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The Effects Microcystin on Reproductive Physiology of Nain (*Cirrhinusmrigala*)

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Abstract - *Microcystines from Microcyustis aeruginosa often becomes the causal factor for death/abnormailities in fishes. These toxins are diverse group of chemical substances, each of which shows specific toxic mechanism. In the present work, effect of crude microcystein on reproductive physiology of Nain Cirrhinus mrigala was studied. The gonadosomatic indices (GSI) on exposure to microcystin at lethal and sub lethal concentrations female and male fish showed significant decrease after long term exposure, there was no effect on short term exposure to the fish. The hepatosomatic indices of control female and male Nain ranged from 1.8-2.4, respectively. Elevated values of HSI of male and female individuals were observed during long term exposure to lethal level of microcystin. The sub lethal level did not induce any significant change in HSI of male or female at any exposure time.*

Keywords*: *Microcyustis aeruginosa, microcystein, Surha lake, Cirrhinus mrigala, GSI, HSI

1. INTRODUCTION

Cyanobacteria are frequent components of many aquatic (fresh, brackish and marine) ecosystem. The cyanobacteria provide an extraordinarily wide-ranging contribution to human affairs in everyday life [1] and are of economic importance [2]. Sometimes, they produce massive growth as a consequence of nutrient enrichment of natural water due to agricultural fertilizer run off or from domestic and industrial effluents. This massive growth of cyanobacterial strains are referred as cyanobacterial blooms. Awareness of cyanobacterial bloom and scums, and health hazards which they can present, is long established [3, 4]. Death or illness of animals and birds after oral intake of cells or the free toxins has been documented well. In many cases the environmental samples examined for toxicity consist almost, or entirely of one cyanobacterial genus or species [5] More than 60 identified toxins of cyanobacteria are regarded as neurotoxins, hepatotoxins, cytotoxins skin irritants and gastrointestinal toxins [6]. Most of the bloom forming cyanobacteria produce various types of toxic substances injurious to human and animal health are commonly known as cyanotoxins. These toxins pose a challenge for water management [7]. Unlike most of the toxic chemicals cyanotoxins only sometimes occur dissolved in water and generally present inside the cell (endotoxin) e.g. Microcystines from *Microcyustis aeruginosa* is known as "fast death factor" (FDF)[8, 9] and commonly known as "very fast death factor" (VFDF). In contrast to the pathogenic bacteria, cyanobacterial cells do not proliferate in human bodies after uptake. These belong to a rather diverse group of chemical substances, each of which shows specific toxic mechanism in vertebrates. Most of the reports of hazardous effect of these blue green algae are from tropical countries but very little attention has been paid in India where the ponds and rivers are worshiped. The present work focus on the effect of cyanotoxin of *Microcystis aeruginosa;* isolated from Suraha Lake, Ballia, Utter Pradesh known for commercial production of fishes; for evaluation of their effect on cellular, biochemical and reproductive physiology of Nain *Cirrhinus mrigala*.

2. MATERIALS AND METHODS

2.1 Experimental Organisms

Microcystsis aeruginosa was isolated from Lake Surha, Ballia, Uttar Pradesh India and were were purified and cultured on B-12 media [10] having following composition (in milligrams per liter of deionized distilled water): $NaNO_3$, 100; K_2HPO_4, 10; $MgSO_4$ $7H_20$, 75; $CaC1_2$ $2H_20$, 40; Na_2CO_3, 20; ferric citrate, 6 (autoclaved separately); disodium EDTA. $2H_2O$, 1; and vitamin B_{12}, 0.1. The pH was adjusted to 9.0. Single cell isolation of the growing isolates was performed by streaking the colonies on agar plates. The fish, Nain (*Cirrhinus mrigala*) were caught from the fish pond of Agriculture farm of SMM Town PG College, Ballia and transferred to glass aquarium in lab 7 days prior to each experiment.

2.2 Toxin Extraction and Testing

The cyanotoxin was extracted by the method described by Siegelman *et al.* (1984). In brief lyophilized cells from pure culture were stirred in 20-200 ml of 5% n-butanol/20% methanol (v/v) in water for 1-2 h at 4 ^oC. The suspension was filtered and concentrated through vacuum evaporation and used as crude toxin.

2.3 Acclimation of *Cirrhinus mrigala*

The fish Nain (*Cirrhinus mrigala*) with mean weight 200 ±25 g were held in Fish tanks (8 individuals $tank^{-1}$) with 96 l of fresh de-chlorinated water at 21 ±2 ^{o}C. The pH of water ranged between pH 7.6 ±0.2, Ca^{2+} 0.60 *mM* l^{-1} and Mg^{2+} 0.3 0.60 *mM* l^{-1}. Fish were fed with commercial fish food and were acclimatized for 7 days before the beginning of the experiments. Acute toxicity tests with toxins from *M. aeruginosa* included the determination of median lethal concentrations at 24, 48, 72 and 96h under static test conditions. The tests were conducted basically as recommended by the committee on methods for toxicity tests with aquatic organisms [11].

2.4 Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI)

At the end of experimental dates, fish from the treated and control groups were weighed and killed. The gonads (Testes and Ovaries and liver of both the groups were isolated from the visceral cavity, peritoneal and visceral tissues cleaned off. The weight of gonads and liver in grams were recorded in fresh condition. The experiment was repeated three times with 6 replicates and parallel control. The gonadosomatic indices (GSI) of both male and female fish were calculated using the formula:

GSI = (Total wet weight of Gonad/ Total weight of fish) x 100

The hepatosomatic indices (HSI) of both male and female fish were calculated using the formula:

HSI = (Total wet weight of liver/ Total weight of fish) x 100

3. RESULTS AND DISCUSSION

3.1 Behaviour of *Cirrhinus mrigala*

The behaviour of the Nain (*Cirrhinus mrigala*) observed during this study was similar to those observed by various workers for teleostean species under influence of several water pollutants [12, 13, 14, 15]. Exposure to lethal concentration of microcystin for long (45-90 days) term showed reduction in GSI values of both the sexes. Similar results have been observed in female carp minnow exposed to three pesticides and in both the sexes of a freshwater teleost, *Clarias batrachus* exposed to dimethoate [16]. It was also suggested that steroid biosynthesis may have been affected under dimethoate stress, which may be acting through hypothalamus-pituitary axis inhibiting the synthesis of GnRH and gonadotropin. The reduced GSI in the present study may be due to lowered gonadal activity under cyanotoxin stress and impairment of the production of steroid hormones which might have arrested the formation of germ cells and caused degeneration or necrosis. All this might have caused loss of germ cells from the gonads, ultimately reducing the weight of gonads, resulting in reduced GSI of exposed male and female *Cirrhinus mrigala.* The teleosts which are characterized by yolk rich oocytes dominated by protein and lipids. are sensitive to lipid-soluble xenobiotics and metal cadmium which interfere the function such as reproduction [17, 18] and oocytes atresia is one of the potential mechanism for the reduction of GSI in fishes (Lam, 1983; Thomas, 1989). Toxicity and oxygen stress has profound effects on the process of reproduction; including puberty, gonadal development and fertility [19]. Studies have shown that mammals, when subjected to high altitude, have delayed puberty and a prolonged sexual maturation period. Very little has been published on the relationship between toxicosis related hypoxia and reproduction in fish. Zhou [20] studied the effects of hypoxia on reproduction of the common carp (*Cyprinus carpio*).Gonad development was reduced when fish were exposed to hypoxia for 8 weeks. The underdeveloped gonads had significant reduction in the number of spermatocytes and the mechanisms underlying the defects are unknown at the moment. Nevertheless, it is known that the physiology associated with reproductive capability is closely related to stress in general.

3.2 Gonadosomatic Index (GSI)

The gonadosomatic indices (GSI) in controls ranged from 14 to 15.3 in females and from 1.1 to 1.9 in males. (Figs 1-5). The 96h exposure to acute concentration of toxin did not show any significant changes in GSI of female and male fish in comparison to control fish (Fig. 1). In general, on exposure to microcystin at lethal and sub lethal concentrations, the GSI of female and male fish showed significant decrease only after long term (45-90 days) exposure, there was no effect on short term (15-30 days) exposure to the fish (Figs. 2A-5A). For example, the GSI of female fish exposed to lethal concentration compared to corresponding control value 14 and 14.2 of control (Fig. 3A). Similar trend was obtained with male fish also. were 13.9 and 11.2 at 30 and 90 day of exposure

3.3 Hepatosomatic Index (HSI)

The hepatosomatic indices of control female and male Nain ranged from 1.8-2.4, respectively (Figs. 1-5). Elevated values of HSI of male and female individuals were observed during long term (45-90 days) exposure to lethal level of microcystin (Figs. 2B and 3 B). The sub lethal level did not induce any significant change in HSI of male or female at any exposure time. For example the HSI of male Nain exposed to sub lethal concentration of microcystin

was 2.1 on day 30 and 90 compared to corresponding control value 2.05 and 2.15 (Fig 5B).

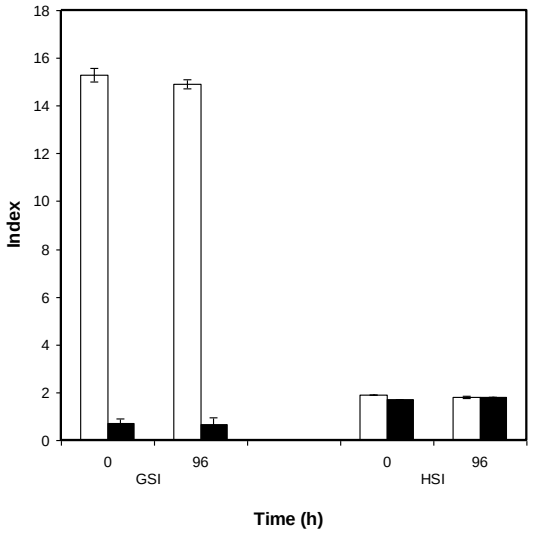

Fig. 1: Changes in gonadosomatic indices (GSI) and hepatosomatic indices (HSI) of the female and male Nain (*Cirrhinus mrigala*) exposed for 96h to acute concentrations (1/5th of 96 h LC_{50} value) of *Microcystis aeruginosa*. Bars represent: ☐ female and ■ male. Data are mean of six replicates; | = S.D.

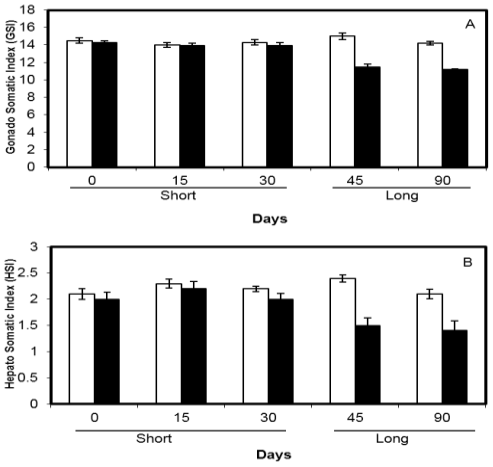

Fig. 2: Changes in Gonado Somatic indices (GSI) and Hepato Somatic indices (HSI) of the female Nain (*Cirrhinus mrigala*) exposed to lethal concentrations (1/10th of 96 h LC_{50} value) of *Microcystis aeruginosa*. A = GSI, B = HSI; Bars represent: ☐ Control and ■ treated. Data are mean of six replicates; | = S.D.

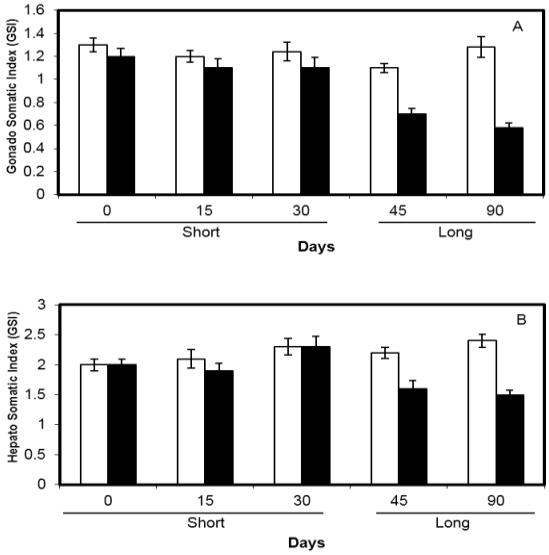

Fig. 3: Changes in Gonado Somatic indices (GSI) and Hepato Somatic indices (HSI) of the male Nain (*Cirrhinus mrigala*) exposed to lethal concentrations (1/10th of 96 h LC_{50} value) of *Microcystis aeruginosa*. A = GSI, B = HSI; Bars represent: ☐ Control and ■ treated. Data are mean of six replicates; | = S.D.

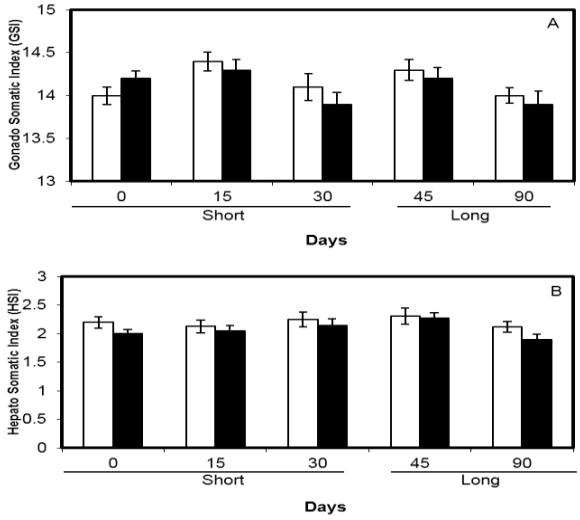

Fig. 4: Changes in Gonado Somatic indices (GSI) and Hepato Somatic indices (HSI) of the female Nain (*Cirrhinus mrigala*) exposed to sub lethal concentrations (1/15th of 96 h LC_{50} value) of *Microcystis aeruginosa*. A = GSI, B = HSI; Bars represent: ☐ Control and ■ treated. Data are mean of six replicates; | = S.D.

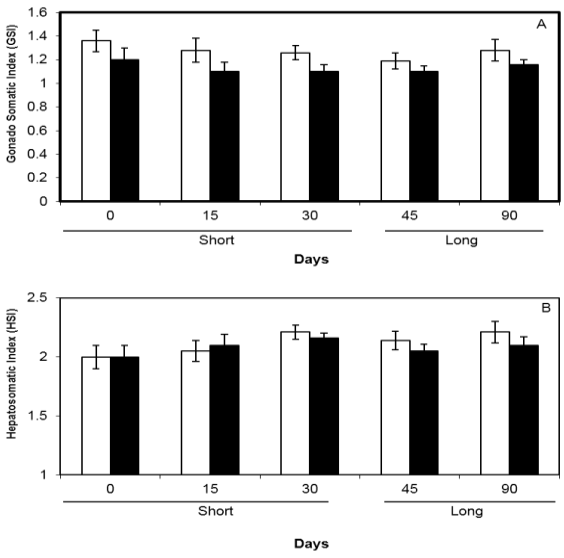

Fig. 5: Changes in Gonado Somatic indices (GSI) and Hepato Somatic indices (HSI) of the male Nain (*Cirrhinus mrigala*) exposed to sub lethal concentrations (1/15th of 96 h LC_{50} value) of *Microcystis aeruginosa*. A = GSI, B = HSI; Bars represent: ☐ Control and ■ treated. Data are mean of six replicates; | = S.D.

3.5 Testis

No information is available on the deleterious effects of cyanotoxins on the testicular structure or on the steroid-secreting cellular site in fish. The results of the present study clearly demonstrate the degenerative changes in testicular lobules. Reduction in spermatogenic activity and haemorrhage in the testes have been reported after toxicant exposure by different workers (Srivastava, 1987; Srivastava and Srivastava, 1994) Impairment of spermatogenic activity was also observed in *Channa punctatus* and *C/arias batrachus* after exposure to mercury [21] . Srivastava and Srivastava [22] and Srivastava *et al.* [23] also reported degeneration of germinal epithelium and cytolysis of sperms after exposure to chlordecone and dyes. Different views have been expressed regarding impairment of spermatogenic activity and degenerative changes in the testes after toxicant action. However, Ram and Sathyanesan [21] suggested inhibition of testicular growth through pituitary gonadal axis. In the present study too, it seems possible that degenerative changes in testicular histology may be attributed to inhibition of, gonadotropin secretion.

3.6 Ovaries

The short and long term treatments of *Cirrhinus mrigala* with microcystins resulted in marked degenerative changes in the ovary during the prespawning phase. These changes included prominent interfollicular spaces and appearance of atretic follicles. The effect of toxins on fish ovary have least been studied so far. However, degenerative changes in fish ovary exposed to other pollutants have been reported by a number of workers [22, 24]. In order to make comparison of the present results, the sporadic findings available for the effects of other toxicants and dyes on fish ovary have been considered. Srivastava *et al*. [23] reported inhibition of ovarian activity in catfish *Heteropneustes tassilis* after pesticides and dyes treatment. They also reported that carbaryl/fenitrothion treatment arrested ovarian activity and caused increase in atretic follicles in *Channa punctatus.* Inhibition of ovarian growth and ovarian steroidogenesis in atlantic croaker after exposure to different chemicals have been observed by Thomas [24]. Inhibition of ovarian growth was also observed in *Channa punctatus* and *C/arias batrachus* [21]. Srivastava and Srivastava [22] have reported degenerative changes and inhibition of ovarian growth in the catfish, *Heteropneustes fossilis* after chlordecone treatment. Arrest of ovarian activity and degenerative changes in oocytes after surfactants treatment of the catfish in the present study may be attributed to inhibition of gonadotropin secretion from pituitary. Similar suggestions were also made by Singh and Singh [25] Ram and Sathyanesan [21].

Toxic cyanobacterial blooms can be associated with fish mortality [26]. Some authors have evaluated the intraperitoneal exposure of such fish species as common carp [27] and rainbow trout [28] to microcystins, with particular emphasis on histopathological aspects. All of these studies showed necrosis and degeneration in liver and kidney [27 and also indicated that microcystins may damage the gill epithelium. Similar changes have been recorded when fish are orally exposed to microcystins. Carps exposed to microcystins underwent damage to renal proximal tubular cells and hepatocytes [28].

CONCLUSION

The accumulation of microcystins in showed growth inhibition and severe damage to hepatocytes in microcystins treated carps. A complicated effect of the toxin may be one of the reasons for histopathological changes of liver in and changes in GSI and HIS of *Cirrhinus mrigala*.

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