis*, showing distinct nuclei and indistinct cell boundary. (Magnification = X 800)

The histological structures of prolactin cells in the fish injected with placebo had not changed throughout the experiment. Also, the prolactin cells in fish treated with microcystin exhibit no structural changes on day 1. From day 3 to day 5, hyperactive prolactin cells were evident by cellular degranulation (Fig. 3). However, on day 10 through day 15, degenerated and vacuolated prolactin cells were observed (Fig. 4).

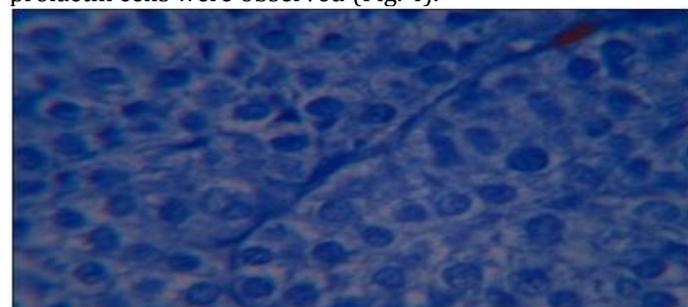


Figure 3. Prolactin cells of 5 days microcystin treated *H. fossilis* showing degranulation. (Magnification = X 800)

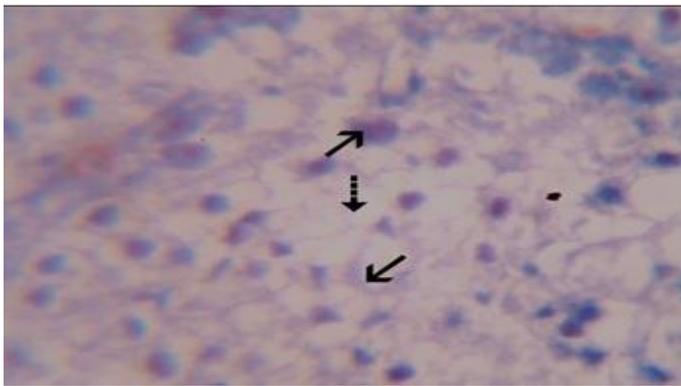


Figure 4. Prolactin cells on 15 days in microcystin treated *H. fossilis* showing degeneration (arrows) and vacuolization (broken arrows). (Magnification = X 800)

The nuclear volume of the placebo treated prolactin cells did not exhibit any structural changes throughout the experiment. However, the nuclear volume of prolactin cells treated with microcystin increased between day 3 to day 10, but the indices were similar to those of the control cells on day 15 (Fig. 5).

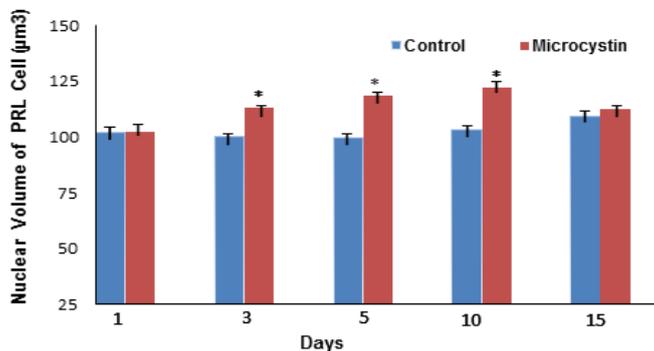


Figure 5. Nuclear volume of prolactin cells in microcystin treated *H. fossilis*.

Each value represents mean \pm S.E. of six specimens. Asterisks indicate significant differences ($P < 0.05$) compared with the controls.

DISCUSSION

The prolactin cells of *H. fossilis* fish in the experimental group consistently exhibited cellular degranulation and increased nuclear volume. There are only few studies published addressing the effects of toxins on the activity of prolactin cells (10-16). Our findings are in agreement with the reports of earlier investigators who also documented the hyperactivity of prolactin cells in fish after exposure to such toxins as cadmium (11), metacid (12), cypermethrin (13) and deltamethrin (14). In contrast, cadmium injection into rainbow trout fish failed to elicit any effect on prolactin cell activity (10). Observations made by previous studies (17-19) lend support to our findings, since they also reported an increase in the level of prolactin in teleosts after exposure to similar toxins. Further, bone demineralization has been reported in cadmium exposed carp (20, 21) and lead exposed fish, *Catlacatla* (22).

In various species of fish, prolactin cells have evoked hypercalcemia (23-27). The mechanism of action of prolactin cells is believed to be through altering the

permeability of gill epithelium (28-30). In the fish injected with microcystin-LR, the hyperactivity of prolactin cells could be attributed to the observed hypocalcemia and hypophosphatemia for maintaining the ionic balance in the blood through its action on the gills, kidney and bones.

CONCLUSIONS

Based on the results, it can be concluded that microcystin-LR affects the physiology of calcium homeostasis in *H. fossilis*. The toxin caused changes in the serum calcium level and altered the histological structures of prolactin cells in freshwater fish, *H. fossilis*. The physiological importance of calcium is well known. Prolactin elicits hypercalcemia in various species of fish by altering the permeability of gill's epithelial cells; hence any alteration in calcium and prolactin cells can cause significant physiological disturbances. We recommend routine inspections be conducted to monitor and eliminate cyanobacteria in the pools and water reservoirs used in fishery industry.

ACKNOWLEDGMENT

The authors are thankful to the University Grants Commission, Gorakhpur University, New Delhi, India, for providing financial assistance [Grant #: F.14-2(SC)/2010 (SA-III)] in support of this study.

CONFLICT OF INTERESTS

The authors declare no conflict of interests in the course of conducting this study.

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