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EFFECT OF WATERBORNE IRON ON PHYSIOLOGICAL RESPONSES OF C. mrigala (Hamilton) FINGERLINGS

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Abstract

Iron is the fourth most abundant element by weight in earth's crust, and is often a major constituent of soils especially clay soils and is trace element, required by all living organisms for many physiological functions, plays a major role in many biochemical processes, but in excess it causes toxicity. High iron content in water bodies of mountainous states is considered as one of the major factor, responsible for low productivity in aquaculture systems. But, till date comprehensive reports on the adverse effect of iron overload in aquatic organisms, especially cultured fishes are scanty. The present study was undertaken to investigate the adverse effects of iron overload in economically important aquaculture fish species *C.mrigala*. Three sub-lethal test concentration of iron (ferrous) i.e. During evaluation of physiological responses treatments with three iron ranges 4.5mgl^{-1},2.1 mgl^{-1}, and 0.035mgl^{-1}(control) combined with six exposure times at 0,6,12,24,48,72 and 120 h with three replicated were used . Blood cells and tissue samples of the control & exposed specimens were sampled at regular intervals of time to assess alterations in parameters like PCV, Hb ,RBC, Blood glucose, lysozyme activity increased significantly($p<0.05$),whereas alkaline phosphate and NBT levels decreased significantly($p<0.05$) with iron stress exposure duration. The observed physiological changes in the present study provide the most comprehensive insight of iron overload stress in *C.mrigala*.

KEYWORDS:

Waterborne Iron, haemato-immuno-physiological changes, *Cirrihinus mrigala*

INTRODUCTION

Among all the natural resources water is one of the important resources and its quality has direct impact on mankind and also aquatic lives (Jabeen, 2012). Unluckily, raised industrial wastes had polluted the natural ecosystem and this nutrient (iron) enlarged momentous contamination stage (Hussain et al., 2011).when the essential metal intake is excessively elevated it can produce toxic effects (Turkmen et al., 2005; Turkmen et. al., 2008). All the aquatic animals require certain amount of inorganic elements for maintaining their homeostatic condition. The inorganic elements which are present and distributed in the body of fish are classified into two groups they are macro elements and trace elements. Macro elements include structural elements such as Ca, P, Mg and electrolytes K, Cl, S occur in concentrations of about 0.1 -2.00% of fish weight, whereas the diet should contain at least of about 100 mg/kg dry diet. Trace elements occur in very minute amount in the environment, few amount of trace elements are incorporated in feed or diet given of about less than 100 mg/kg of diet such as Fe, Cu, Mn, Zn, Co, Mo, Cr, Se, F, I, Ni, Li, Si, V, Ag to meet the requirement (Lall et al., 2002).These inorganic elements play a significant role in several complex biochemical mechanisms and also control and regulate the uptake, storage and excretion of electrolytes such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- where as these electrolytes are essential in the osmotic and ionic regulation of extra- and intracellular fluids in fish. It's very essential to identify the nature and level of toxicity of heavy metals for the aquatic life (Azmat and Javed, 2011).

Iron is also one of the important trace metal that plays major role in many biochemical process including electron transfer, gene regulation, binding and transport of oxygen and regulation of cell growth. Excessive uptake of iron can be toxic or disturbances in its regulation which is related to its ability to catalyze reactive oxygen species (ROS) formation via the Fenton reaction. The deleterious effects of iron include DNA damage, lipid peroxidation (LPO) and oxidation of proteins (Valko et al., 2005). Moore (1991) reported that iron in an inorganic form contaminates water and alters pond production, chemistry and increases toxicity.

Different forms of iron available in fish are in muscle as myoglobin, in blood as haemoglobin and in small quantities as transferring and ferritin in serum. Myoglobin is structurally similar to haemoglobin but functionally it plays an intermediate role between haemoglobin and tissue hemins, transferrin an iron binding protein serves as a principle carrier of

iron in blood serum plays important role in iron metabolism whereas ferritin and hemosiderin are iron storage proteins which occurs widely in liver and spleen (Naser, 2000).

In fish there are two potential sites for iron uptake, dietary borne (intestinal) or waterborne (brachial epithelium). The nutritional value of waterborne iron is not elucidated. Gills play a vital role in iron homeostasis, the concentration of metals in gills reflects their concentration in water where the fish lives, whereas the concentration in liver represents storage of metals in the water. Deficiency of iron leads to microcytic hypochromic anaemia in excess levels. It is toxic in nature, so to avoid the excess toxic iron in fish and thus to judge the effect of iron one should know the requirement of iron.

Among all Indian major carps *Cirrihinus mrigala* is commercially important species and is easily prone to diseases and infections and culture is very common all over the country. It is essential to know the effect of certain heavy metals onto aquatic systems to maintain the production levels. In the present study fingerlings of *C. mrigala* was selected for evaluating the toxic effects of iron (Fe) on them.

RESEARCH METHODOLOGY

Toxicity and Patho-physiological Responses of *C. mrigala* fingerlings in an Environment with and without Waterborne Iron.

Toxicity test is classified to short–term toxicity(Range finding toxicity test) and long-term toxicity, In range-fining toxicity tests, materials of unknown toxicity is conducted (24 h or 48 h), to determine approximate concentration range to include in definitive short-term tests. The overall objective of the long-term, partial-life-cycle toxicity test is to determine no-observed-effect concentration (NOEC) or chronic value (ChV) of toxicants. This test is also used to determine the effects on growth, survival of spawn or fry, behaviour, bioaccumulation etc. (APHA, 2005). LC_{50} Value of *Cirrihinus mrigala* due to waterborne iron was calculated and then further all the physiological, behavioral changes were observed. A lower concentration of 1/10 i.e.,2.15-2.10 mg^{-1} (T_1) and 1/20 i.e., 4.55-4.50 mg^{-1} (T_2) of LC_{50} value of iron was selected for long term definitive test During this period of time changes in various haematological parameters such as total erythrocyte count (EC), total leucocyte count (LC), packed cell volume (PCV), total hemoglobin, NBT, lysozyme activity, globulin, glucose, alkaline phosphate assay were examined at regular intervals of time i.e. 0 h, 6h, 12h, 24h, 48h, 72h, 120h.

Collection of Blood, Plasma and Serum

Blood samples were directly drawn from caudal vein of fish using heparinised 2ml tuberculin syringe. Blood was sampled from five fish of each control and metal exposed groups during each exposure period of 0h, 6h, 12h, 24h, 48h, 72h, 96h, 120h.

The blood parameters such as Total erythrocyte count (EC), Total leucocyte count (LC), Packed cell volume (PCV) and Total Hemoglobin was by the method of Schaperclaus (1991), Blood glucose by glucose diagnostic kit (Coral Clinical systems, India) was used which is based on Trinder (1969) GOD/POD method, Alkaline phosphatase activity was measured using a diagnostic kit (Accurex Biomedical, India) based on Biuret method (Strikland et al., Anal .Chem.33, 1961). Henri, R. J. et al, *"Clinical Chemistry-Principles and techniques"* Harper & Row, II Ed.(1974). Lysozyme activity of blood serum , Nitroblue tetrazolium (NBT) assay was determined as described by Anderson and Siwicki (1995). All measurements were performed in triplicate and the results were expressed as mean±S.D. The comparison of the control and experimental groups was statistically analyzed by Chi-square test and the validity of investigation was expressed as probability (p) values. Values of $p<0.05$, $p<0.01$ and $p<0.001$ were considered as less significant, significant and highly significant, respectively.

Results

96 h LC_{50} value of Fe for *Cirrihinus mrigala* fingerling was found to be 42.83 ± 3.57 mg l^{-1} The mortality difference among concentrations was significantly ($p>0.05$). LC_{50} values were increasing with decreasing time. Various LC_{50} and toxic factors of 24, 48, 72, and 96 h are presented in Table 6. Chi-square test did not show any significant difference between predicted value and experimental dose (concentration) during determination of Fe concentration and mortality i.e., with an increase in concentration mortality of fingerling was increased.

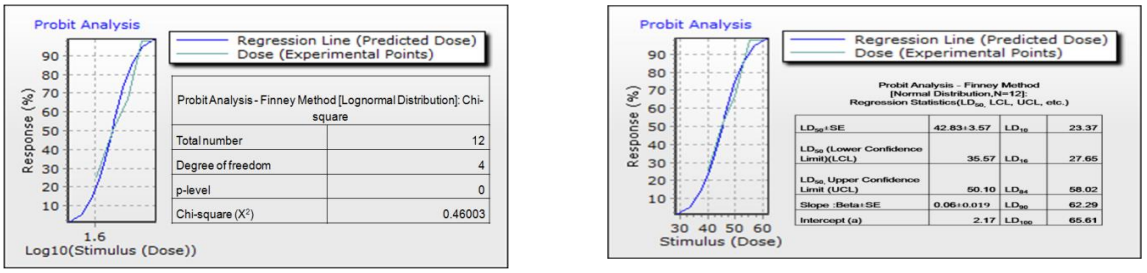

Fig.1 Chi square and lethal concentration values for *C. mrigala*, exposed to Fe (Output taken from Biostat 2009 Software).

Effect of Waterborne Iron Stress on Haematological Parameters

Different haematological parameters were measured at different sampling hour (0 h, 6 h 12 h, 48 h, 72 h, and 120 h) exposed to three different waterborne iron concentrations (Control, T1 and T2).

Physico-chemical parameters of water during the experimental period

Parameter	Control	Treatment 1 (T1)	Treatment 2 (T2)
Temperature (^{0}C)	30.00-29.00	30.00-29.00	30.00-29.00
pH	6.90-6.800	6.90-6.00	6.90-5.70
DO (mg l^{-1})	5.50-5.00	5.50-4.50	5.50-4.00
Total carbon dioxide (mg l^{-1})	0.04-1.20	0.04-1.75	0.04-2.15
Total alkalinity	100-101	100-97.00	100-95.00
Total iron (mg l^{-1})	0.03-0.04	2.15-2.10	4.55-4.50

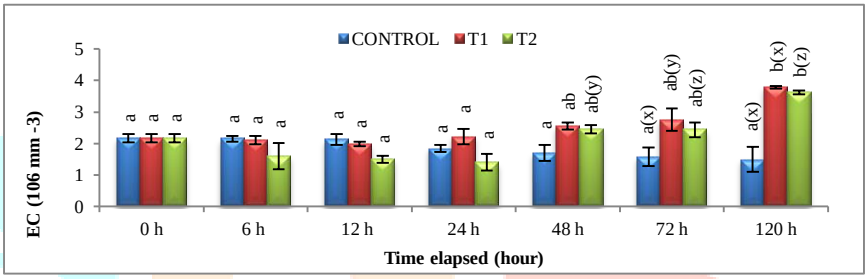

Fig. 2 EC count (EC10^6mm^{-3}) of different experimental groups at various sampling hours.
'a, b' represent significant changes ($p<0.05$) within each treatment with sampling hour; 'x, y, z' represent significant differences ($p<0.05$) between treatments at particular sampling hour.

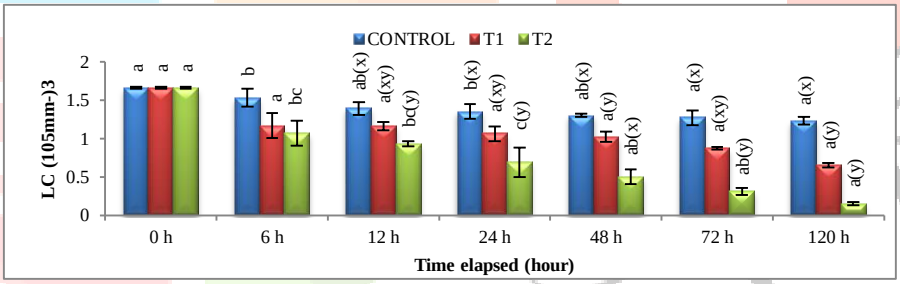

Fig .3 LC count (LC10^5mm^{-3}) of different experimental groups at various sampling hours.
'a, b' represent significant changes ($p<0.05$) within each treatment with sampling hour; 'x, y, z' represent significant differences ($p<0.05$) between treatment at particular sampling hour.

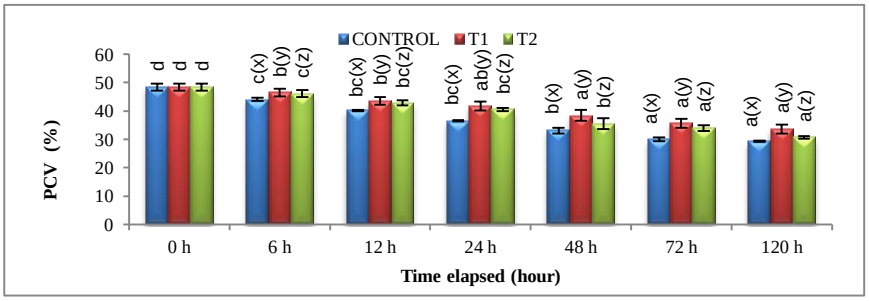

Fig. 4 PCV (%) of different experimental groups at various sampling hours.
'a, b, c' represent significant changes ($p<0.05$) within each treatment with sampling hour; 'x, y, z' represent significant differences ($p<0.05$) between treatment at particular sampling hour.

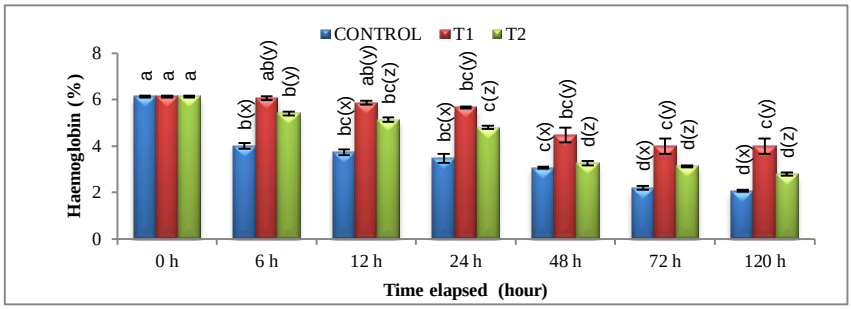

Fig. 5 Hb (%) of different experimental groups at various sampling hours.
'a, b, c, d' represent significant changes ($p<0.05$) within each treatment with sampling hour; 'x, y, z' represent significant differences ($p<0.05$) between treatments at particular sampling hour.

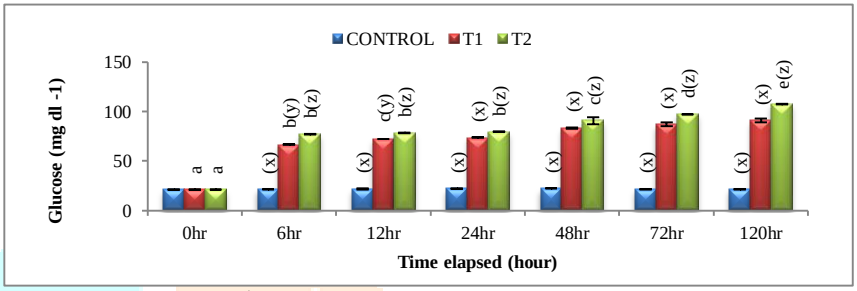

Fig.6 Glucose (mg dl^{-1}) of different experimental groups at various sampling hours.
'a, b, c, d, e' represent significant changes ($p<0.05$) within each treatment with sampling hour; 'x, y, z' represent significant differences ($p<0.05$) between treatments at particular sampling hour.

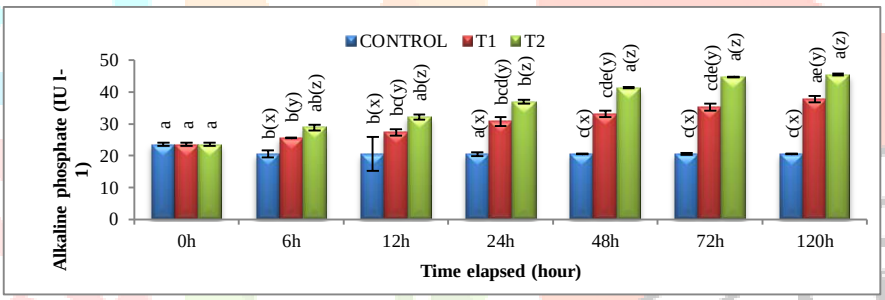

Fig.7 ALP (IU l^{-1}) of different experimental groups at various sampling hours.
'a, b, c, d, e' represent significant changes ($p<0.05$) within each treatment with sampling hour; 'x, y, z' represent significant differences ($p<0.05$) between treatments at particular sampling hour.

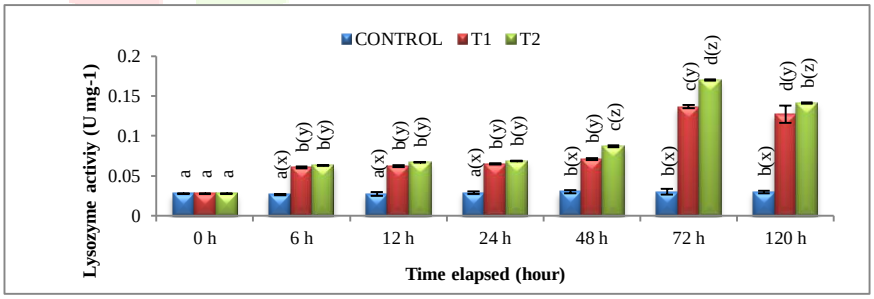

Fig.8 Lysozyme activity (U mg^{-1}) of different experimental groups at various sampling hours.
'a, b, c, d' represent significant changes ($p<0.05$) within each treatment with sampling hour; 'x, y, z' represent significant differences ($p<0.05$) between treatments at particular sampling hour.

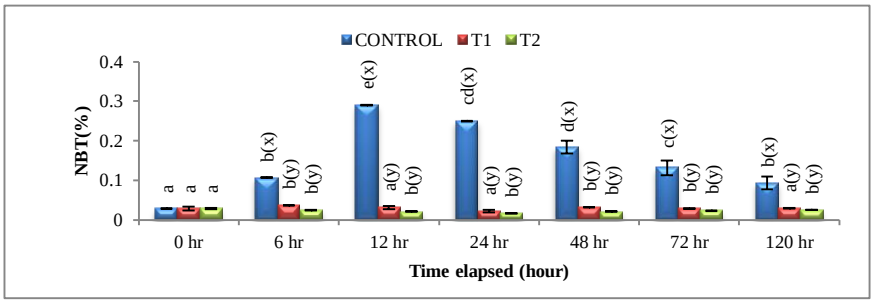

Fig.9 NBT (%) of different experimental groups at various sampling hours.
'a, b, c, d, e' represent significant changes ($p<0.05$) within each treatment with sampling hour; 'x, y, z' represent significant differences ($p<0.05$) between treatments at particular sampling hour.

The total erythrocyte count (EC) varied from 1.40 ± 0.26× 10^6 mm^{-3} in T2 at 24 h to 3.78 ± 0.03× 10^6 mm^{-3} at 120 h in T1.The total leukocyte count (LC) varied from 0.15 ± 0.02×10^5 mm^{-3} at 120 h in T2 to 1.65 ± 0.01×10^5 mm^{-3} at 0 h in all the treatments including control. The packed cell volume (PCV) varied from 29.30 ± 0.22% at 120 h in control to 48.43 ± 1.27% at 0 h in all the treatments including control. The haemoglobin varied from 2.06 ± 0.11% at 120 h in control to 6.13 ± 0.11% at 0 h in all the treatment groups including the control. Blood glucose varied from 21.09 ± 0.13 mg dl^{-1} at 0 h in all the treatment groups including control to 107.40 ± 0.08 mg dl^{-1} at 120 h in T2.Alkaline phosphatase (ALP) varied from 20.53 ± 0.15 IU l^{-1} at 120 h in control to 45.55 ± 0.11 IU l^{-1} at 120 h in T2 group. The lysozyme activity varied from 0.02 ± 0.00 U mg^{-1} at 0 h in all the treatment groups including control to 0.17 ± 0.03 U mg^{-1} at 72 h in T2. Nitroblue Tetrazolium (NBT) varied from 0.01 ± 0.00 % at 120 h in T2 to 0.10 ± 0.01% at 0 h in all the treatment groups including control.

Discussion

The primary aim of the study was to evaluate the effect of waterborne iron on patho-physiological responses of *Cirrihinus mrigala* (Hamilton) fingerlings. Based on the results from the Range finding experiment through which LC_{50} has been calculated and then experiment was conducted with two sub-lethal concentration of waterborne iron i.e., LC50/10 (4.5 mg l^{-1}) and LC50/20 (2.1mg l^{-1}) along with control having negligible waterborne iron concentration of 0.03 mg l^{-1}.

Effect of Waterborne Iron on *C. mrigala* Fingerlings.

The present study during Iron stressed condition the total EC and LC were increased and decreased respectively with respect to control significantly ($p<0.05$) and this is an agreement with the finding of Vinodhini *et al.*(2009) in common carp when exposed to heavy metals showed elevated levels of red blood cells and with Remya *et al.* (2015), they demonstrated that nano particles of iron oxide on *Labeo rohita* has a significant role in increasing and decreasing in the counts of red blood cells and white blood cells respectively. In the present study the Hb content following exposure to iron stressed condition indicated elevated levels in T1,T2 which were significantly ($p<0.05$) higher than control was observed. Whereas within the treatments the Hb content has decreased significantly ($p<0.05$) from 6 h. The present study is not in a compliance with Martinez and Souza, 2002; Das *et al.*,2006; they reported that a significant reduction in the Hb content following exposure to acidic pH indicated a reduced blood oxygen carrying capacity. However, significantly ($p<0.05$) elevated levels of PCV content was observed in between treatments T1and T2 but not in control after 24 h of sampling. Whereas within the treatment significantly ($p<0.05$) reduced PCV was observed after 48 h of sampling.

Blood biochemical parameters with significant variations ($p<0.05$) as compared to control with time exposure were observed for the levels of blood glucose and ALP. The ranges of serum biochemistry vary from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age, and sex of the fish (Jawad *et al.*, 2004). In the present study increasing trend was found in blood glucose level after iron stress. These alterations are may be due to the stimulation of hypothalamo-pituitary axis (HPA) and sympathetic nervous system resulting in liberation of catecholamines and gluco-corticoids (Prabhakaran *et al.*, 2005). Blood glucose level was significantly higher ($p<0.05$) in fingerlings exposed to iron stress after 6 h as compared to control in both in between and within treatments and the level of glucose content was more in the group exposed to iron concentration of 4.5 mg l-1 as compared to 2.1 mg l-1. Thus the increase in blood glucose level is proportional to the amount of iron in the water and the increased blood glucose level is may be due to an increased depletion of liver glycogen (Ojolick *et al.*, 1995), to mobilize more energy by the fish to maintain their homeostasis during stress. The present study is an agreement with the finding of Vinodhini *et al.* (2009) in common carp when exposed to heavy metals showed elevated levels of blood glucose and total cholesterol.

Alkaline phosphatise is a P-stress marker enzyme that catalyses the hydrolysis of phosphorus compounds and the transfer of phosphoric groups to an acceptor molecule. The rate of catalytic activity of the enzyme is inversely proportional to the concentration of inorganic phosphate in the ambient environments (Dyhrman and Palanik, 1999). This enzyme could serve as a good indicator of intoxication because of its sensitivity to metallic salts (Boge *et al.*, 1992). In the present study significant ($p<0.05$) elevated levels of alkaline phosphatase was observed in both the T1 and T2 but not in control after 6 h exposure to iron. It is in agreement with the study of Mukherjee *et al.* (2007) who reported that even a small range in alternation of pH of more than 0.3 units/day act as pH shock, leading to alternation in alkaline phosphatase activity. These

results are also in consistent with other results previously observed in *C. punctatus* (Sharma, 1990) and in other species (Verma *et al*., 2007), exposed to endosulfan stress.

Respiratory burst activity (measured by NBT) is one of the most important bactericidal mechanisms in fish. However insignificant difference ($p>0.05$) among and in between the groups at different sampling time was observed. However, a decrease in NBT values after stress was observed in this study and it is not in a compliance with the findings of Yin *et al.* (2006) in *Oreochromis niloticus*, after giving stress. Siwicki and Studnicka (1992) reported a 35 % decrease in the percentage of active (NBT-positive) phagocytes, and a 20 % decrease in myeloperoxidase activity in common carp subjected to a chemical stress. Significantly ($p<0.05$) increasing trend of lysozyme was found in both i.e., in between and within treatments T1, T2 under stressed condition compare to control, this result is in line with the observation of Balfry *et al*. (1997) identified a significant increasing level of lysozyme activity in red and wild-type *Oreochromis niloticus* following *Vibrio pararahaemelyticus* challenge.

CONCLUSION

In the present study, fingerlings of mrigala showed 96 h LC50 at 42.83 ± 3.57 mg l-1 of iron. PCV, RBC, Hb, , Blood glucose level, ALP level, Lysozyme activity has been increased in iron stressed condition, whereas LC, and NBT,has been decreased. Significant changes in haematological, biochemical, immunological parameters were observed within treatments i.e., iron stressed condition at various sampling hours during the experimental period.

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