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Histological alteration in the ultimobranchial gland of catfish *Heteropneustes fossilis* in response to microcystin– LR treatment

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Abstract
Freshwater catfish *Heteropneustes fossilis* (both sexes, average body weight 25-35g) were collected and acclimatized. The experimental fish were intraperitoneally injected with Microcystin-LR (2.5 µg/25 g) at the initiation of the experiment. Fish were sacrificed after 1, 3, 5, 10 and 15 days. The ultimobranchial gland (UBG) of control *H. fossilis* exhibits no change throughout the experiment. In microcystin injected fish (group B), there is no significant change has been observed in the nuclear volume of ultimobranchial cells after day 1. The nuclear volume of ultimobranchial cells exhibits a progressive decrease from day 3 to day 10. However, on day 15, the nuclear volume of ultimobranchial cells of microcystin injected fish almost becomes normal. The degeneration of ultimobranchial cells are discerned in microcystin injected fish (group B) on day 10 and day 15. It can be concluded from the present study that microcystin- LR is effective in inducing histological changes in the ultimobranchial gland of fish and causing physiological disturbances.

Keywords: Microcystin-LR, calcium and phosphate, ultimobranchial cells, *H. fossilis*

1. Introduction
Several species of cyanobacteria produce a group of toxins known as microcystins. These toxic cyanobacteria have been reported from marine, brackish water and freshwater habitat throughout the world [1]. Microcystin consists of a seven-membered peptide ring, which is made up of 5 non-protein amino acids and two protein amino acids. The two protein aminoacids (L-aminoacids) at position 2, 4, and methylation/demethylation on Me-Asp (Methyl-Aspartic acid) and Mdha (N-methyldehydroalanine), [2, 3] distinguish the various microcystins from one another, while the other amino acids are more or less constant between variant microcystins. Using amino acid single letter code nomenclature each microcystin is designated a name depending on the variable amino acids which complete their structure. About 70 structural analogues of microcystins have been identified [4, 5]. Microcystins are monocyclic hepatopeptide hepatotoxins (liver toxins) composed of seven amino acids with the molecular weight varying in range of 909-1115 [6]. This is the most frequently encountered group of cyanobacterial toxins observed in the environment [4, 7]. Microcystin can accumulate in the tissue of organism. After exposure to cyanobacterial blooms, microcystin have been found accumulated in the tissues of bivalves [8, 9, 10], snails [11, 12], shrimp [11, 12, 13] and frogs [12]. Calcitonin has been localized in ultimobranchial gland (UBG) of fish [14-23]. The structure and amino acid sequence of calcitonin has been determined in two teleosts – salmon and eel [16, 17, 20, 24, 25] and in an elasmobranch – ray [23]. There exists several reports on the impact of environmental toxicants on fish, e.g., behavioral responses [26, 27] histopathology of vital organs [28, 29, 30, 31] and hematological anomalies [32, 33, 34] but there exist no information regarding the impact of microcystin toxin on ultimobranchial gland of fish. Hence, it was aimed in the present investigation to study the toxic effect of microcystin-LR on histological changes in the ultimobranchial gland of a freshwater catfish *Heteropneustes fossilis*.

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 250L plastic

pool during the experiment. Small mesh dip net of soft material was used for gentle handling of fish for experiment. Care was taken to minimize stress to the fish. Dead fish were removed immediately.

Preparation of dose

The dose administered to *H. fossilis* in present study was selected on the basis of doses (either similar or near about) given earlier to fishes [35] and mice [36].

Experimental design

Microcystin was dissolved in ethanol (1 ml) and diluted with 0.6% saline to prepare the stock solution (100µg/50 ml). 100 fish were used in the experiment and divided into two groups each containing 50 fish and employed as follow:

Group A: Fish from this group served as control and injected intraperitoneally with 0.6% saline (vehicle).

Group B: The fish from this group were intraperitoneally injected with Microcystin-LR (2.5 µg/25 g) at the initiation of the experiment.

Biochemical estimations

Fish were sacrificed (under slight anesthesia with MS222) from group A and B after 1, 3, 5, 10 and 15 days. Blood was collected after sectioning of caudal peduncle and sera was separated by centrifugation at 3,500 rpm and analyzed for calcium (calcium kit, RFCL Limited, India) and inorganic phosphate levels (inorganic phosphorous reagent kit, RFCL Limited, India) and expressed as mg/100 ml. All samples were analyzed.

Preparation for histological slides

The area adjoining the heart along with the oesophagus were removed and fixed in aqueous Bouin's fluid.

Tissues were routinely processed in graded series of alcohols, cleared in xylene and embedded in paraffin. Serial sections were cut at 6 µm. Ultimobranchial glands were stained with hematoxylin-eosin (HE).

Nuclear volume

Nuclear indexes (maximum length and maximum width) of ultimobranchial cells, corpuscles cells and prolactin cells were taken with the aid of ocular micrometer and then the nuclear volume was calculated as- volume = $4/3\ \pi\ ab^2$

where 'a' is the major semiaxis and 'b' is the minor semiaxis. In the gland, when there are degenerating nuclei, only the indexes of intact nuclei were measured.

Statistical analysis

All data were presented as the mean ± 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 its specific time control group.

Results

There was no perceivable change in serum calcium level in group A throughout the experiment. The serum calcium level in microcystin-LR injected *H. fossilis* (group B) remained unchanged at day 1. The levels indicated a progressive decrease from day 3 to day 5 which tend to recover from day 10 till the end of the experiment (day 15) (Fig. 1). In group A, the serum phosphate levels remained unaffected throughout the experiment. In group B, the first perceivable change has been noticed in serum phosphate levels which show a decrease at day 3. This decrease progressed till day 5. Thereafter the levels showed a tendency to become normal from day 10 to day 15 (Fig. 2).

The ultimobranchial glands (UBG) of vehicle-injected (control) *Heteropneustes fossilis* are embedded in interseptum between pericardial and abdominal cavities. Some time, the ultimobranchial gland is also seen in oesophageal musculature (Fig. 3). The ultimobranchial gland is surrounded by thick connective tissue sheath and it is made up of follicles and cords. The cells are eosinophilic having indistinct boundaries. The UBG of control fish exhibits no change throughout the experiment (Fig. 4). In microcystin injected fish (group B), there is no change in the nuclear volume of ultimobranchial cells on day 1. From day 3 to day 10 the nuclear volume exhibits a progressive decrease (Fig. 5). However, on day 15 the nuclear volume of ultimobranchial cells becomes almost normal. On day 10 and day 15 degeneration of ultimobranchial cells are discerned (Fig. 6).

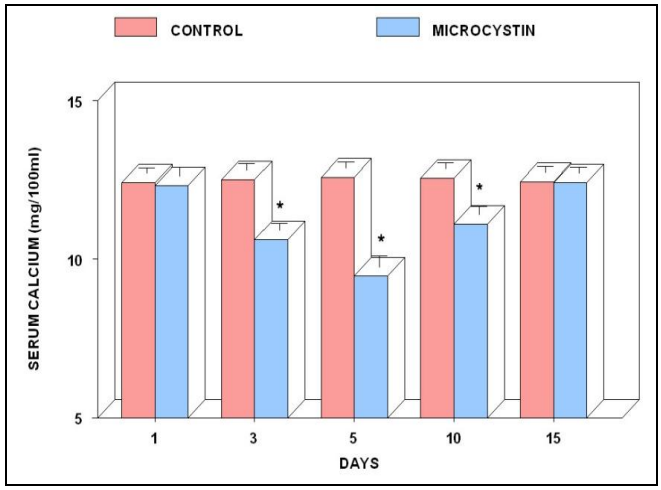

Fig 1: Serum calcium levels of microcystin treated *Heteropneustes fossils*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($P< 0.05$) from control.

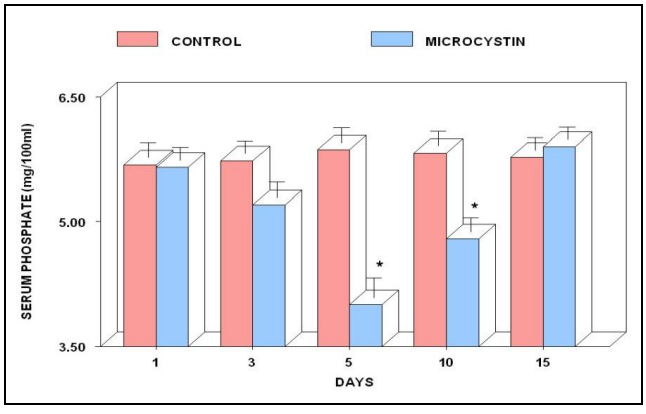

Fig 2: Serum phosphate levels of microcystin treated *Heteropneustes fossils*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($P< 0.05$) from control.

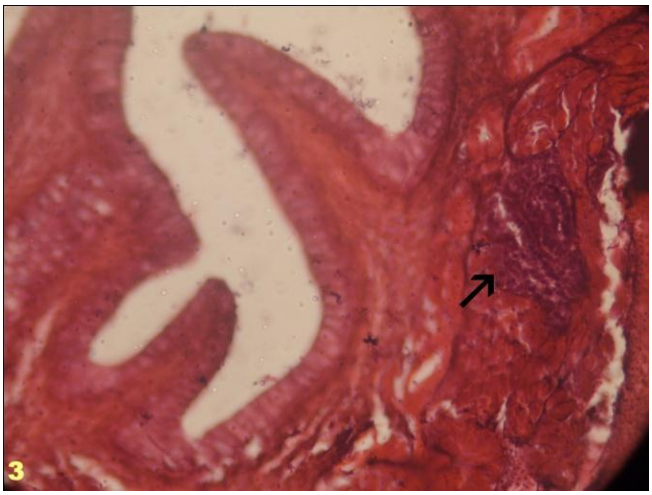

Fig 3: Ultimobranchial gland (arrow) of normal *Heteropneustes fossilis* embedded within the oesophageal musculature (broken arrow). HE X 50.

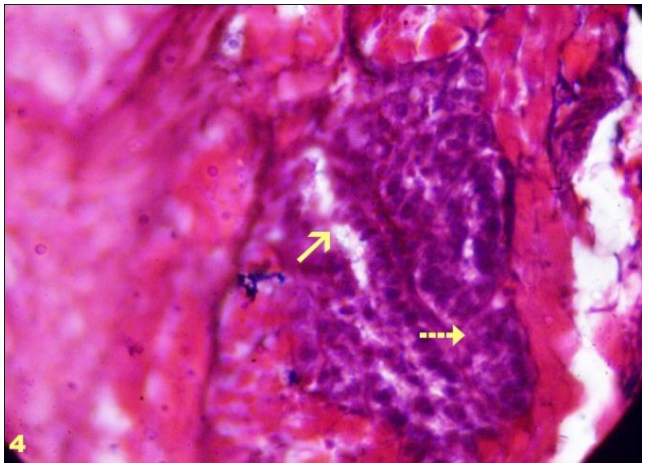

Fig 4: Ultimobranchial gland of normal *Heteropneustes fossilis* exhibiting follicles (arrow) and cell cords (broken arrow). HE X 200.

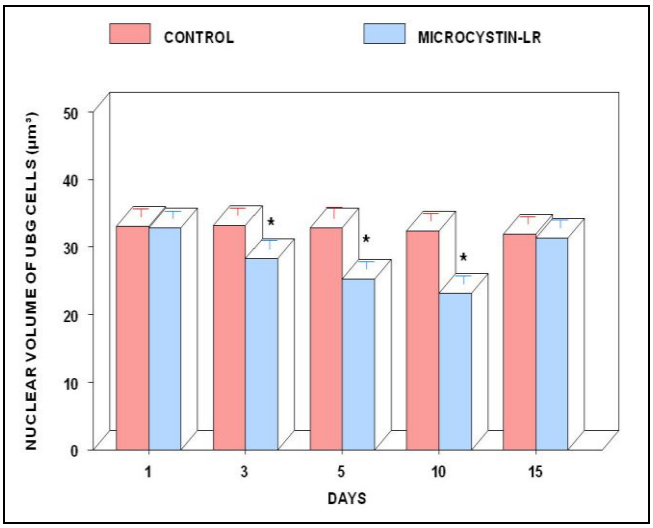

Fig 5: Nuclear volume of ultimobranchial cells of microcystin treated *Heteropneustes fossils.* Values are mean ± SE of six specimens. Asterisk indicates significant differences ($P < 0.05$) from control group**.**

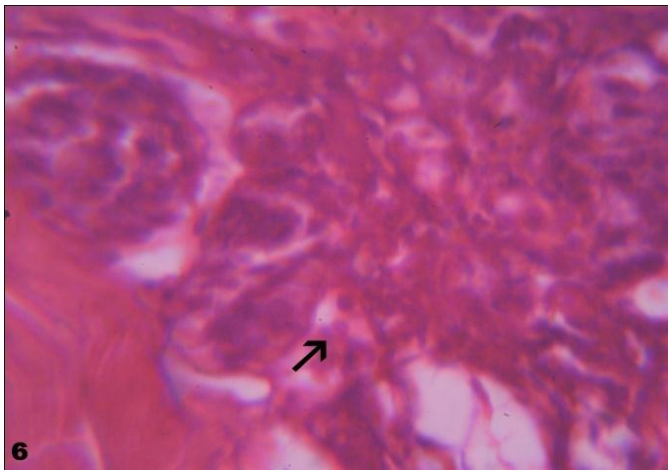

Fig 6: Ultimobranchial gland of microcystin treated *Heteropneustes fossils* exhibiting degeneration (arrows). HE X 500.

Discussion

In the present study ultimobranchial glands contain single cell type. The ultimobranchial gland of teleosts has been reported to contain a single cell type Srivastav *et al.* [37, 38]; Rai *et al.* [39]; Agrawal [40], two cell types Peignous-Deville *et al.* [41]; McMillan *et al.* [42]; Hooker *et al.* [43]; Swarup and Ahmad [44]; Zaccone [45] or even three cell types Takagi and Yamada [46]. Ultrastructural studies have recorded that UBG contain granular (secretory) and non-granular (supporting) cells (Hooker *et al*. Robertson) [43-22]. The granular cells (columnar in nature) are filled with numerous electron-opaque granules concentrated near the basement membrane and the lumen. These cells contain less electron dense ovoid nuclei, rough endoplasmic reticulum and Golgi profiles near the nuclei. Microtubules are found usually in the apex of those cells which are in contact with the lumen. Throughout the granular cells, mitochondria are distributed and microfilaments are occasionally seen (Hooker *et al.*) [43].

The UBG of fish *H. fossilis* injected with Microcystin-LR (MCLR) exhibited inactivity which is evident by decreased staining response and nuclear volume of UBG cells. More over the UBG of treated fish also showed vacuolization and degeneration. The inactivity of UBG could be explained on the basis of hypocalcemia caused by microcystin-LR treatment. There exists no report regarding the activity of UBG of fish after exposure to microcystins. The observed inactivity of UBG of MCLR treated fish derives support from studies of other investigators who have also noticed inactivity of UBG after exposure of fish to various toxicants--deltamethrin Srivastav *et al.* [37], (metacid Mishra *et al.*) [47], cypermethrin (Mishra *et al.*) [48], cadmium (Rai *et al.*) [39], *Nerium indicum* (Prasad, *et al.* [49]), *Euphorbia royleana* (Prasad, *et al.*) [50], *Azadirachta indica* (Kumar *et al.*,) [51] and mercuric chloride Agarwal [40]. The inactivity/hypoactivity of UBG has been also noticed in response to calcitonin induced hypocalcemia by other investigators in teleost *Anguilla Anguilla* (Peignoux-Deville *et al.*) [41], *Gasterostes aculeatus* (Wendelaar Bonga) [52], *Clarias batrachus* (Srivastav *et al.*) [53], *Amphipnous cuchia* (Tiwari) [54], *Heteropneustes fossilis* (Srivastav *et al.*) [38]. These studies lend support to the present finding regarding inactivity of UBG due to hypocalcemia after MCLR injections. The observed hypocalcemia resulted in to continuous disuse of UBG which provoked degeneration and vacuolization in gland.

Conclusion

It can be concluded from the present study that microcystin LR is effective in inducing histological changes in the ultimobranchial gland of fish and causing physiological disturbances.

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