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Research Article

Impact of Lead Nitrate on Haematological Parameters of *Cyprinus carpio* (Common Carp)

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ABSTRACT

Alterations in the chemical composition of aquatic environment by various pollutants like pesticides, fertilizers, heavy metals, detergents and discharge of effluents from various industries induce changes in the behavioural, physiological and biochemical aspects of inhabitants, particularly fishes. Water pollution by pesticides has been increasing alarmingly due to their indiscriminate use for eradication of various pests to protect agricultural crops. These pesticides drained into aquatic environments during monsoon period and their residue pose an untold threat to aquatic fauna. The present work is one such attempt to investigate the effect of heavy metal lead nitrate on haematological parameter of *C. carpio*. RBC, Hb and Ht decreased significantly when exposed to sublethal concentration (4.45 ppt) of lead nitrate. But the WBC count showed an increasing trend as the concentration of lead nitrate increased in the medium.

Key words: *C. carpio*, lead nitrate, RBC, WBC, Hb, Ht.

INTRODUCTION

Heavy metals are being introduced into environments through industrial processes, sewage disposal, soil leaching and rainfall[31]. Due to modern developments, various effluents are constantly affect the healthy growth of living organisms mostly fishes of economic importance. Toxic effects of heavy metals on animals are of two types depending upon the duration of exposure viz. chronic toxicity and acute toxicity. Chronic effects have been reported on survival, growth and reproduction of fish and other organisms[6,12]. Acute effects include mortality, respiratory distress, behavioural and biochemical changes etc.,[19]. Heavy metal pollutants cause massive fish kill and destruction of other aquatic life [17].

Fish in close association with their aquatic environment and any changes in this environment would be reflected in alterations in their haematological studies[13]. Stresses and pollutants generally cause rapid changes in blood characteristics of fish [18, 23]. Copper and Lead are stable and persist environmental pollutants and are considered as strong toxic metals to aquatic organism. These pollutants cause serious effects on growth, physiology and survival rate of aquatic organism especially fish[16].

With increasing emphasis on pisciculture and greater awareness of the pollution of natural water resources, haematological studies in fish have assumed greater significance. The aim of this work was to study the effect of heavy metal lead nitrate on haematological parameters of freshwater fish *C. carpio*.

MATERIALS AND METHODS

EXPERIMENTAL FISH

C. carpio Var. *Communis* is an exotic fish to India and most extensively cultivated species. It is generally called as scale carp and it is commercially important. It was introduced in India in 1939 and in 1957 from Sri Lanka and Thailand. *C. carpio* is voraciously omnivorous. It is a fast growing fish on artificial fish feed. Hence this is considered as one of the important species for fish culture species in India. This carp is also known as a sanitary fish as they serve as biological control for maintaining the ecological balances in fish ponds.

COLLECTION AND MAINTENANCE

For the present work juveniles of *C. carpio* were procured from Tamil Nadu Fisheries Development

Corporation, Azhiyar, Tamil Nadu and transported to laboratory in polythene bag filled with aerated water. The fish were acclimated in the glass aquaria (30 X 20 X 20 cm) at 28 ± 2°C. During the laboratory condition the fish were fed ad-libitum with freshly chopped pieces of goat liver. After 15 days of acclimation the fish were used for experimental study.

Standard chemical (Lead nitrate) was procured and stock solution (ppt) was prepared. From the stock solution 4.45 ppt concentration of heavy metal was prepared. Fingerlings of *C. carpio* were exposed to sublethal concentrations of heavy metal (4.45 ppt) for 4 days in a cylindrical glass aquaria (30 X 15 X 20 cm) containing 10 litre of test medium. Food was not provided to the fishes during the experiment. The medium was renewed daily to give constant effect of heavy metal on fish. The fish thus reared in heavy metal concentrations were taken for haematological studies after the desired period.

METHODS

COLLECTION OF BLOOD SAMPLE

Blood sample was collected from the experimental fishes of each group at a time on 1st , 2nd ,3rd and 4th day of experiment. The fishes were collected and gently wiped with a dry cloth to remove water. Caudal peduncle was cut with a sharp blade and the blood was collected in a watch glass containing EDTA and anticoagulant (6% Ethylene Diamine Tetra Acetic Acid). The blood was mixed well with the EDTA.

RBC COUNTING

For RBC count, a method devised[32] and latter modified[7] was followed. The standard RBC diluting pipette and a 1: 200 dilution was used for the RBC count. Yokayama's fluid was used for dilution. The diluting fluid consisted of two parts and it was prepared by mixing 800 mg of Nacl, 40 mg of Kcl, 10 ml of formalin, 250 mg of dextrose, 50 mg of NaHCO3 and 40 ml of distilled water with an equal part of wright's stain.

Blood was drawn in the pipette up to the 0.5 mark. The tip of the pipette wiped with a filter paper to adjust the volume exactly to 0.5 mark and then filled with the diluting fluid up to 101 mark. Partial rotation of the pipette while it was being filled assured the complete mixing of the blood and diluting fluid and prevented clotting with it ends grasped between the thumb and second finger, the pipette was then shaken for 5 minutes, which ensured the through mixing of the blood with diluting fluid.

The improved neubaur counting chamber was used for counting the cells. A glass cover slip was placed over the 'H' groove in the chamber. Then the blood was allowed to enter the chamber by simply touched the edge between the coverslip and the chamber where the blood spread under the coverslip by capillary action.

The counting area is divided into 25 squares of 16 small squares. Only 5 squares were taken to count RBC marked as E1-E2. Two minutes time was given for the corpuscles to settle down. For counting the total number of RBC, the total number of cells in the five squares was multiplied by 10^6 which gives the total number of RBC per mm^3 of blood.

WBC COUNTING

For counting the total number of WBC the pipette with white bead was used. The number of cells present in the four large corner squares marked by capital letter 'L' was counted and multiplied by 10^3 which gives the total number of WBC per cubic millimetre of blood.

HAEMOGLOBIN CONTENT(Hb)

Haemoglobin determination is the quickest means for detecting anaemia. However, many factors are known to influence the haemoglobin level. The Sahli hellige method was followed for haemoglobin determination. Sahli's pipette was filled slightly above the 20 mm mark; the pipette was wiped with a filter paper or cotton to remove excess blood and the volume was adjusted to exactly 20 mm^3 by blotting the tip. The blood was expelled into a calibrated (transmission) test tube containing 2 ml of 0.1 N Hcl. The pipette was rinsed several times in the acid solution. The sample was allowed to stand for 15 minutes.

The principle behind the method is the conversion of haemoglobin to acid haematin. The acid haematin was then diluted with distilled water till colour matched with colour of the standard in haemoglobinometer. The height of the column at which the match obtained gives the value of haemoglobin in g%.

HAEMATOCRIT (Ht)

After the determination of Hb, the capillary tubes inside the wintrobe tubes were centrifuged at 3000 rpm for half an hour. After centrifugation three layers, i.e. bottom layer of packed erythrocytes, middle puffy coat and upper plasma layer were noted. The haematocrit value was estimated by using the following formula

$$L1 / L2 \times 100$$

Where L1 is the length of RBC column, L2 is the total length of the column (RBC + plasma + buffy coat). Haematocrit is expressed in percentage.

RESULTS

The blood parameters like total RBC count, WBC count, haemoglobin content and haematocrit were estimated in the blood of *C. carpio* exposed to Lc50 value of lead nitrate (4.45 ppt) concentrations are presented in Table 1. The total RBC count showed a decreasing tendency. The RBC count was 3.45 million/cu.mm in the blood of control fish and this count was decreased to 3.20 millions/cu.mm in the Lc50 (4.45 ppt) concentration of lead nitrate at 96 hrs of exposure (Table 1). The decreasing trend was a function of exposure periods. The percentage change in the RBC count was also calculated and presented in Table 2 and Figure 1.

The WBC count exhibited a different trend when compared to the RBC count. It exhibited a slight increasing trend as the increasing number of days (Table 1). The WBC count, which was 4645 cells/cu.mm in control and increased gradually to 4900 cells/cu.mm when exposed to Lc50 (4.45 ppt) concentration of lead nitrate at 96 hrs of exposure period. The increasing trend was a function of exposure periods. The percentage change in the WBC count is expressed in Table 2 and Figure 2.

The haemoglobin content of blood of *C. carpio* was 7.98 % in control medium. The Hb content decreased when exposed to Lc50 (4.45 ppt) concentrations of lead nitrate (Table 1). This was estimated as 6.0 in the blood of fish exposed to lead nitrate at 96 hrs of exposure period. The reduction in Hb content is presented in Table 2 and Figure 3. The reduction in Hb count was gradually increasing with increasing exposure periods. The decreasing trend in Hb content is comparable to that of RBC count in the blood of fish exposed to different durations.

Haematocrit also showed a declining trend in Lc50 (4.45 ppt) concentration of lead nitrate and with increasing exposure periods (Table 1). This trend is comparable to the trend of RBC count. The percentage change in haematocrit in the fish exposed to lead nitrate at different exposure periods was also calculated and expressed in Table 2 and Figure 4.

DISCUSSION

The effects of Lc50 (4.45 ppt) of lead nitrate on haematological parameters of *C. carpio* at different durations of exposures (24, 48, 72 and 96 hrs) are generally gradual and at 96 hrs of exposure it is very significant. The RBC and Hb content reduced, such reduction may be due to damage and destruction of blood cells[8, 10]. Stressors and pollutants generally caused changes in blood parameters[2]. A clear cut evidence of reduction in RBC count and Hb content has been reported in *L. rohita*[14, 26, 29], *C. mrigala*, *C. carpio* respectively when exposed to lead.

The reduction in RBC count either by haemolysis or erythropoietic disorders and also the reduction in Hb contents by haemopoietic disorders lead to anaemic condition in fish. The anaemic condition in fishes is attributed to an inhibition on erythrocyte production or haemodilution[15]. The anaemia may affect the general well being of the fishes. The haemoglobin properties was altered by heavy metals such as cadmium, chromium, nickel and lead by losing their oxygen binding property, which finally causing erythrocytic damage[30]. The results are in accordance with earlier reports stated that a significant decrease in RBC's haemoglobin and packed cell volume of freshwater fish exposed to heavy metals [28, 24]. The cyprinid fish *L. rohita* exposed to lead, the RBC, WBC, Hb and Ht decreased significantly ($p< 0.01$) [1].

In the present investigation the total WBC exhibited a very slight increase when the fish exposed to Lc50 (4.45 ppt) concentration of lead nitrate at 96 hrs of exposure. This increase is considered as an adaptive mechanism. This may be due to the direct stimulation of the immunological defense mechanism against stressor[11]. Such increase in WBC count may be due to lymphocytosis and immune response in lead exposed fish[25, 21, 22, 5].

There is no alteration in WBC count in fish exposed to waterborne lead (at concentrations of 0.3- 10 mg/l). In a study done by Witeska M et al., the WBC maintained a level similar to control[29].

Decrease in haematocrit content of blood is in accordance with the decrease in RBC count. The similar results was obtained in Coho salmon and in *Anabas testudineus* respectively[18, 20]. A decrease in RBC and Hb but not Ht in *Tilapia zilli* exposed to 8.3 mg/l of pb9. But a decrease in RBC, Hb and Ht was observed in *Oreochromis aureus* treated with 10 mg/l of pb [4]. RBC and packed cell volume decreased significantly when *C. carpio* exposed to combined heavy metals [27].

The study of haematological parameters gave the valuable information about the effect of water pollutants in fish[23].

CONCLUSION

Fish act as biological indicator of aquatic pollution. Analysis of biochemical components of fishes very helpful for the study of water quality and therefore ensures safety food for people.

Table 1

Effect of Lc50 (4.45 PPT) of lead nitrate on haematological parameters of *C. carpio* at different durations of exposure. Each value is the mean (± SD) of at least 3 estimations.

Haematological Parameters	Concentration (PPT)	Alterations of Haematological parameters at different durations of exposure. Each value is the mean (+ SD) of atleast 3 estimations.			
		24 h	48 h	72 h	96 h
RBC	Control	3.45 ± 0.30	3.45 ± 0.30	3.40 ± 0.20	3.40 ± 0.15
Millions/cu.mm	Experiment	3.40 ± 0.20	3.35 ± 0.15	3.30 ± 0.20	3.20 ± 0.18
WBC	Control	4645 ± 80	4646 ± 110	4640 ± 90	4640 ± 80
Cells/cu.mm	Experiment	4700 ± 90	4765 ± 100	4850 ± 110	4900 ± 105
Haemoglobin	Control	7.98 ± 0.005	7.98 ± 0.006	7.990 ± 0.007	7.98 ± 0.005
g/dl	Experiment	7.50 ± 0.007	7.00 ± 0.008	6.50 ± 0.009	6.00 ± 0.006
Haematocrit	Control	39.70 ± 0.77	39.70 ± 1.10	39.50 ± 0.90	39.50 ± 1.10
(%)	Experiment	39.00 ± 0.95	38.50 ± 1.15	38.00 ± 1.25	37.50 ± 1.20

Table 2

Percentage change in Haematological parameters of *C. carpio* when exposed to Lc50 of lead nitrate at different periods of exposure.

Haematological parameters	Percentage change of haematological parameters at different duration of exposure			
	24 h	48 h	72 h	96 h
RBC	(-) 1.450	(-) 2.898	(-) 2.941	(-) 5.882
WBC	(+) 1.184	(+) 2.561	(+) 4.526	(+) 5.603
Haemoglobin	(-) 6.015	(-) 12.280	(-) 18.648	(-) 24.812
Haematocrit	(-) 1.763	(-) 3.023	(-) 3.797	(-) 5.063

(+) = indicates increase
(-) = indicates decrease

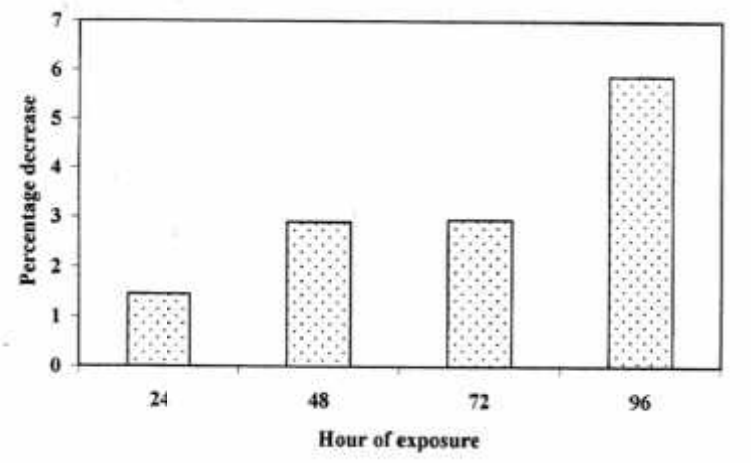

Fig. 1

Percentage decrease in haematological parameter (RBC) of lead nitrate treated *C.carpio* at different durations of exposure

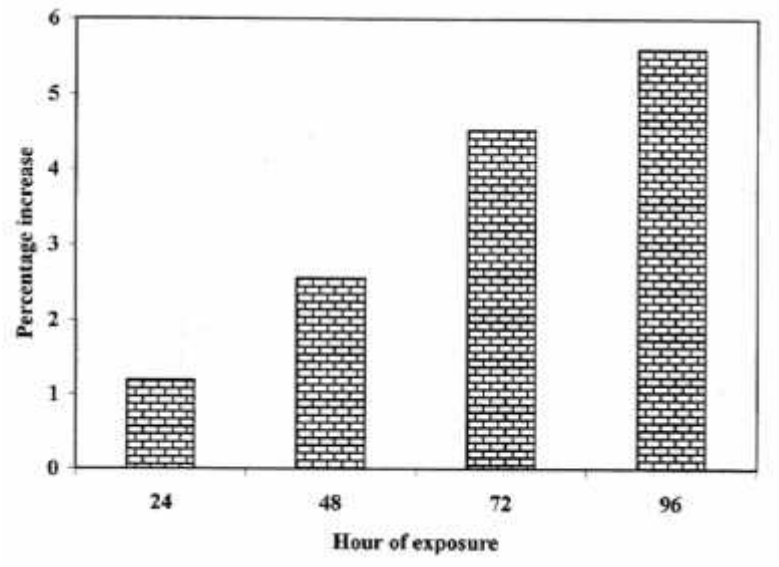

Fig. 2
Percentage increase in haematological parameter (WBC) of lead nitrate treated *C.carpio* at different durations of exposure

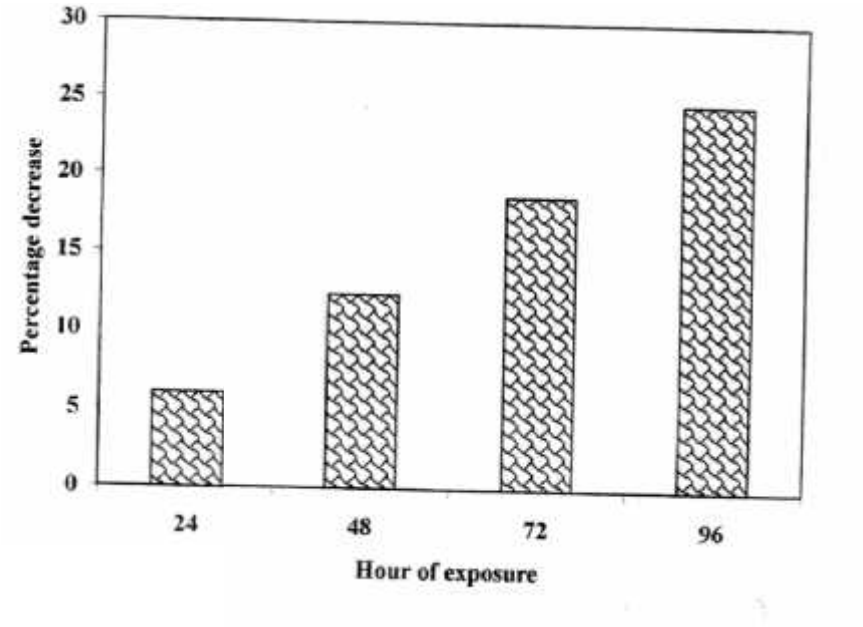

Fig. 3
Percentage decrease in haematological parameter (Hb) of lead nitrate treated *C.carpio* at different durations of exposure

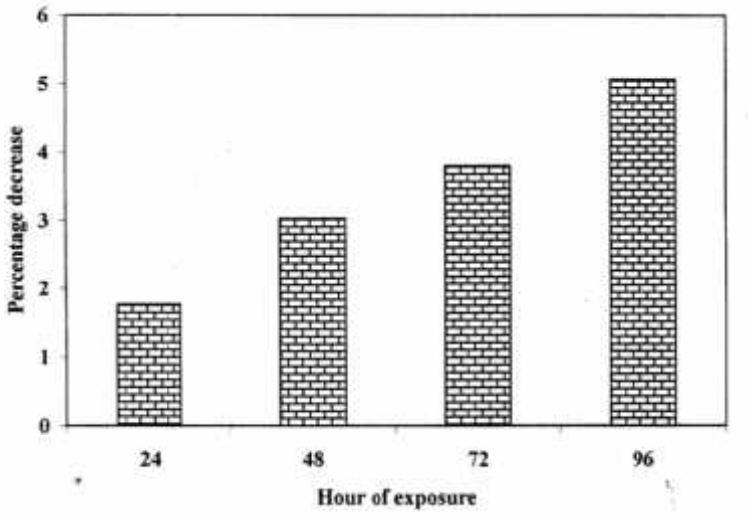

Fig. 4
Percentage decrease in haematological parameter (Haematocrit) of lead nitrate treated *C.carpio* at different durations of exposure

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