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Protective effect of $ZnCl_2$ on toxicity produced by Microcystin-LR on Serum Calcium and Phosphate levels of freshwater catfish *Heteropneustes fossilis*

Chandra Prakash, ManiRam Prasad, Abhishek Kumar, Diwakar Mishra, Archana Chaudhary and Sunil K. Srivastav*

Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur 273009, India

*Corresponding Author E-mail: sunilsrivastav@hotmail.com

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ABSTRACT

The present study was aimed to investigate the protective effect of $ZnCl_2$ on toxicity produced by microcystin-LR on freshwater teleost fish Heteropneustes fossilis. Fish were divided into Group A, B, C and D. Group A served as control. Fish from group B were injected microcystin-LR (2.5 µg/25 g) intraperitoneally at the initiation of the experiment and after 5, 10, 20 and 30 days. In group C, fish were treated similar to group B and kept in freshwater containing 5 mg $ZnCl_2$/L. Fish from group D were kept in freshwater containing 5 mg $ZnCl_2$/L. fish were sacrificed and blood samples were collected on 5, 10, 20, and 30 days and analyzed for serum calcium and inorganic phosphate levels. In group B decrease in serum calcium levels was recorded on day 10, 20 and 30. No significant change in the serum calcium levels was noticed in group C and D. In group B, serum phosphate levels decrease on day10, 20 and 30. No significant change in the serum phosphate levels was noticed in group C and D.

Keywords: *Microcystin-LR, Serum Calcium, Serum Phosphate, Heteropneustes fossilis, $ZnCl_2$.*

INTRODUCTION

Cyanobacteria commonly known as (Blue - green algae) produce toxins that may present a hazard for drinking water safety and for other organisms, these toxins are structurally diverse and their effects range from liver damage, including liver cancer to neurotoxicity[8]. Cyanobacterium blooms have been detected in fresh water ponds and lakes all over the world. They are able to survive under a wide range of environmental conditions and few of them produce potent toxins. Cynobacteria are capable of producing two kinds of toxin, the cyclic peptide - hepatotoxin and the alkaloid-neurotoxin.Different species of freshwater cyanobacteria (namely Microcystis (order: Chroococcales), Anabaena (order: Nostocales) and Oscillatoria (order: Oscillatoriales)} produce toxins. The most common toxin produced by cyanobacteria (Microcystis). Microcystin-LR is a specific and potent hepatotoxin and is natural component of most water ecosystems.

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The development of microcystin toxins of water blooms decreases water quality from management of water, hygiene and fishery[27]. The microcystins have been reported to affect fish health, behaviour and growth.

Microcystin toxicity affect in endocrine and physiological aspect of fishes[42]. It is responsible for the loss of ion homeostatic produced by the inhibitory action of the toxins on the ion pump of gill chloride cells[13]. Microcystin toxin acts as a phosphatase inhibitor and causes liver disease in humans and animals[9, 6].

Environmental toxicants such as pesticides[36], pulp mill effluents[11] and metals[1] have been reported to provoke oxidative stress in aquatic organisms but naturally occurring toxicants such as microcystins (MCs) are also capable of doing so[41, 20]. Anthropogenic activities lead to the eutrophication of the water, which means that these algae can grow in massive quantities. This produces cyanobacterial bloom[21]. The intact cells as well as the toxins released after cellular lysis can be responsible for the toxic effects observed in both animals and humans[5, 26] and have also been associated with fish kills[43]. In response to the increase in health-related problems on a global scale, the World Health Organisation (WHO) has established safe guidelines for drinking water at 1.0µg MC-LR/L [9].

MATERIALS AND METHODS

Collection and handling of fish

Adult freshwater teleost *Heteropneustes fossilis* (both sexes body weight 25 – 35 g) were collected locally. Healthy fish showing no external signs of injury and disease were selected for experiments and were acclimatized to laboratory conditions (under natural photoperiod 11:35–12:40; temperature 28.46 ± 2.5 °C; pH 7.24 ± 0.8; hardness 132.34 ± 5.72 mg L^{-1} as CaCO3; dissolved oxygen 7.88 ± 0.34 mg L^{-1} and no free chlorine. During acclimatization the fish were fed daily with wheat flour pellets and ground dried shrimps, 2–3 times per day. The fish were not fed 24 h before and during the experimental period. The study was approved by the Animal Research Ethical Committee of DDU Gorakhpur University.

Procurement of extract and dose

In the present study microcystin- LR (purchased from Enzo Life Sciences, USA, Product No. ALX-350-012-C500; isolated from *Microcystis aeruginosa*) was used. Microcystin was dissolved in ethanol (100 µg/ ml) and diluted with 0.6% saline to prepare the stock solution (100 µg /50 ml). 200 fish were taken for the experiment and divided into four groups-A, B, C, D containing 50 fish each group and employed as follow:

Group A: Fish from this group served as control and were given intraperitoneal injection of 0.6% saline (vehicle) at the initiation of experiment and on 10 and 20 days.

Group B: Fish from this group were intraperitoneally injected with microcystin-LR (2.5µg/25g) at the initiation of the experiment and on 10 and 20 days.

Group C: Fish were treated same as group B and kept in freshwater containing 5 mg $ZnCl_2$/L.

Group D: Fish from this group were injected similarly as in group A and kept in freshwater containing 5 mg $ZnCl_2$/L.

Fish were sacrificed on 5, 10, 20 and 30 days after initiation of the experiment.

Biochemical Estimations

fish were sacrificed (under slight anesthesia with MS 222) from groups A, B, C and D after 5, 10, 20, and 30 days and blood was collected after sectioning of caudal peduncle and sera were separated by centrifugation at 3500 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 in duplicate.

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 the serum calcium level in group A fishes throughout the experiment. The serum calcium level of microcystin-LR injected *H. fossilis* (group B) show no change up to day 5. The level exhibited a decrease from day 10 to day 30 (close of experiment). In microcystin-LR injected fish kept in ZnCl2 (group C), the serum calcium level showed no perceivable change throughout the experiment. In group D fishes kept in ZnCl2 no change in serum calcium level was observed throughout the experiment (Fig. 1).

In Group A (control) fishes, the serum phosphate level remain unchanged throughout the experiment. In MCLR injected fish (group B) there was no change in phosphate level at day 5. However, the level decreased from day 10 to day 30 (close of the experiment). In microcystin-LR injected fish kept in ZnCl2 (group C), the serum phosphate level exhibited no significant change throughout the experiment. In group D fishes kept in ZnCl2 there was no change in phosphate level throughout the experiment (Fig. 2).

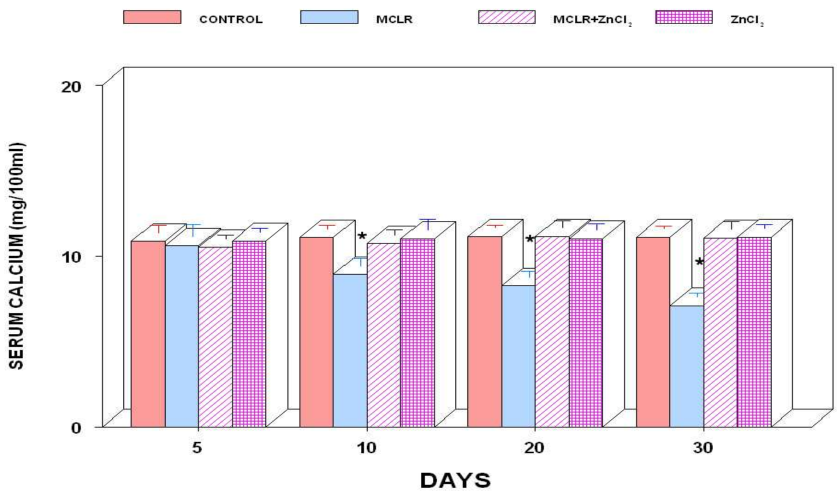

Fig. 1: Serum calcium levels of saline or microcystin treated *Heteropneustes fossils* kept either in freshwater or kept in water containing $ZnCl_2$. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($P< 0.05$) from control

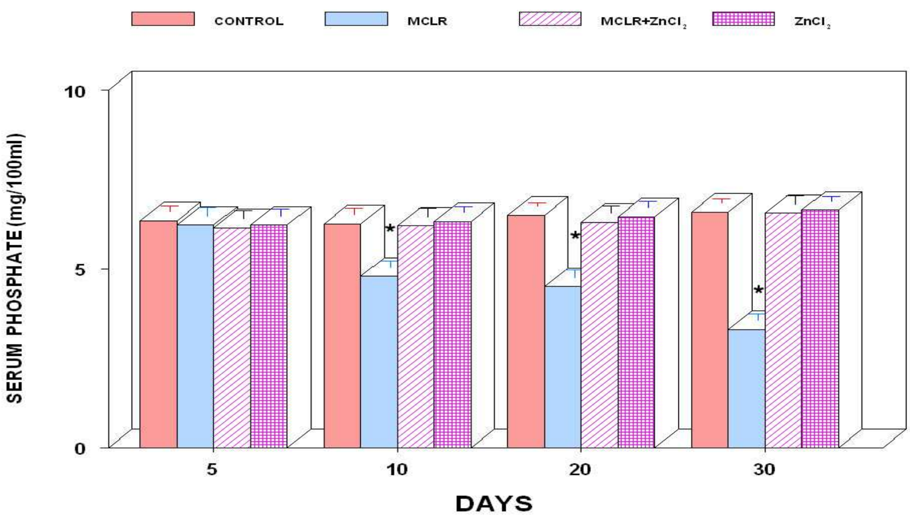

Fig. 2: Serum phosphate levels of saline or microcystin treated *Heteropneustes fossils* kept either in freshwater or kept in water containing $ZnCl_2$. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($P< 0.05$) from control

DISCUSSION

Heteropneustes fossilis subjected to microcystin-LR treatment exhibited hypocalcemia on day 10, 20 and 30. There was no change throughout the experiment in the serum calcium and phosphate levels in the fishes treated either with $ZnCl_2$ or $ZnCl_2$ +MCLR. This is in agreement with the studies of [15] as they have found a decrease in calcium level after 67 days in Silver carp under the influence of natural cyanobacteria population. However, they have noticed an increase in blood calcium level after 30 days of exposure to cyanobacteria population. Calcium level has also found a decrease after 7-28 days following exposure of Silver carp, *Hypopthalmichys molithrix* to cyanobacterial water bloom[15]. The present study is first to report hypocalcemia in *H. fossilis* as the fish were injected with microcystin-LR[15] have used natural cyanobaterial bloom. This study also derives support from the studies of earlier investigators who have also noticed hyocalcemia after exposure of fish to other pestisides--botanical pesticides[17,18,30,31], cypermethrin,[28,23,24] deltamethrin[40], aldrin[37], cadmium[32,35], propoxur and formothion[38].

Contrary to this study[3] have not found any change in plasma calcium levels in purified microcystin (MCLR 90.8µg/l) treated tilapia for 24 h. However, these authors have recorded hypocalcemia after treating tilapia with extract of *Microcystis aeruginosa* CYA43, (27mg/l) for 24 h. These authors have noticed an inhibited whole body Ca^{++} influx after treating tilapia to extracts of *Microcystis aeruginosa*, CYA43, but similar effect was not noticed after treatment with purified MCLR.

H. fossilis treated with cyanobacteria depicted decrease in serum phosphate levels on day 20 and 30. Increase the serum phosphate level has been recorded after 30 days in Silver carp, *Hypothalmichthis molithrix* after the treatment of cyanobacteria [15]. A decrease in serum phosphate levels after 7-28 days of exposure of the Silver carp, *Hypothalmichthis molithrix,* to cyanobacteria has also been reported[16]. Hypophophatemia has also been noticed by earlier investigators after exposure to-botanical pesticides – azadirachtin[17], *Euphorbia tirucalli*[18], *Nerium indicum*[30], *Euphorbia royleana*[31], synthetic pyrethriod – deltamethrin[39], cypermethrin[22] and heavy metal – cadmium[34].

The observed hypocalcemia and hypophosphatemia in the present study could be explained by the discturbances in the histological structures of kidney caused by microcystin. Degeneration of glomeruli and tubular epithelial cells have noticed in the kidney of MC-LR exposed carps[33]. Kidney lesions have also been recorded by other investigators. The renal change induced by microcystin is generally restricted to the proximal tubule in the posterior part of kidney[4, 12, 19]. Reported that microcystin caused degenerative changes in tubuli, glomeruli and interstitial cells of tilapia[2]. Kidney damage in fishes after microcystin exposure has been noticed [4, 12]. In *Oncorhynchus mykiss* coagular tubular necrosis and dilatation of Bowman's space have been noticed[19]. The observed hypocalcemia and hypophoshphatemia noticed in cyanobacteria exposed *H. fossilis* could be due to the possible damage in the kidney structure which resulted into reduced absorption of these electrolytes thus enhancing the efflux of these electrolytes in urine.

The gills in fish are responsible for maintaining the gaseous exchanges as well as ionic, osmotic and acid-base balance. Thus changes in gill structures may cause respiratory and electrolytic disturbances. Reported that reduction in respiratory surface caused by pollutants hampers the exchanges of gases and other gill functions[29]. Poleksic and Mitrovic [29] noticed hyperplasic, function of secondary lamellae, epithelial discalation, aneurism and dilation of sanguineous capillaries of the gills of fingerlings of *Brycon cephalus* after exposure to algal extract. Histopathological changes in the gills have noticed of microcystin exposed tilapia[25]. Gupta and Guha[14] noticed epithelial cells lifting, necrosis, lamellar fusion, hyperplasic, lamellar aneurism and hypertrophy in the gills of microcystin treated catfish. The hypocalcemia observed in the present study after exposure to microcystin of *H. fossilis* can be attributed to the changes noticed in the gills of microcystin treated fish which caused reduced surface of gills thus altering the absorption of the electrolyte through gills.

CONCLUSION

We conclude that microcystin-LR exposure to the fish *H. fossilis* alters the blood electrolytes- (calcium and phosphate) inducing hypocalcemia and hypophosphatemia of the fish, thus causing physiological disturbances which might affect seriously the normal vital functions, when $ZnCl_2$ was added in media containing fish the disturbed electrolyte (Calcium and Phosphate) showed recovery indicating Zn^{++} acted as a protective agent against MCLR toxicity.

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