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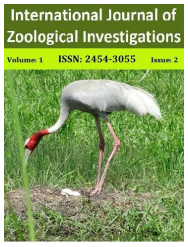

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Corpuscles of Stannius of Freshwater Catfish *Heteropneustes fossilis* after Exposure to Microcystin-LR

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Abstract: Freshwater catfish *H. fossilis* (both sexes, body weight 25-35g) were collected and acclimatized. Microcystin was dissolved in ethanol (1 ml) and diluted with 0.6% saline to prepare the stock solution (100µg/50 ml). 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. Blood was collected after sectioning of caudal peduncle and sera were separated by centrifugation at 3,500 rpm and analyzed for calcium and inorganic phosphate levels. Corpuscles of Stannius along with the adjoining portion of kidney were removed from the fish 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 and stained with aldehyde fuchsin (AF) and HE stains. There is no histological change in the AF-positive cells of CS following microcystin treatment up to day 3. AF-positive cells of corpuscles of Stannius have shown accumulation of secretory granules up to day 5. Degeneration in AF-positive cells has been noticed on day 15. The nuclear volume of AF-positive cells in microcystin injected specimens decreases from day 3 to day 10. However on day 15 it increases reaching close to the normal value. The nuclear volumes of AF-negative cells of CS remain unchanged in vehicle-injected fish throughout the experiment. A significant decrease in nuclear volume of AF-negative cells has been noticed in microcystin injected fish from day 10 to day 15.

Keywords: Corpuscles of Stannius, microcystin-LR, *Heteropneustes fossilis,*

Introduction

In environmental water cyanobacteria occur naturally but several factors promote the formation of massive blooms the enrichment of water by nutrients such as phosphate and nitrate (eutrophication), play a major role in the proliferation of cyanobacteria in an aquatic system (Chorus and Bartram, 1999).

During day time microcystin produce oxygen by photosynthesis. The water body becomes oxygen-depleted when water blooming occurs due to the high density of algae, because oxygen is consumed by the algae themselves at night and other microorganisms also utilize oxygen to degrade dead algae cells (Okino, 1973).

Microcystis aerusinosa is species of cyanobacteria (blue-green algae) that cause water bloom. Contamination by cyanobacterial blooms is a worldwide problem relevant to the freshwater and marine environment but also to the

practice of fish farming causing serious water pollution. Microcystin-LR these hepatotoxins of their ability to cause acute poisonings to those aquatic organisms, wildlife, domestic animals, and humans that drink algae in the water (Carmichael 1996). The cyclic heptapeptide microcystin, first isolated from the freshwater cyanobacterium *Microcystis aeruginosa*, is a potent hepatotoxin (Tillett *el al*., 2000). Microcystin into the extracellular environment has in the past been attributed to the death and lysis of cyanobacterial blooms (Sivonen and Jones, 1999). Microcystin responsible for, many exposure routes to causing death and illness in wildlife, birds, fishes, and livestock (Chorus, 2001 Carmichael, 2001; Mohamed et al., 2003; Chaudhary *et al*., 2015). Microccystin-LR effect to sublethal doses has been linked to the high incidence of certain types of liver cancer (hepatocellular carcinoma) in several communities in China (Yu, S. 1989).

Microcystins are a group of closely related toxic cyclic heptapeptides secreted by freshwater cyanobacteria (Jochimsen et al., 1998; Carmichael et al., 2001). Among over 80 microcystin variants found from *Microcystis*, *Anabaena*, *Oscillatoria* (*Planktothrix*), *Nostoc* and *Anabaenopsis*, micorocystin- LR (MW=995.2) containing leucine (L) and arginine (R) in position 2 the first identified microcystin-LR species known to be most toxic (Campbell et al., 1994; McElhiney and Lawon, 2005). The development of rapid and sensitive methods for the determination of MC-LR at levels reported that World Health Organization (WHO) [5], Microcystin–LR, Toxin-producing cyanobacteria that are widely distributed in freshwaters.

Many studies report that microcystins to induce pathological changes among salmonids and cyprinids. Moreover, differences in sensitivity have been shown in various fish species. Toxic effects are found not only in the liver but also in the kidney, gastrointestinal tract and gills (Tencalla et al., 1994; Fischer and Dietrich, 2000; Li et al., 2003). In the general, studies histopathological and ultrastructural changes in various fish species acute exposure to *M. aeruginosa* respects to those seen in mammal (Zurawell et al., 2005).

Materials and Methods:

Freshwater catfish *H. fossilis* (both sexes, 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.

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 were 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.

Preparation for histological slides

Corpuscles of Stannius along with the adjoining portion of kidney were removed

from the fish 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 and stained with aldehyde fuchsin (AF) and HE stains.

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.

Result

The serum calcium level in microcystin-LR injected *H. fossilis* 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).

There is no histological change in the AF-positive cells of CS following microcystin treatment up to day 3. AF-positive cells of corpuscles of Stannius have shown accumulation of secretory granules up to day 5 (Fig. 1). Degeneration in AF-positive cells has been noticed on day 15 (Fig. 2).

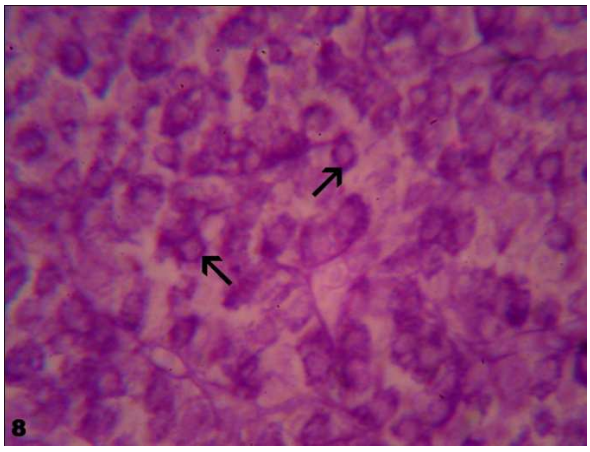

Fig. 1: Corpuscles of Stannius of 5 days microcystin treated *Heteropneustes fossilis* showing accumulation of secretary granules (arrows) in AF-positive cells. AF X 500.

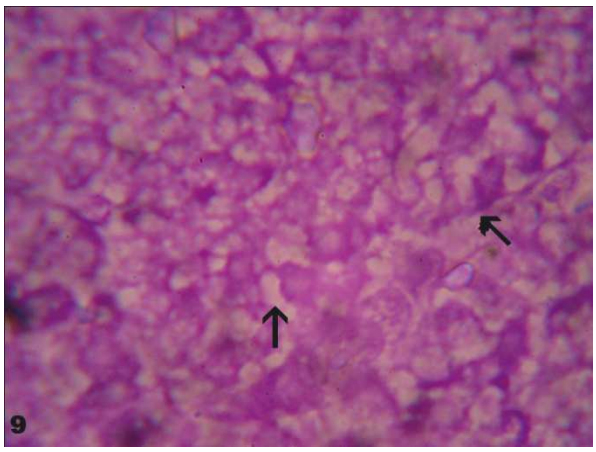

Fig. 2: Corpuscles of Stannius of 15 days microcystin treated *Heteropneustes fossilis* showing degeneration (arrows) in AF-positive cells. AF X 500.

The nuclear volume of AF-positive cells in microcystin injected specimens decreases from day 3 to day 10. However on day 15 it increases reaching close to the normal value (Fig. 3).

The nuclear volume of AF-negative cells of CS remain unchanged in vehicle-injected fish throughout the experiment. A significant decrease in nuclear volume of AF-negative cells has been noticed in microcystin injected fish from day 10 to day 15 (Fig. 4).

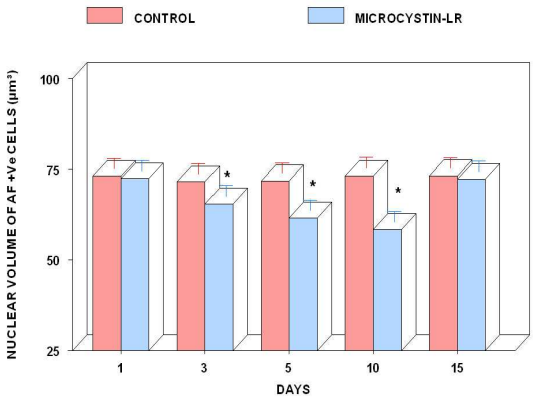

Fig. 3: Nuclear volume of AF-positive cells of microcystin treated *Heteropneustes fossilis.* Each value represents mean ± S.E. of six specimens. Asterisk indicates significant differences ($P<0.05$) from control.

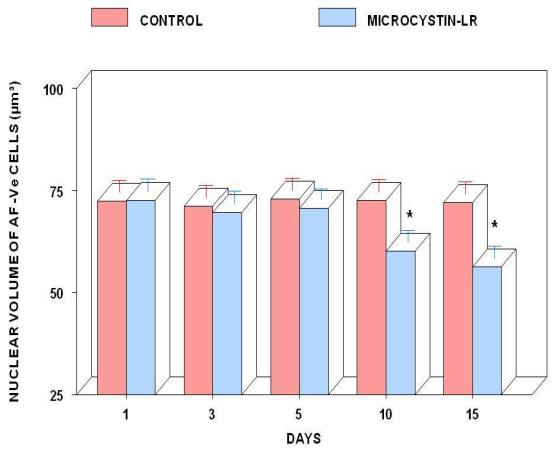

Fig. 4: Nuclear volume of AF-negative cells of microcystin treated *Heteropneustes fossilis.* Each value represents mean ± S.E. of six specimens. Asterisk indicates significant differences (P<0.05) from control.

Discussion

In the present study the AF-positive cells of CS of MCLR-injected fish exhibits increased accumulation of secretory granules and a decrease in nuclear volume. Accumulation of secretory granules and decrease in nuclear volume of AF-positive cells have been recorded earlier by few investigators in response to exposure of fish to toxicants (Srivastav *et al*., 2009b, 2010b; Mishra *et al*., 2009, 2010b; Agarwal, 2013; Prasad *et al*., 2014). The AF-positive cells of CS have been reported to regulate branchial calcium uptake in fish through secretion of hormone i.e. stanniocalcin (a hypocalcemic hormone) (Meats *et al*., 1978; Wendelaar Bonga, 1980; Wendelaar Bonga and Pong, 1986, 1991; Pong and Pang, 1986; Srivastav and Srivastav, 1988; Singh, 1990; Tiwari, 1993; Tseng *et al*., 2009; Agarwal, 2013; Prasad *et al*., 2014).

The increased granulation in the AF-positive cells of MCLR treated fish can be explained on account of inhibition of the hormonal release and continued biosynthesis of stanniocalcin. Present study derives support from the earlier reports describing accumulation of AF-positive granules in CS in response to experimentally induced hypocalcemia in teleosts kept in ambient acalcic freshwater (Tiwari, 1993; Singh and Srivastav, 1996). In mammals calcitonin cells (responsible for the secretion of a hypocalcemic factor-CT) have also been reported to accumulate secretory granules in response to experimentally induced hypocalcemia (Gittes *et al*., 1968; Leitz and Donath, 1970; Biddulph and Maibenco, 1972; Swarup *et al*., 1980).

References

Agarwal K (2013) Heavy metals: Impact on fish calcium regulation. Ph.D. Thesis, University of Gorakhpur, Gorakhpur, India.

Biddulph DM and Maibenco HC (1972) Response of hamster thyroid light cells to plasma calcium. Anat. Rec. 173: 25-43.

Campbell DL, Lawton LA, Beattie KA and Codd GA (1994) Comparative assessment of the specificity of the brine shrimp and microtox assays to hepatotoxic (microcystin-LR-containing) cyanobacteria Environ. Toxicol. Water Qual. 9: 71–77.

Carmichael WW (1996) Toxic Microcystis and the environment. In Toxic Microcystis (M. F. Watanabe, H. Harada,W. W. Carmichael, and H. Fujiki, eds.) pp. 1–11. CRC Press, New York, USA.

Carmichael WW, Azevedo SM, An JS, Molica RJ, Jochimsen EM, Lau S, Rinehart KL, Shaw GR and Eaglesham GK (2001) Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ. Health Perspect. 109: 663–668.

Carmichael WW (2001) Health effects of toxin producing cyanobacteria: ‘‘The cyanoHABs’’. Hum Ecol Risk Assess 7:1393–1407.

Chaudhary A, Prakash C and Srivastav SK (2015) Biochemical changes in blood of freshwater catfish *Heteropneustes fossilis* exposed to microcystin-LR. Int. J. Zool. Invest. 1: 72-76.

Chorus I and Bartram J (1999) Toxic Cyanobacterium in Water: A Guide to their Public Health Consequences, Monitoring and Management. London: E & FN Spon (published on behalf of the WHO)

Chorus I (2001) Introduction: Cyanotoxins - research for environmental safety and human health. In: Cyanotoxins - Occurrence, Causes, Consequences (ed. Chorus I.), pp. 1-4. Springer-Verlag, Berlin.

Fischer WJ and Dietrich DR (2000) Pathological and biochemical characterization of microcystin – induced hepatopancreas and kidney damage in carp (Cyprinus carpio). Toxicol Appl Pharmacol 164: 73-81.

Gittes RF, Toverud SV and Cooper CW (1968) Effects of hypercalcemia and hypocalcemia on the thyrocalcitonin content of rat thyroid glands. Endocrinol. 82: 83-90.

Jochimsen EM, Carmichael WW, An JS, Cardo DM, Cookson ST, Holmes CE, Antunes MB, Melo Filho D A, Lyra TM, Barreto VS, Azevedo SM and Jarvis WR (1998) Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. N. Engl. J. Med. 338:873-878.

Leitz H and Donath K (1970) Cytochemical evidence for the presence of hormonal peptides in thyroid C cells. In "Calcitonin 1969", (Taylor, S. ed.), pp. 227-239, Proc. 2nd Intern. Symp. London, William Heinemann Medical Books Ltd., London.

Li X, Liu Y, Song L and Liu J (2003) Responses of antioxidant systems in the hepatocytes of common carp (Caprinus carpio L.) to the toxicity of microcystin-LR. Toxicon 42: 85-89.

McElhiney J and Lawon LA (2005) Detection of the cyanobacterial hepatotoxins microcystins. Toxicol. Appl. Pharmacol. 203: 219–230.

Meats M, Ingleton PM, Chester Jones I, Garland HO and Kenyon CJ (1978) Fine Structure of the corpuscles of Stannius of the trout,*Salmo gairdneri*, structural changes in response to increased environmental salinity and calcium ions. Gen. Comp. Endocrinol. 36: 451-461.

Mishra D, Srivastav SK, Suzuki N and Srivastav AK (2009) Corpuscles of Stannius of a freshwater teleost, *Heteropneustes fossilis* in response to metacid-50 treatment. J. Applied Sci. Environ. Management. 13: 69-77.

Mishra D, Tripathi S, Srivastav SK, Suzuki N and Srivastav AK (2010) Corpuscles of Stannius of a teleost, *Heteropneustes fossilis* following intoxication with a pyrethroid (cypermethrin). North-West. J. Zool. 6: 203–208.

Mohamed ZA, Carmichael WW and Hussein AA (2003) Estimation of microcystins in the freshwater fish Oreochromis niloticus in an Egyptian fish farm containing a Microcystis bloom. Environ Toxicol 18:137–141.

Okino T (1973) Studies on the blooming of *Microcystis aerusinosa*. Jpn. J. Bot. 20: 381-402.

Pang PKT and Pang RK (1986) Hormones and calcium regulation in *Fundulus heteroclitus*. Am. Zool. 26: 225-234.

Prasad M, Kumar A, Srivastav SK, Suzuki N and Srivastav AK (2014) Cyto-architectural alterations in the corpuscles of Stannius of stinging catfish, *Heteropneustes fossilis* after exposure to a botanical pesticide *Nerium indicum*. Iranian J. Toxicol. 8: 1017-1024.

Singh S and Srivastav Ajai K (1996) Vitamin D_3 induced histological changes in the corpuscles of Stannius of a freshwater catfish, *Heteropneustes fossilis* kept either in artificial freshwater, calcium-rich freshwater or calcium-deficient freshwater. Okajimas Folia Anat. Japonica 73: 75-82.

Singh S (1990) Studies of endocrine glands regulating calcium and inorganic phosphorus homeostasis in *Heteropneustes fossilis*. Ph.D. Thesis, University of Gorakhpur, Gorakhpur, India.

Sivonen K and Jones G (1999) Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. E & FN Spoon, London, United Kingdom.

Srivastav AK and Srivastav SP (1988) Corpuscles of Stannius of *Clarias batrachus* in response to 1, 25 dihydroxyvitamin D_3 administration. Zool. Sci. 5: 197-200.

Srivastav AK, Srivastava SK, Mishra D, Srivastav SK and Suzuki N (2009) Effects of deltamethrin on serum calcium and corpuscles of Stannius of freshwater catfish, *Heteropneustes fossilis.* Toxicol. Environ. Chem. 91: 761-772.

Srivastav AK, Srivastava SK, Tripathi S, Mishra D and Srivastav SK (2010) Chlor pyriphos based commercial formulation: alterations in corpuscles of Stannius of catfish. Int.J. Environ. Health 4: 323-332.

Swarup K, Tewari NP and Srivastav AK (1980) Response of calcitonin cells, pararthyroid glands and bone to prolonged calcitonin administration in the Indian palm squirrel *Funambulus pennanti* (Wroughton). Acta Anat. 106: 180-191.

Tencalla FG, Dietrich DR and Schlatter C (1994) Toxicity of Microcystis aeruginosa peptide

toxin to yearling rainbow trout (Oncorhynchus mykiss). Aquat Toxicol 30: 215-224.

Tillett D, Dittmann E, Erhard M, von Do¨hren H, Bo¨rner T, and Neilan BA (2000) Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC 7806: an integrated peptide-polyketide synthetase system. Chem. Biol. 7:753–764.

Tiwari PR (1993) Endocrinal regulation of calcium in teleost. Ph.D. Thesis, University of Gorakhpur, Gorakhpur, India.

Tseng DY, Chou MY, Tseng YC, Hsiao CD, Huang CJ, Kaneko T and Hwang PP (2009) Effects of Stanniocalcin 1 on calcium uptake in zebrafish (*Denio rerio*) embryo. Am. J. Physiol.-Reg. Int. Comp. Physiol. 296: R549-R557.

Wendelaar Bonga S and Pang PKT (1986) Stannius corpuscles. In "Vertebrate Endocrinlogy: Fundamentals and Biomedical Implications", vol. 1, (Pang, P.K.T. and Schreibman, M.P., eds.), pp. 436-464, Academic Press, London.

Wendelaar Bonga SE and Pang PKT (1991) Control of calcium regulating hormones in the vertebrates: Parathyroid hormone, calcitonin, prolactin and stanniocalcin. Int. Rev. Cytol. 128: 139-213.

Wendelaar Bonga SE (1980) Effect of synthetic salmon calcitonin and low ambient calcium on plasma calcium, ultimobranchial cells, Stannius bodies and prolactin cells in the teleost *Gasterosteus aculeatus.* Gen. Comp. Endocrinol. 40: 99-108.

WHO (2004) Guidelines for Drinking Water Quality, 3rd ed., World Health Organization, Geneva, Switzerland

Yu S (1989) Drinking water and primary liver cancer., p. 30–37. *In* Z. Tang, and S. Xia (ed.), Primary liver cancer. China Academic Publishers, New York, N.Y.

Zurawell RW, Chen H, Burke JM and Prepas BR (2005) Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environment. J. Toxicol. Environ. Health B 8: 1–37.