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RESEARCH ARTICLE

TOXICOLOGICAL EVALUATION OF CYANOBACTERIUM *ANABEANOPSIS ABIJATAE* IN TILAPIA (*OREOCHROMIS MOSSAMBICUS*)

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ABSTRACT

Sub-chronic exposure of Tilapia (*Oreochromis mosambicus*) to *Anabeanopsis abijatae*, a toxic cyanobacterium was investigated with emphasis on the biochemical indices and histopathology (liver and gill). A single dose of two different concentrations (100µg/kg and 200µg/kg) of extracted toxins were intraperitoneally injected in Tilapia. Serum biochemical assays with commercial kits (Liquid Gold reagents) indicated that the levels of glucose, cholesterol, bilirubin, triglycerides and the activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were significantly increased as compared to the control levels. Histopathology of liver revealed congestion of parenchymatic cells with hepatic necrosis and vacuolation of cartilage with inflammatory cell infiltration was observed in gills.

INTRODUCTION

The eutrophication of lakes and reservoirs leads to water blooms of cyanobacteria in many countries throughout the world. This is of great concern to society, because blooms not only decrease water quality, but also increase the risk of toxicity to both animals and humans because they produce toxins (Li *et al*.,2005). Cyanobacterial blooms are often dominated by hepatotoxins, i.e. microcystins, nodularins, cylindrospermopsin and sometimes by neurotoxins, i.e, saxitoxin and anatoxin. There are more than eighty microcystin analogs, among which microcystin-LR is the most widely investigated cyanobacterial peptide toxin as it predominates cyanobacterial blooms in rivers and lakes (Sivonen and Jones, 1999). The main toxin producing cyanobacteria are *Microcystis, Aphanizomenon, Oscillatoria, Anabeana, Anabeanopsis, Synechocystis, Nostoc, Lyngbya, Scytonema, Nodularia, Gleotrichia* and *Cylindrospermopsis*. To date, atleast 46 cyanobacterial species are known to produce toxins, thus it is not surprising that approximately 75% of cyanobacteria samples taken from surface waters have been shown to contain toxins. *Anabeanopsis abijatae* is one of the important genera amongst the widespread toxin- producing cyanobacteria in temperate and tropical climates (Frazoa *et al*.,2010). Microcystin cause acute poisonings to aquatic organisms, wildlife, domestic animals and humans that drink or ingest the algae in the water (Carmichael, 1996).

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Moreover chronic exposure of humans to low microcystin concentrations may promote cancer. The International Agency for Research on Cancer (IARC) has classified Microcystin-LR as carcinogenic to humans (Group 2B). Aquatic animals such as zooplankton, fish, and molluscs consumed by humans have been reported to bioaccumulate microcystins (Magalhaes *et al*., 2003). Cyanobacteria are an important dietary component of many tropical cichlids (e.g. Tilapia) and cyprinids (e.g. silver carp) (Zurawell *et al*., 2005). Both Tilapia and Silver carp are important to humans because of their roles in aquatic ecosystems as direct consumers of phytoplankton, their importance as food fish and their potential for the biological management of cyanobacterial blooms (Chen *et al*., 2006). Intraperitoneal exposure of cyanobacterial toxins may cause significant changes in biochemical indices and plasma enzyme activities. Microcystin cause liver tissue damage in fish, demonstrated by a significant increase of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) (Navratil *et al*., 1998: Vajcova *et al*., 1998). Biochemical indices of blood and plasma in fish are affected by many endogenous and exogenous factors. The changes of biochemical indices in fish could be caused by chemical factors (Kopp and Hetesa, 2000; Luskova *et al*., 2002), age, health, body condition (Svetina *et al*., 2002) and nutrition (Serpunin *et al*., 1995) and stress (Dobsikova *et al*., 2006). The objective of present investigation was to assess the impact of sublethal exposure of microcystin from *A.abijatae* in terms of biochemical profile and histopathological changes.

MATERIALS AND METHODS

Cultivation and Identification of *Anabeanopsis abijatae*

A.abijatae, isolated from a water reservoir in Madurai was cultivated in BG-11 media at 25°C. A photoperiod of 12:12 (light:dark cycles) with light intensity of 1500-2000 lux was maintained throughout the growth period. Cyanobacterium was identified morphologically using phycological books (John *et al*., 2002 and Desikachary,1959) and by molecular approach based on PCR amplification and sequencing of the 16S rRNA gene.

Toxin extraction

Algal cells (30-40th days of growth) were ruptured by sonication and frozen overnight at 20°C after adding the solvent mixture of butanol, methanol and milli-Q water (5:20:75). It was then centrifuged at 5000 rpm for 10 mins. The supernatant was dried by evaporation and resuspended in 0.9 % saline for intraperitoneal injection.

Experimental setup and acclimation of fish

Oreochromis mossambicus (Tilapia), a common freshwater and brackish water cichlid fish with a mean weight of 25-30 g was used for the toxicity bioassay. They were obtained from a fish hatchery and transported to the laboratory where they were held in circular plastic tanks (6 fishes/ tank). The aquariums were set up with continuos aeration and water was changed on alternate days. Water temperature was not maintained in the fish tanks since earlier studies in this laboratory indicated only minor daily fluctuation in temperature i.e. 28±1.5°C. The fish were fed once a day with a balanced fish diet prepared in the laboratory.

Toxicity bioassay using Tilapia

Fishes were injected intraperitoneally with 0.1 ml of extracted toxin at a sub lethal dose of 100µg/kg and 200 µg/kg (Gupta and Guha, 2006) and control fishes received only the 0.9% saline solution. The fishes were bled at an interval of 3, 6, 24 and 72 hrs. Blood was taken out from the caudal area using a tuberculin syringe and centrifuged at 400g for 15 min at 4°C. The resulting serum was subjected to biochemical analyses using Liquid Gold reagents kits in Nexgen semiautomatic analyser. Biochemical indices such as glucose, cholesterol, triglycerides, total bilirubin, alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were determined. At the end of seven days (experimental period), the fishes were sacrificed and their liver and gill were excised for fixation in bouin's fluid. Histopathological changes in liver and gills were observed under light microscope.

RESULTS AND DISCUSSION

Along with worldwide eutrophication, cyanotoxins are of environmental concern and bioaccumulation in fish is associated with it. This study was focused on the effects of *A.abijatae* on biochemical and histopathological changes in Tilapia.

Fig 1 Glucose concentration in blood serum of control and treated fish, O.mossambicus

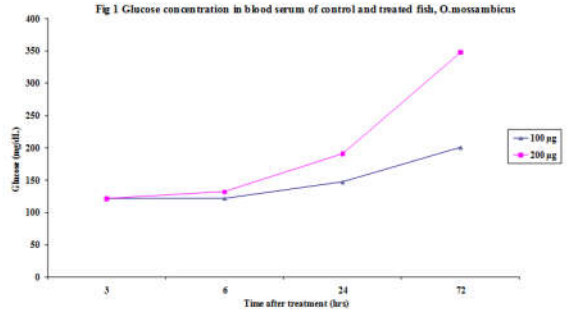


Fig 2 Cholestrol concentration in blood serum of control and treated fish, *O.mossambicus*

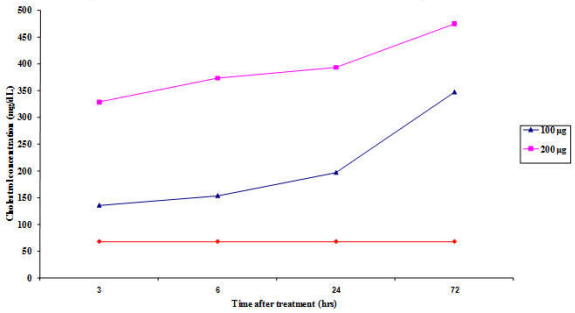


Fig 3 Triglyceride concentration in blood serum of control and treated fish, O.mossambicus

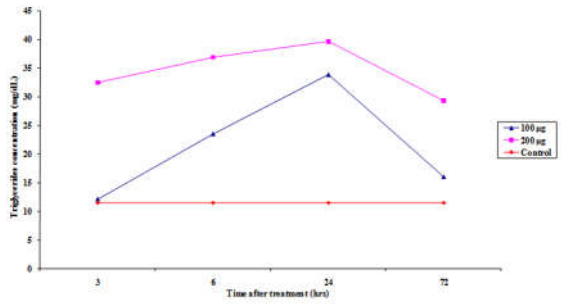


Fig 4 Total bilirubin concentration in blood serum of control and treated fish, *O.mossambicus*

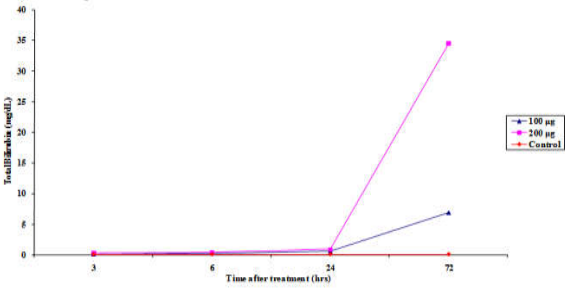
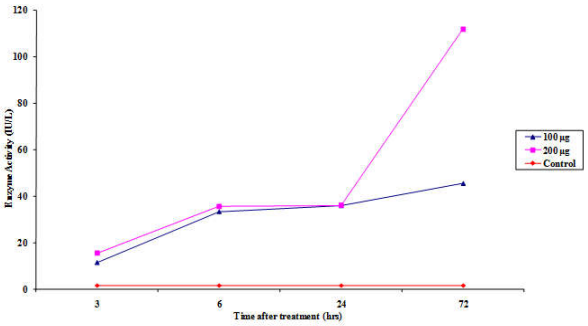
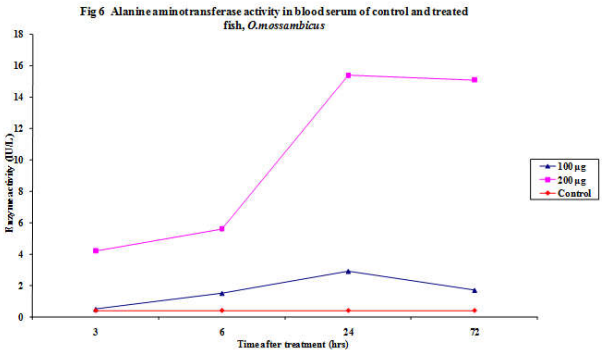
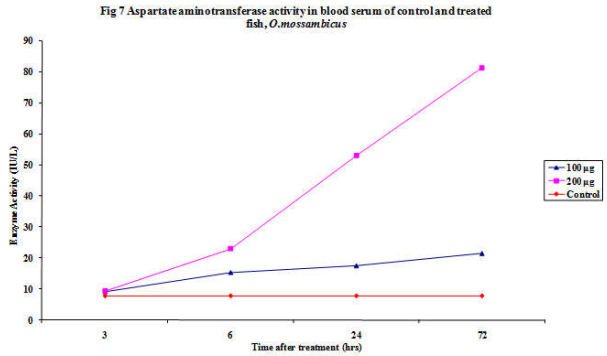


Fig 5 Alklaine phosphatase activity in blood serum of control and treated fish, *O.mossambicus*



The changes in biochemical parameters correspond well with the results reported by other authors (Rabergh *et al*., 1991 and Carbis *et al*., 1996). The effect of toxin injection on the concentration of glucose in blood serum is shown in Fig 1. In





triglycerides in the blood of *O.mossambicus* was 11.5 mg/dL in the control fish. It increased gradually but decreased after 72 h in both the doses. Maximum triglyceride concentration in blood was 33.9 mg/dL in 100 µg/kg and 39.7mg/dL in 200µg/kg in treated fish. After 72 h of treatment, the concentration was 16.0 mg/dL in 100 µg/kg and 29.3 mg/dL in 200 µg/kg in treated fish (Fig 3). An increase in cholesterol, and triglycerides in blood was observed which may be due to structural damage of liver. Similar increase in cholestrol and triglyceride level was observed in Bloch injected with microcystin-LR (Gupta and Guha, 2006).

Bilirubin is the main pigment that is formed from the breakdown of heme in red blood cells. Liver damage indicated by elevated serum aspartate aminotransferase and alanine aminotransferase activity reduces hepatic function, which may be associated with an increase in serum bile acid and bilirubin concentration (Best *et al*., 2001). The effect of toxin injection on the concentration of bilirubin in blood is shown in the Figure 4. In both the doses (100 µg/kg & 200 µg/kg), the concentration increased upto 72 h. In control, the concentration of bilirubin was 0.1 mg/dL, which increased in treated fish in 24 h and reached upto 6.9 mg/dl in 100 µg/kg and 34.6 mg/dl in 200 µg/kg. The results are in confirmation with Carbis *et al*., (1996) who showed an increase in bilirubin concentration in Cyprinus carp after due to microcystin intoxication.

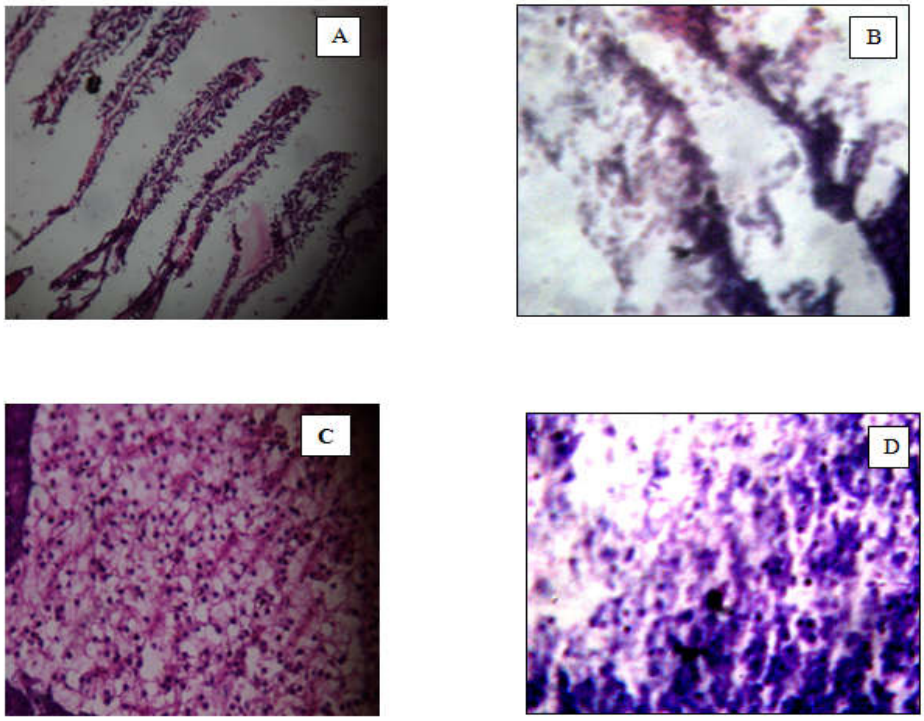


Fig 8. Histopathological changes in liver and gill of Tilapia injected intraperitoneally to toxic extracts of *Anabeanopsis abijatae* at a dose of 200 µg kg^{-1} (A) Gill of control Tilapia fish, (B) Gill of treated Tilapia fish showing congestion and vacuolation of respiratory epithelium, (C) Liver of control Tilapia fish, (D) Liver of treated Tilapia fish) showing necrosis in areas of hepatocytes.

both doses, the concentration of glucose increased gradually till 72 hrs. Elevated glucose concentration was observed in *O.niloticus* exposed to cyanobacterial water bloom (Palikova *et al*., 2010). Cholesterol concentration in the blood of control fish was 68.0 mg/dL. It increased in the treated fish gradually and reached up to 475.2 mg/dL in 200 µg/kg and 347.1 in 100 µg/kg after 72 hrs of treatment. (Fig 2). The concentration of

(iu/l). In both concentrations (i.e. 100 µg/kg and 200 µg/kg), the enzyme activity was highest compared to control (Fig 7). Maximum activity was observed after 24 h and it increased till 72 hrs. Significant increase in ALT and AST was reported in silver carp *Hypohthalmichthys molitrix* after intraperitoneal application of pure microcystin (Vajcova *et al*., 1998). Increase in blood plasma enzymes (ALT and AST) were

observed in carp after two hours of intraperitoneal injection of toxin and finally hepatocyte necrosis was also noticed (Rabergh *et al*., 1991). Elevated concentration of ALT and AST in juvenile carp (*C.carpio*) on exposure of fish to different natural populations of cyanobacterial water blooms was observed (Kopp and Hetesa, 2000).

Mild to severe histopathological changes were present in the livers and gills of treated fish. In control fish, no histological changes were observed in any of the studied organs. No fish died during the experiment. The microscopic examination of Hematoxilin and Eosin stained sections of treated fish, showed dilations along the central vein in the liver parenchyma and congestion was also noticed (Fig 8). The hepatocytes showed areas of necrosis. Intraperitoneal injection of extracted toxin caused less damage to the gills of *O.mossambicus*. Stained gill sections showed congestion of respiratory epithelial cells, vacuolation of cartilage with inflammatory cell infiltration. The histopathological findings in *O.mossambicus* indicated that cyanobacteria produce potent toxins which alter the architecture of hepatocytes and may impair their function. The congested blood vessels in liver indicated clear evidence of haemorrhages. Vacuolization of hepatocytes indicated an imbalance in the rate of synthesis of substance(s) in the parenchymal cells and their rate of release into the systemic circulation (Molina *et al*., 2005). In summary, results indicated that the acute intraperitoneal exposure of Tilapia to cyanobacterial extracts induced toxic reaction in liver and gill of fish with subsequent disturbances in biochemical indices, which is of toxicological importance for humans and livestock. A modern water-treatment plant using pre- and intermediate ozonation steps in conjunction with on-line O_3 measurements and different filtration steps, and regular monitoring of cyanobacterial cell densities in raw water can provide safe drinking water.

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REFERENCES

Best,J.H., Eddy.F.B and Codd,G.A. 2001. Effects of purified microcystin-LR and cell extracts of *Microcystis* strains PCC 7813 and CYA 43 on cardiac function in brown trout (*Salmo trutta*) alevine, *Fish Physiol Biochem,* 24:171-178.

Carbis,C.R., Rawlin,G.T., Mitchell,G.F., Anderson,J.W and McCauley,I. 1996. The histopathology of carp, *Cyprinus carpio* L., exposed to microcystins by gavage, immersion and intraperitoneal administration, *J Fish Dis,* 19: 199-207.

Carmichael,W.W. 1996. Toxic Microcystis and the environment. In toxic *Microcystis* (Watanabe MF, Harada.H, Carmicheal.WW and Fujiki.H eds) CRC Press, New York, USA. 1-272.

Chen,J., Xie,P., Zhang,D., Ke,Z and Yang,H. 2006. In situ studies on the bioaccumulation of microcystins in the phytoplanktivorous silver carp (*Hypophthalmichthys molitrix*) stocked in Lake Taihu with dense toxic *Microcystis* bloom, *Aquaculture*, 261**:** 1026-1038.

Desikachary,T.V. 1959 How to identify cynaophyta? ICAR monograph on algae.

Dobsikova,R., Svobodova,Z., Blahova,J., Modra,H and Velisek,J. 2006. Stress response to long distance transportation of common carp (*Cyprinus carpio* L.) *Acta Vet Brno* 75:437-448.

Frazoa, B., Martins, R and Vasconcelos,V. 2010 Are Known Cyanotoxins Involved in the Toxicity of Picoplanktonic and Filamentous North Atlantic Marine cyanobacteria? *Marine drugs*, 8(6):1908-1919.

Gupta,U.S and Guha,S. 2006. Microcystin toxicity in a freshwater fish, *Heteropneustes fossilis* (Bloch), *Current Science,* 91(9):1261-1270.

John,D.M., Whitton,B.A and Brook,A.J. 2002. The fresh water algal flora of the British Isles- An identification guide to freshwater and terrestrial algae, Cambridge University Press.

Kopp,H and Hetesa,J. 2000 Changes of haematological indices of juvenile carp (*Cyprinus carpio L*.) under the influence of natural populations of cyanobacterial water blooms, *Acta Ver Brno* 69:131-137.

Li,X., Xie, P and Chen,J. 2005. In vivo studies on toxin accumulation in liver and ultrastructural changes of hepatocytes of the phytoplanktivorous bighead carp i.p. injected with extracted microcystins, *Toxicon*, 46:533-545.

Luskova,V., Svoboda,M and Kolarova,J. 2002. The effect of Diazinon on blood plasma biochemistry on carp (*Cyprinus carpio L*.), *Acta Vet Brno,* 71:117-123.

Magalhaes,V.F., Marinho,M.M., Domingos,P., Oliveira,A.C., Costa,S.M and Azevedo,L.O. 2003. Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ) 42:289-295.

Mazorra,M.T., Rubio,J.A and Blasco,J. 2002. Acid and alkaline phosphatase activities in the clam Scrobicularia plana: kinetic characteristics and effects of heavy metals, *Comp Biochem Physiol,* 131: 241-249.

Molina,R., Moreno,I., Pichardo,S., Jos,A., Moyano,R and Monterde,J.G. 2005. 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.

Navratil,S., Palikova,M and Vajcova,V, 1998. The effect of pure microcystin LR and biomass of blue green algae on blood indices of carp (*Cyprinus carpio* L.), *Acta Vet Brno*, 67: 273-279.

Palikova,M., Kopp,R., Mares,J., Navratil,S., Kubicek,Z., Chmelar,L and Pikula,J. 2010. Selected haematological and biochemical indices of Nile tilapia (*O.niloticus*) reared in the environment with cyanobacterial water bloom, *Acta vet Brno*, 79:63-71.

Rabergh,C.M.I., Bylund,G and Eriksson,J.E. 1991. Histopatholgical effects of Microcystin LR a cyclic peptide toxin from the cyanobacterium (blue green algae) *Microcystis aeruginosa* on common carp (*Cyprinus carp L*.), *Aquatic Toxicology,* 20:131-146.

Serpunin,G. 1995. The connection between carp haematological indicators and its growth rate and its well-fedness. In: Proceedings of the 4th Ichtyohaematolgical Conference, Hluboka nad Vltavou, *Czech Republic,* 21-22.

Sivonen,K and Jones,G. 1999. Cyanobacterial toxins. In toxic cyanobacteria in water: A guide to their Public Health

Consequences, Monitoring and management (Chorus and Bartram, eds) E and FN Spon, New York, 41-111.

Svetina, A., Matasin,Z., Tofant.A., Vucemilo.M and Fijan,N. 2002. Haematology and some blood chemical parameters of young carp till the age of three years. *Acta Vet Hung*, 50(4):459-467.

Vajcova,V., Mavratil,S and Palikova,M. 1998. The effect of intraperitoneally applied pure microcystin LR on haematological, biochemical and morphological indices of silver carp (*Hypophthalmichthys molitrix Va*l.), *Acta Vet Brno* 67:281-287.

Zurawell,R.W., Chen,H., Burke,J.M and Prepas,B.R. 2005. Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environment, *J Toxicol Environ Health* B, 8(1): 1-37.

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