RESEARCH ARTICLE

Protective effect of dietary *Spirulina platensis* on haematological parameters of *Labeo rohita* exposed to sublethal concentration of mercuric chloride

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ABSTRACT

A considerable number of contaminants of environment contain heavy metals as their constituents. Mercury is considered to be one of the most toxic metals. Industries discharge heavy metals recklessly in to the environment. The consumption of heavy metalsparticularly mercury through food chain via fish by human being cause severe disorder in the physiology. Heavymetals causing serious damages to entire ecosystem including fishes. To reduce the toxicity of heavy metals of fishes, it is one of the serious and major problems in fish culture. There is a lack of study about the effects of phytoplankton especially micro algae on fishes. The protective effects of *Spirulina* applied on other animal's however it has yet to be applied for fishes. The effect of spirulina supplementation on reduction of mercuric chloride toxicity based on haematological parameters was studied in a freshwater fish, *Labeo rohita*. The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days. The results showed that sublethal exposure of *Labeo rohita* fed with *spirulina* free diet (T1 groups) significantly reduced the hamotological parameters than those exposed to sublethal level of mercuric chloride and fed *Spirulina* supplementation diet (T2- T5 groups).

Keywords: Protective effect, *Spirulina platensis*, Haematologial parameters, Mercuric chloride, *Labeo rohita.*

INTRODUCTION

The natural aquatic systems is getting extensively contaminated with heavy metals released from domestic, industrial and other anthropogenic activities (Velez and Montoro, 1998, Conacher *et al.,* 1993). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al.,* 2007; Vosyliene and Jankaite, 2006). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.,* 2004). Recent investigations have shown that a small amount of metal is sufficient to bring about severe biochemical, physiological and haematological consequences.

Effect of *Spirulina* in channel catfish *Ictaluruspunctatus,* (Duncan and Klesius, 1996) found that fish fed *Spirulina* had a lower percentage of erythrocytes and a higher percentage of lymphocytes than fish fed a control diet. There was no difference in thrombocytes and macrophages in the *Spirulina* and control diet. However, peritoneally elicited phagocytes from fish fed *Spirulina* showed enhanced phagocytosis to zymosan and increased chemotaxis to *Edwardsiellaictaluri* exoantigen (5.9 times). *Spirulina* also enhanced production of antibodies to key limpet hemocyanin (KLH) but not to *E ictaluri*. (Hayashi *et al*., 1993*),* who showed in mice that *Spirulina* increased the antibody response to a thymus-dependent antigen but not to a thymus independent antigen. Inastudy by (Lee, 1999) the activity of granulocytes and hyaline cells was enhanced significantly in tiger prawns *Penaeusmonodon* supplied with a feed containing as low as 0.1% (w/w) dry *Spirulina*. The increase in phagocytic activity of haemocytes was a function of *Spirulina* content and time. Prawns fed with *Spirulina* couldclear *Vibrio parahaemolyticus*, a pathogen of prawns, from the hemolymphat half the time taken by control prawns fed with basal diet.

In the present study, experiments were designed to investigate the impact of dietary *Spirulina* supplementation on the haematological, and alleviation of mercuric chloride in carp, *Labeorohita*. In the present investigation, focus on damage induced by inorganic mercuryin the Indian majorcarp *Labeo rohita,* and protective role done *Spirulina Plantensis*.

MATERIALS AND METHODS

The live major carp, *Labeorohita*were obtained from a Girna river dams near Chalisgaon city. They were acclimatized in laboratory condition for more than two weeks. The temperature, PH, Salinity and dissolved oxygen of the water were found to be 27 ± 1Ċ, 7.55 ± 0.1, 0.76 ± 0.09% and 7.20 ± 0.12 ml/l respectively. During the acclimatization, water was changed daily and fish were fed ad libitim with pelletised diet containing 35% protein. Acclimatized fish (1.30 ± 0.10g) were exposed to different concentrations (0, 0.03, 0.06, 0.09., 0.12, 0.15, 0.18 ppm) of mercuric chloride $HgCl_2$ obtained from Merk India Ltd. (Mumbai, India) and mortality was observed for 96 h. A static bioassay method was adopted for the determination of 96 h median lethal concentration Probit analysis was followed for the calculation of 96 hours $LC_{50.}$ Control group of fish was maintained in mercury free freshwater.

Feed: In the present experiment, 35% protein diet was used as basal diet for *Spirulina patensis* supplementation. The intergradient of dried fish meal, ground oil cake, cod liver oil, egg yolk, tapioca flour, vitamins and mineral mixtures were used to prepare the 35% protein diet, with appropriate proportion by square method. (Hardy, 1980). In addition to the control diet, five diets (0, 2, 4, 6, and 10 %) were prepared with different *Spirulina patensis* levels. The experimental diets were by adding the appropriate level of *Spirulina patensis* with chosen intergradient to boiled water, mixed well and steam cooked for 15-20 min. After moderate cooling, pellets (2mm) were prepared with operated pelletizer and dried in sunlight. After drying diets were separated stored in refrigerator.

Active and healthy fish (1.30 ± 0.10 g) were chosen from the acclimatization tank and starved for 24 h prior to the commencement of experiment. The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days. Triplicates were maintained for each group.

Group-I: served as control and reared in mercuric chloride free freshwater and fed with *Spirulina patensis* free diet. Test animals belonging to 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} groups were exposed to 0.12 ppm of mercuric chloride.

Group-II: individuals was fed with *Spirulina patensis* free diet, however 3^{rd} , 4^{th} , 5^{th} and 6^{th} groups were fed with 2, 4, 6, and 10% *Spirulina patensis* diets respectively.

The experimental groups 1, 2, 3, 4, 5, and 6 are designated as C, E1, E2, E3, E4 and E5 respectively. The experiment was conducted in glass aquaria containing 100L water. The water was not changed during the experiment but was aerated for 14 h to avoid depletion of oxygen. The hydrobiological parameters like dissolved oxygen, temperature, PH, salinity and hardness of water were estimated during non-aeration period. Two series of experiment were conducted in the present study.

Exp-II: Like the first series of experiment, a parallel experiment was conducted simultaneous- sly for 7, 14, 21 days to study the impact of dietary *Spirulina*

patensis on selected parameters in *Labeo rohita.* Test animals fed ad libitum with chosen experimental diets to respective exposures twice a day at 07:00 and 18:00 hrs. for 1h each. Test animals were starved for 24h prior to the conclusion of the experiment for the estimation of haematological parameters.

Three fish were removed from each experimental group at the end of the experiment; blood was collected and analyzed for selected haematological parameters. Blood was collected in watch glass containing required amount of 6% EDTA as an anticoagulant from three experimental fish at a time by cutting the caudal peduncle using a sharp knife. Haematological parameters were estimated according to routine clinical method RBC was counted by using an improved Neubauer counting chamber. Haemoglobinometer was used to determine the haemoglobin content of blood.

RESULTS AND DISCUSSION

The haemoglobin concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible. Short-term exposures to low concentrations of heavy metals mostly induce an increase in these hematological indices. Increase in RBC number and hematocrit level was reported in *Mystusvittatus* exposed to sub-lethal and lethal concentrations of copper (Singh and Singh, 1982).

Some workers also reported a fall in RBC count, haemoglobin percent and packed cell volume and decrease in MCH, MCHC and MCV in freshwater fishes exposed to cadmium, zinc and nickel indicating anemia, erythropenia and leucopoiesis (Vincent *et al.,*1996). The TEC, hemoglobin percent and mean cell hemoglobin (MCH) were appreciably declined in *Labeo rohita* exposed to chromium reflecting the anemic state of the fish which could be possibly due to iron deficiency and its consequent decreased utilization for hemoglobin synthesis. This is in accordance with a similar study on *Labeo rohita*, which also reported hypo chromic microlytic anemia under lead chloride stress, Reddy *et al.* (1998). Anemia in fish is an early manifestation of acute and chronic intoxication of chromium. Further, a significant decrease in TEC, hemoglobin per cent, MCH and hematocrit were also reported in *Channa punctatus* exposed to both copper and chromium and this decrease is more pronounced in fishes exposed to chromium suggesting that the metal induces acute anemia under toxic conditions (Singh., 1995). The anemia could be probably due to structural alterations of heme leading to disturbed haemoglobin synthesis and also the inhibitory effect of mercuric chloride on the enzyme system in the synthesis of haemoglobin cannot be ruled out. (Johansson-Sjobeck and Larrsson, 1979).

Table-1: Effect of supplementation of dietary *Spirulina platensis* on Red blood corpuscles (RBC) count ($\times 10^6$ mm^3) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride for 21 days.

Sr. No.	Treat.	g % *S.p.*	Red blood corpuscles count ($\times 10^6$ mm^{-3})		
			7 days	14 days	21 days
1	Control	0%	2.58±0.13	2.61±0.18	2.56±0.02
2	T_1	0%	1.45±0.10** -43.57	1.41±0.1* -46.15	1.34±0.11*** -47.85
3	T_2	2%	1.48±0.05** -42.41	1.63±0.13* -37.54	1.64±0.06*** -36.18
4	T_3	4%	1.51±0.06** -41.24	1.73±0.14^{NS} -33.71	1.80±0.12** -29.96
5	T_4	6%	1.56±0.04** -39.29	2.00±0.13^{NS} -23.37	2.1±0.07** -18.28
6	T_5	10%	2.00±0.11^{NS} -22.17	2.06±0.23^{NS} -21.07	2.08±0.11* -19.06

i) Each value are mean ± S.D. of three estimations.
ii) (+) or (-) signs indicate % variation over control.
iii) Values are significant at * = P<0.05, *** = P<0.001, NS = Non significant.

Table 2: Effect of supplementation of dietary *Spirulina platenis* on Haemoglobin content (%) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride.

Sr. No.	Treat.	g% S.p.	Haemoglobin content (g %)		
			7 days	14 days	21 days
1	Control	0%	8.58±0.42	8.51±0.32	8.41±0.37
2	T_1	0%	4.92±0.09** -42.65	4.40±0.55** -48.29	4.03±0.46** -52.08
3	T_2	2%	5.27±0.35** -38.57	5.51±0.32** -35.25	5.80±0.62* -31.03
4	T_3	4%	5.72±0.33** -33.33	6.27±0.39* -26.32	6.33±0.27* -24.73
5	T_4	6%	6.38±0.44* 25.64	6.86±0.19* -19.38	6.80±0.76^{NS} -18.29
6	T_5	10%	6.82±0.60^{NS} -20.51	7.05±0.57^{NS} -17.15	7.00±0.67^{NS} -13.79

i) Each value are mean ± S.D. of three estimations.
ii) (+) or (-) signs indicate % variation over control.
iii) Values are significant at * = P<0.05, *** = P<0.001, NS = Non significant.

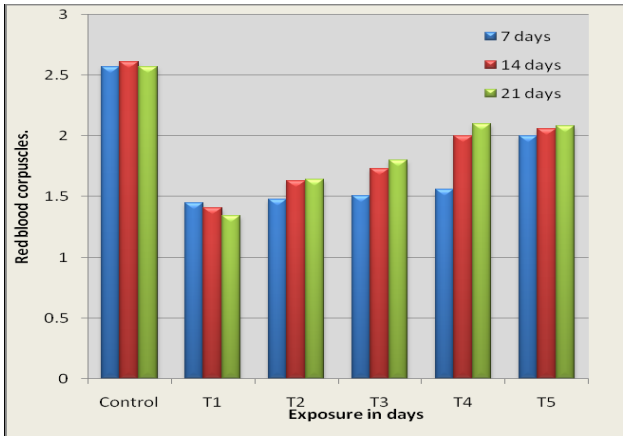

Fig1: Variations in effect of supplementation of dietary *Spirulina platensis* on Red blood corpuscles (RBC) count (×10^6 mm^{-3}) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride for 21 days.

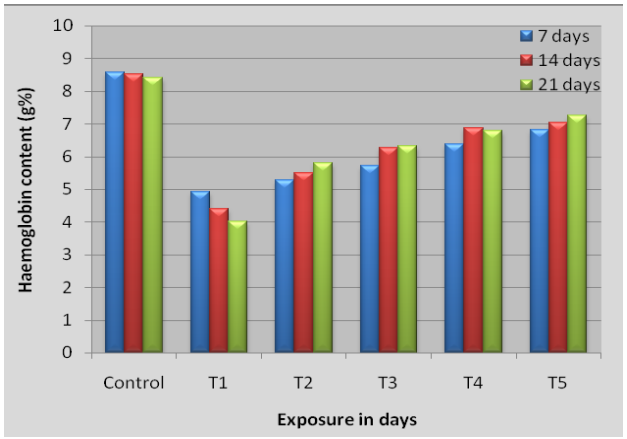

Fig 2: Variations in effect of supplementation of dietary *Spirulina platenis* on Haemoglobin content (%) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride.

In the present wok the sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet resulted in significant decrease in RBC count but RBC count were increased in the mercuric chloride with *Spirulina platensis* diet from 2% to 10% and the RBC count was increased from 1.64 to 2.08 (×10^6 mm^{-3})as the percent dose of *Spirulina platensis* increased. Haemoglobin content was decreased in the sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet but it was significantly increased in the mercuric chloride with *Spirulina platensis* diet from 2% to 10% and the haemoglobin content was increased from 5.80 to 7.00 g % as the percent dose of *Spirulina platensis* increased. In mercuric chloride exposed *Labeo rohita* and it may be due to the reduction of RBC count and HB content which reflected on tissue respiration. This may be evidently reflected on overall oxygen consumption of animals exposed to mercuric chloride.

The present study revealed that, the haematological parameters were improved in mercuric chloride exposed *Labeo rohita* fed *Spirulina* supplementation diets as suggests the protective role of *Spirulina* against mercuric chloride toxicity in

Labeorohita has 14% phycocynin pigment and it stimulates the erythropoesis (EPO) hormone production for hematopoesis. Phycocynin pigment also regulates the production of white blood cells even when bone marrow stem cells are damaged by toxic chemical or radiation reported by (Henrikson, 1994). (Sharma and Sharma, 2005), reported that *Spirulina* added feeds improved the tolerance of Poecilla reticulate when exposed to an azodye methyl red by considerable reduction in the cytotoxic effects on RBC'S count at higher concentration of the dye. Hayashi et al 1996 made similar findings and reported enhancement and proliferation of haematopoietic cells in the bone marrow.

CONCLUSION

The present study shows that, the dietary supplementation of *Spirulina* reduced the metal toxicity in mercuric chloride exposed *Labeo rohita* and improved the haematological parameters like RBC count and haemoglobin content significantly as the percent dose of *Spirulina platensis* was increased in a short period of time.

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