Int J Pharm Bio Sci 2015 Jan; 6(1): (B) 1147 - 1153

Research Article **Toxicology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

MICROCYSTIN-LR ATTENUATES BLOOD CALCIUM AND PHOSPHATE LEVELS IN STINGING CATFISH *HETEROPNEUSTES FOSSILIS*

CHANDRA PRAKASH, ABHISHEK KUMAR, MANIRAM PRASAD, SUNIL K. SRIVASTAV AND AJAI K. SRIVASTAV*

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

ABSTRACT

The present study was aimed to investigate the effects of microcystin–LR (MCLR) on the blood electrolytes of *Heteropneustes fossilis*. The fish were injected microcystin- LR (2.5 µg/25 g) intraperitoneally at the initiation of the experiment. Blood samples were collected and the serum calcium and phosphate levels were estimated at 1, 3, 5, 10, 15 days. There is no change in serum calcium level of microcystin-LR injected *H. fossilis* at day 1. The levels indicate a progressive decrease from day 3 to day 5 which tend to recover from day 10 till the end of the experiment (day 15). The first perceivable change has been noticed in serum phosphate levels, which show a decrease at day 3. This decrease progresses till day 5. Thereafter, the levels show a tendency to become normal from day 10 to day 15. It is concluded from this study that microcystin–LR exposure to the fish *H. fossilis* alters the blood calcium and phosphate levels of the fish, thus causing physiological disturbances which might adversely affect the normal vital functions, reproduction, growth rate and their survival in nature.

KEYWORDS: **Microcystin- LR, Serum Calcium, Phosphate, *Heteropneustes fossilis***

AJAI K. SRIVASTAV
Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur 273009, India

***Corresponding author**

INTRODUCTION

Nowadays, cyanobacterial blooms have become a major environmental threat which posses a serious global public health problem. Cynotoxins are toxic metabolites produced by cyanobacteria[1, 2]. In freshwaters the toxins producing cyanobacteria are distributed widely which includes the planktonic nitrogen fixing genera of *anabena*, *Aphanizomenon*, *Nostoc*, *Cylindrospermopsis* and the non nitrogen fixing genera such as *Microcystis*, *Planktothrix* and *Oscillatoria*[3]. Microcystins are the most abundant group among cyanobacteria, which after being consumed either through water or food can cause health hazards to aquatic organisms, wildlife, livestock and humans[4-7]. Fish being the main inhabitants of aquatic environments are continuously exposed to microcystins[8]. Fish can consume cyanobacteria either through food or may absorb these toxins directly through water[7, 9-12]. Microcystins can affect mainly the liver, gill, kidney and other organs like intestine, heart and spleen[7, 13-15].To the best of our knowledge, there exists no report regarding the effects of microcystin-LR on calcium and phosphorous contents of blood in fish, hence the present study has been conducted to evaluate such an effect of microcystin-LR on the serum calcium and phosphorous levels of the fish *Heteropneustes fossilis*.

MATERIALS AND METHODS

2.1 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 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.

2.2 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 (1 ml) and diluted with 0.6% saline to prepare the stock solution (100 µg /50 ml). 90 fish were taken for the experiment and divided into two groups, each containing 30 fish and employed as follow:

Group A: This group served as control.

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

2.3 Biochemical Estimations

Six fish were sacrificed (under slight anesthesia with MS 222) from each group (A and B) after 1, 3, 5, 10, and 15 days. Blood samples were collected after sectioning of the caudal peduncle. The 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.

2.4 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. Two-way Analysis of Variance (ANOVA) was used for multiple group comparisons.

RESULTS

3.1 Serum calcium levels

There was no perceivable change in serum calcium levels 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) (Figure 1). ANOVA indicated that the levels of serum calcium were significantly different between groups (between intervals F= 12.40, p< 0.0001; between treatment F= 78.23, p< 0.0001).

3.2 Serum Phosphate levels

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 (Figure 2). ANOVA indicated that the levels of serum phosphate were significantly different between groups (between intervals F= 7.71, p<0.0001; between treatment F= 34.77, p< 0.0001).

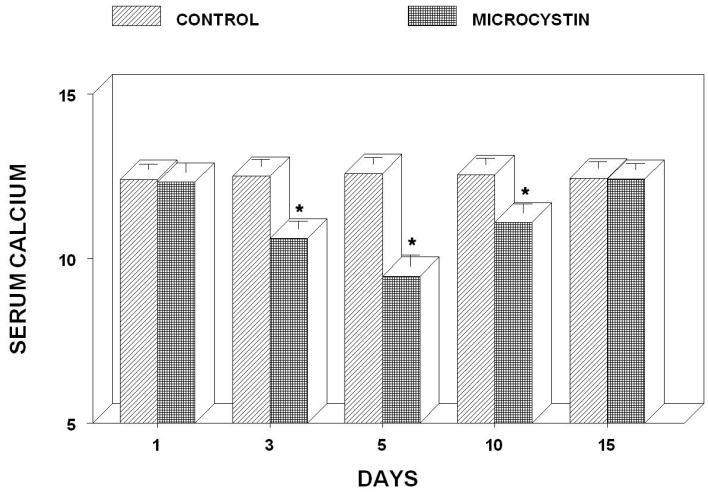

Figure 1
Serum calcium levels of microcystin treated Heteropneustes fossils. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

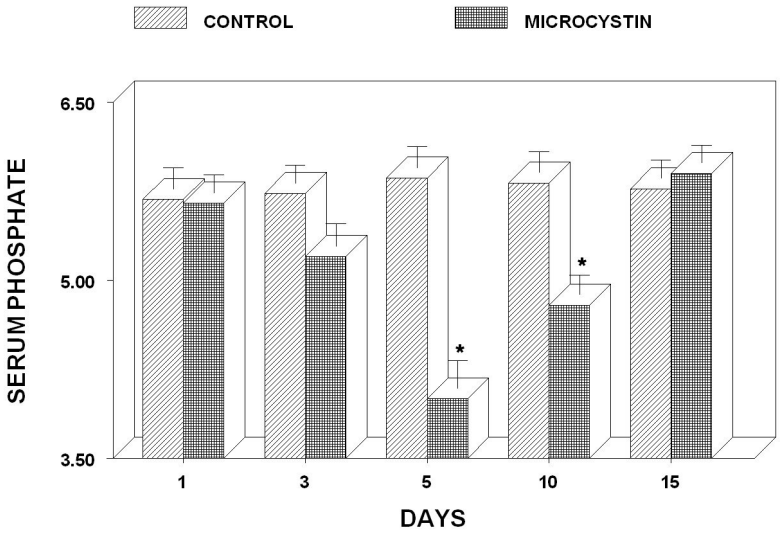

Figure 2
Serum phosphate levels of microcystin treated Heteropneustes fossilis. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

DISCUSSION

In the present study the microcystin-LR treatment induced hypocalcemia in the freshwater catfish, *H. fossilis*. To the best of our knowledge, there exist no studies regarding the effects of microcystin-LR on the blood calcium and phosphate content of the fish, hence the present study could not be compared with similar studies. The hypocalcemia recorded after exposure of the fish to microcystin-LR derived support from the studies of earlier investigators who have also noticed hypocalcemia in the fish treated with various environmental toxicants – cypermethrin[16-18], deltamethrin[19], cadmium[20, 21], botanical pesticides[22-25]. However, elevated calcium levels of fish have been noticed after exposure to various pesticides[26-28]. Contrary to above reports, no effect has been observed in blood calcium content in DDT treated flounders *Platichthys flesus*[29], methoxychlor treated northern puffer *Sphaeroides maculates*[30], cadmium exposed *Oncorhynchus niloticus*[31], and bifenthrin treated rainbow trout *Oncorhynchus mykiss*[32]. Microcystin-LR treated *Heteropneustes fossilis* exhibited hypophosphatemia. Earlier to this study, the effects of microcystin-LR have not been evaluated on the serum phosphate level of the fish. Hypophosphatemia has been noticed by earlier investigators after exposure to various environmental toxicants[22-25,33,34]. Contrary to this, hyperphosphatemia is noticed in fish after exposure to various toxicants[35-38]. The disturbed net electrolyte influx either at the gill and/or renal function could be attributed to hypocalcemia observed in microcystin exposed *H. fossilis*. Degenerative changes in gills have been reported after microcystin exposure to fishes[39], which may reduce surface for ionic permeability and caused a reduction in blood calcium levels. Several investigators observed degenerative changes in the kidney of fish exposed to microcystin[6,7], which could be attributed to the observed hypocalcemia and hypophosphatemia in microcystin exposed H. fossilis. Kidney degeneration might have resulted into increased efflux of these ions thus, causing hypocalcemia and hypophasphatemia in microcystin treated *H. fossilis*. Absorption of phosphate has been

reported to be exclusively via the gut[40]. Keeping this in view, the observed hypophosphatemia in *H. fossilis* could be attributed to redistribution of electrolytes between intracellular or extracellular compartments and/or impairment of renal function. In cadmium exposed fish, kidney damage has also been attributed as the cause of observed hypocalcemia [41-44].

CONCLUSION

We conclude that microcystin–LR exposure to the fish *H. fossilis* alters the blood electrolytes (hypocalcaemia and hypophosphatemia) of the fish, thus causing physiological disturbances which might affect seriously the normal vital functions, reproduction, growth rate and their survival in nature.

ACKNOWLEDGMENT

One of the authors (SKS) is thankful to University Grants Commission, New Delhi for providing financial assistance [Grant No. - 34-455/2008 (SR)] for this study.

REFERENCES

1. Ressom R, Soong FS, Fitzgerald J, Turczynowicz L, El Saadi O, Roder D, Maynard T, Folconer IR. Health effects of toxic cyanobacteria (blue green algae). National Health and Meedical Council, Australian Government Publishing Service, Canberra. (1994)
2. Blaha L, Babica P, MarŠălek B. Toxins produce in cyanobacterial water bloom-toxicity and risks. Interdicip. Toxicol. 2, 36-41. (2009)
3. Welker M, Brunke M, Preussel K, Lippert I, von D″ohern H. Diversity and distribution of *Microcystis* (Cyanobacteria) oligopeptide chemotypes from natural communities studied by single colony mass spectrometry. Microbiol. 150, 1785-1796. (2004).
4. Duy TN, Lam PK, Shaw GR, Connell DW, Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. Rev. Environ. Contam. Toxicol. 163, 113–186. (2000).
5. Malbrouck C, Kestemont P. Effects of microcystins on fish. Environ. Toxicol. Chem. 25, 72–86. (2006).
6. Atencio L, Moreno I, Prieto AI, Moyano R, Molina AM, Camean AM. Acute effects of microcystins MC-LR and MC-RR on acid and alkaline phosphatase activities and pathological changes in intraperitoneally exposed Tilapia fish (*Oreochromis* sp.). Toxicol. Pathol. 36, 449-458. (2008)
7. Mitsoura A, Kagalou I, Papaioannou N, Berillis P, Mente E, Papadimitriou T. The presence of microcystins in fish *Cyprinus carpio* tissues: a histopathological study. Internat. Aquat. Res. DOI: 10.1186/2008-6970-5-8. (2013).
8. Ernst B. Investigations of the impact of toxic cyanobacteria on fish Dissertation, University of Kostanz. (2008).
9. Tencala F, Dietrich D, Schlatter C. Toxicity of Microcystin *aeruginosa* peptide toxin to yearling rainbow trout (O*ncorhynchus mykiss*). Aquat. Toxicol. 30, 215-224. (1994).
10. Fisher W, Dietrich D. Pathological and biochemical characterization of 4 MC-inducd hepatopancreas and kidney damage in carp (*Cyprinus carpio*). Toxicol. Appl. Pharmacol. 164, 73-81. (2000).
11. Phillips MJ, Roberts RJ, Stewart JA, Codd GA. The Toxicity of the cyanobacterium *Microcystin aeruginosa* to rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 8, 339-344. (1985).
12. Smith JL, Boyer GL, Zimba PV, A review of Cyanobacterial odours and bipoactive metabolites: impacts and management alternatives in aquaculter. Aquacult. 280, 5-20. (2008).

13. Cabis CR, Mitchell GF, Anderson JW, McCauley I. The effects of microcystins on the serum biochemistry of carp, *Cyprinus carpio* L, when the toxins are administered by gavage, immersion and intraperitoneal routes. J. Fish Dis. 19, 151–159. (1996).
14. Rabergh CM, Bylund G, Eriksson JE. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) *Microcystis aeruginosa*, on common carp (*Cyprinus carpio* L.). Aquat, Toxicol. 20, 131–146 (1991).
15. Molina R, Moreno I, Pichardo S, Jos A, Moyano R, Monterde JG, Camean A. Acid and alkaline phosphatase activities and pathological changes induced in Tilapia fish (*Oreochromis* sp.) exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. Toxicon. 46, 725–735 (2005).
16. Pandey R, Malviya A, Das VK, Toxicity of cypermethrin, effects on serum electrolytes (Ca+2, Mg+2and Pi) levels and recovery response in freshwater catfish *Heteropneustes fossilis* Bloch. J. Environ. Biol. 30, 437–440 (2009).
17. Mishra D, Rai R, Srivastav SK, Srivastav Ajai K. Histological alterations in the prolactin cells of a teleost *Heteropneustes fossilis* after exposure to cypermethrin. Environ. Toxicol. DOI: 10.1002/tox.20562. (2010a).
18. Mishra D, Tripathi S, Srivastav SK, Suzuki N, Srivastav Ajai K. Corpuscles of Stannius of a teleost, *Heteropneustes fossilis* following intoxication with a pyrethroid (cypermethrin). North-West. J. Zool. 6, 203–8. (2010b).
19. Srivastav, Ajai K, Srivastava SK, Mishra D, Srivastav SK. Deltamethrin-induced alterations in serum calcium and prolactin cells of a freshwater teleost, *Heteropneustes fossilis*. Toxicol. Environ. Chem. 92, 1857–64. (2010).
20. Pratap HB, Fu H, Lock RAC, Wendelaar Bonga SE. Effect of water borne and dietary cadmium on plasma ions of the teleost *Oreochromis mossambicus* in relation to water calcium level. Arch. Environ. Contam. Toxicol. 18, 568–75. (1989).
21. Rai R, Mishra D, Srivastav SK, Srivastav Ajai K. Ultimobranchial gland of a freshwater teleost, *Heteropneustes fossilis* in response to cadmium treatment. Environ. Toxicol. 24, 589–93. (2009).
22. Kumar A, Prasad M, Mishra D, Srivastav SK, Srivastav Ajai K. Botanical pesticide, azadirachtin attenuates blood electrolytes of a freshwater fish *Heteropneustes fossilis*. Pest. Biochem. Physiol. 99, 170- 173. (2011a).
23. Kumar A, Prasad M, Mishra D, Srivastav SK, Srivastav Ajai K. Effects of *Euphorbia tirucalli* latex on blood electrolytes (calcium and phosphate) of a freshwater air-breathing catfish *Heteropneustes fossilis* Toxicol. Environ. Chem. 93, 585-592. (2011b).
24. Prasad M, Kumar A, Mishra D, Srivastav SK, Srivastav Ajai K. Alterations in blood electrolytes of a freshwater catfish *Heteropneustes fossilis* in response to treatment with a botanical pesticide, *Nerium indicum* leaf extract. Fish Physiol. Biochem. 37, 505-510. (2011a).
25. Prasad M, Kumar A, Mishra D, Srivastav SK, Srivastav Ajai K. Blood electrolytes of a freshwater catfish *Heteropneustes fossilis* in response to treatment with a botanical pesticide, *Euphorbia royleana* latex. Integ. Zool. 6, 150-156. (2011b).
26. Dalela RC, Rani S, Kumar V, Verma SR. In vivo hematological alteration in a freshwater teleost *Mystus vittatus* following subacute exposure to pesticides and their combination. J. Environ. Biol. 2, 79–86. (1981).
27. Sharma ML, Agarwal VP, Awasthi AK, Tyagi SK. Hematological and biochemical characteristics of *Heteropneustes fossilis* under the stress of congo red (diphenyl diszabine pthionic acid). Toxicol. Lett. 14, 237–40. (1982).
28. Suzuki N, Tabata MJ, Kambegawa A, Srivastav AK, Shimada A, Takeda H, Kobayashi M, Wada S, Katsumata T,

Hattori A. Tributylin inhibits osteoblastic activity and disrupts calcium metabolism through an increase in plasma calcium and calcitonin levels in teleosts. Life Sci. 78, 2533–41. (2006).
29. Haux C. Effects of DDT on blood plasma electrolytes in the flounder *Platichthys flesus* L. in hypotonic brackish water. Ambio 8, 171–3 (1979).
30. Eisler R. US Bur. sport fish. In Pesticides and ecology, ed. R.P. Edwards, 213–51. Wildlife Technology Paper No. 17, 15 (1967).
31. Oner M, Atli G, Canli M. Changes in serum biochemical parameters of freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, and Zn) exposures. Environ. Toxicol. Chem. 27, 360–366. **(**2008).
32. Velisek J, Svobodova Z, Piackova V. Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*). Vet. Med. 54, 131–7. (2009).
33. Mishra D, Srivastav S, Srivastav SK, Srivastav Ajai K. Toxicity and behavioural responses of a freshwater catfish, *Heteropneustes fossilis* to a synthetic pyrethroid (cypermethrin). J. Adv. Zool. 23, 39–42. (2002).
34. Rai R, Srivastav Ajai K. Effects of cadmium on the plasma electrolytes of a freshwater fish *Heteropneustes fossilis*. J. Ecophysiol. Occupat. Health 3, 63–70. (2003).
35. Colvin HJ, Phillips AT. Inhibition of electron transport enzymes and cholinesterase by endrin. Bull. Environ. Contam. Toxicol. 3, 106–15 (1968).
36. Larsson A, Bengtsson BE, Svanberg O. Some hematological and biochemical effects of cadmium on fish. In Effects of pollutants on aqautic organisms, ed. A.M.P. Lockwood, 35–45. London: Cambridge University Press. (1976).
37. Gill TS, Pande J, Tewari H. Effects of endosulfan on the blood and organ chemistry of freshwater fish *Barbus conchonius* (Ham.). Ecotoxicol. Environ. Safe. 21, 80–91 (1991).
38. Singh NN, Das VK, Singh S. Effect of aldrin on carbohydrate, protein and ionic metabolism of a freshwater catfish, *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol. 57: 204-210 (1996).
39. Eler MN, Campagna AF, Minillo A, Ribeiro MAP, Espindola ELG. Water quality, toxicity and gill lesions caused by intraperitoneally administered water-bloom crude extract in *Brycon cephalus* (Gunter, 1896; Characidae) from fee-fishing ponds in Sao Paulo state, Brazil. Acta Limnol. Bras. 21, 89-100 (2009).
40. G. Flik, S.E. Wendelaar Bonga, Z. Kolar, N. Mayer-Gostan and J.C. Fenwick. Environmental effects of Ca2+ uptake in the chichlid teleost *Oreochromis mossambicus* . In : Fish Culture, Promociones Universitarias, Barcelona, (1985) p A 6.4.
41. Koyama J, Itazawa Y. Effects of oral administration of cadmium on fish. I. Analytical results of the blood and bones. Bull. Jap. Soc. Sci. Fish 43, 523–6 (1977).
42. Roch M, Maly EJ. Relationship of cadmium induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity. J. Fish Res. Board Can. 36, 1297–1303 (1979).
43. Haux C, Larsson A. Long term sublethal physiological effects on rainbow trout, *Salmo gairdneri*, during exposure to cadmium and after subsequent recovery. Aquat. Toxicol. 5, 129–142 (1984).
44. Akram M, Hafeez MA, Nabi G. Histopathological changes in the kidney of a freshwater cyprinid fish, *Barilius vagra*, following exposure to cadmium. Pak. J. Zool. 31, 77–80 (1999).