International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979, Impact Factor: RJIF 5.72
www.allsubjectjournal.com
Volume 4; Issue 5; May 2017; Page No. 257-260

Toxicological impact of heavy metal, chromium on heamatological parameters in fresh water teleost fish *catla catla*

Dr. P Ranganatham

Department of Zoology, The Adoni Arts and Science College, Adoni, Andhra Pradesh, India

Abstract
The fingerlings of a fresh water teleost fish, *Catla catla* exposed to sublethal concentrations of chromium for 1, 8, 16, 32 days of time brought changes in the hematological parameters like RBC and WBC count were studied in the present investigation. The heavy metal, Chromium, an industrial effluent; an aquatic pollutant enters into the food chain of ecosystems responsible for changes in the hematological parameters of the animals such as the fresh water fishes.

Keywords: chromium, catla catla, hexavalent chromium, hematology

1. Introduction

The significance of blood parameters in clinical biochemistry, population genetics and medical anthropology is well established. Recent studies have proved that they may be used as valuable indicators of disease or stress in animals (Calabrese *et al.*, 1975). Haematological indices are very important parameters for the evaluation of fish physiological status. For so many years the study of hematological parameters has been used as diagnosing tool in investigating disease, physiological and metabolic alterations (Bansal *et al*., 1979) [3]. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners, and diseases (Luskova, 1997; Golovina, Trombicky, 1989; Golovina, 1996; Zhiteneva et al., 1989) [11, 8, 7, 18].

Hematological investigation on fishes has confirmed that the variations in the blood give indication of health condition and environmental changes (Scharperclaus, 1986; Nath et al., 2006). The study of blood parameters has gained momentum in recent years in view of their importance in assessing the condition of fish and their responses to environmental changes (Bayne et al., 1980). Blood alterations caused due to damage to the haemopoitic organs in these organisms may also be associated with pathological conditions related to water borne pollutants (Reichenbach and Klinke, 1966; Gardner and Yevich, 1970; Sadd, 1973; Dawson, 1975). Hematological indices vary from animal to animal and in the same animal at different stages of life. In fish, the hematological parameters are reported to vary under stress (Chakrabarthi et al., 1965). Mahajan and Dheer (1980) have shown that changes in relative population of certain cell types in the peripheral blood of fish particularly neutrophils, thrombocytes and erythrocytes when considered together could serve as good indicators of pollution stress at sublethal levels.

Hematological parameters of fish are closely related to the response of fish to environmental and biological factors. Hematological parameters can provide information on nutrient status, digestive function, and routine metabolic level of fishes. Fishes may be confronted with stress factors such as varied water qualities, pollution, and disease. Fishes can adapt themselves to bad environmental conditions by changing their physiological activities. A number of hematological indices such as RBC, WBC are used to assess the functional status of the blood stream and have been used as an indicator of metal pollution in aquatic environment.

Materials and Methods

Catla catla (Hamilton, 1822), the Indian major carp is an economically important edible fish, having a great commercial value, occurs abundantly in fresh water tanks and ponds, collected from the department of fisheries, Anantapur, Andra Pradesh, and were immediately transported in big fish containers in the laboratory Then they were released into large cement tanks contained of chlorinated tap water. The fish were fed with commercial fish pellets having around 40% protein content, and allowed to acclimatize for 15 days. Then the fish were isolated into batches having weight of 10±2gms were maintained in static water without any flow. Water was renewed every day to provide fresh water rich in oxygen. The quality of dechlorinated tap water used for the experiment was analysed and various parameters such as dissolved oxygen - 6.8mg/l, alkalinity-130mg/l, hardness-125mg/l and pH-7.3 were measured and maintained. Water temperature was maintained between 22 ± 3ºC as recommended by APHA during experiment. Water was aerated once a day to prevent hypoxic conditions. As the level of toxicity reported to vary with the interference of extrinsic and intrinsic factors like temperature, salinity, P^H, hardness of water, exposure period, density of the animals, size, sex etc., (Sivaramakrishna et al., 1991), and precautions were taken throughout this investigation.

Lethal concentration (LC50) of chromium chloride (trivalent and hexavalent) to fish *Catla catla* was determined by "Probit method" of Finney (1971). Based on the fact that the effect of a metal on fish becomes consistent within 96 hour of exposure (Eisler, 1977), LC_{50S}/96 hours of trivalent and hexavalent chromium are considered as lethal concentrations. So, about

1/10 th of the 96 h LC_{50} lethal concentration was taken as sublethal concentration i.e., 59.68mg/l, 100 mg/l(Cr as 35.40mg/lit) were the lethal concentrations, 5.96 mg / l of trivalent chromium and 10 mg /l(Cr as 3.54 mg/lit) of hexavalent chromium respectively was taken as the sublethal concentration for further studies.

For experimentation, 200 healthy fishes were taken and divided into two batches, each batch again divided into 5 groups and each group contains 20 fishes. Batch-1 was exposed for sublethal concentration (1/10 of LC50) of trivalent chromium and Batch-2 was exposed for sublethal concentration of hexavalent chromium. The I-group fishes of two batches was controlled unexposed, II-Group exposed for 1 day, III-Group for 8 days, IV-Group for 16 days and V-Group for 32 days of exposure.

Catla catla exposed to sublethal concentrations for 1, 8, 16 and 32 days of trivalent and hexavalent chromium, at the end of each exposure period the healthy fishes were taken out, the blood from the control and treated fingerling was collected from incision at the caudal vein region into the heparinized capillary tubes for hematological studies. The treated and control blood samples were used to estimate the hematological parameters.

RBC count was made with a Neubauer crystalline counting chamber as described by Samuel (1977). The RBC number was determined in *Catla catla* at different sublethal exposure periods like 1, 8, 16 and 32 days including control medium. The RBC number was determined in 6 individual fish at each exposure period including control medium. The red blood cell number was made with Nuebauer Crystalline counting chamber. The blood collected was diluted with Hayem's fluid (5gm of sodium sulphate, 1gm of sodium chloride and 0.5 gm of mercuric chloride dissolved in 200 ml of distilled water) and the RBC number was represented in millions per cubic millimeter (mm3). The Hayem's fluid was taken upto '0' mark in the RBC pipette and it was mixed thoroughly by rotating the pipette and the mixture was allowed to stand about 2-3 minutes for uniform mixing. The counting chamber and cover slip were cleaned and the coverslip was placed over portified area. Again the solution was mixed gently and the stemful of solution was expelled and a drop of fluid is allowed to flow under coverslip by handling the pipette at an angle of 90. It was allowed to stand for 2-3 minutes till the RBC are settled. Afterwards, the portified area of the counting chamber was focused under the microscope and the RBC are counted in five small squares of the RBC columns (the RBC were counted in the outer four corner squares and the central square) under high power and the number of RBC per cubic millimeter (mm3) are calculated using the following formula. (Number of cells × Dilution factor (200) × Depth factor) / Area counted= millions/mm3.

WBC count was estimated by Samuel (1977). The blood is mixed with a weak acid solution, it haemolyses the red blood cells leaving only WBC's. As the number of WBC are usually more, blood samples are diluted in the thumb pipette taking blood upto 0.1 mark and diluted it upto 11 mark with the diluting fluid known as Shaw's solution. It has two solutions as follows. White Blood Cell count fluid: (Shaw's solution, 1930).

Solution A: Sodium chloride (0.900 gm), Neutral red (0.025gm), Distilled water (100 ml)

Solution B : Crystalline violet (12.0 mg), Sodium citrate(3.8 gm), Formaldehyde solution (40% w/v) 0.4 ml), Distilled water(100 ml)

Both the solutions were filtered and mixed in equal volume before starting the experiment. Thus hundred fold dilution of the original sample was obtained. The diluted blood was changed into Neubauer's chamber taking the precautionary measures and the number is expressed in thousand's per cubic millimeter using the following formula.

$$\text{White Blood Cells / cu mm} = \frac{\text{Number of white blood cells counted} \times \text{correction}}{\text{Volume} \times \text{correction for dilution}}$$

Results and Discussion

For comparative assessment, the differences obtained in relation to controls in each parameter at the said exposure periods were converted as mean values and percentages of the corresponding controls, the mean values and percent values were also given in the respective table-4 and plotted graphs of mean values against exposure periods in Figures 3 and 4(RBC count), 5 and 6 (WBC count).

From the results obtained the data is presented in Table-4. It is seen that, relative to controls, the number of RBC significantly increased at day 1 and significant decrease at day 8 and then gradually increased at day 16 and 32 in the fish exposed to trivalent chromium in the order 1>8<16<32. In the fish exposed to hexavalent chromium also a significant decrease in the RBC number was observed at day 1 and 8 followed by a significant increase at day 16 and at day 32 in the order 1>8<16<32. RBC count, Relative to controls, the mean values and percent values were also given in the respective Table-4 and plotted graphs of mean values against exposure periods in Figures 4 and 5.

From the results obtained the data is presented in Table-4. It is seen that, relative to controls, the number of WBC significantly increased at day 1 and decreased at day 8 and then increased at day 16 and decreased at day 32 in the fish exposed to trivalent chromium in the order 1>8<16>32. In the fish exposed to hexavalent chromium, relative to controls, a significant decrease in the WBC number was observed at day 1 and 8 and gradually increased at day 16 and 32 nearly reached at normalcy at day 32, in the order 1>8<16<32. WBC count, Relative to controls, the mean values and percent values were also given in the respective Table-4 and plotted graphs of mean values against exposure periods in Figures 6 and 7.

The hematological parameters like RBC and WBC count were studied in the present investigation in the fish exposed to trivalent and hexavalent chromium. In the present study, In trivalent chromium exposed fishes, the initial increase in RBC count at day 1 may indicate the enhanced erythropoiesis which is triggered as initial typical toxic stress of trivalent chromium and decreased at day 8 is observed may be due to reduction in the rate of production and /or increase in the destruction of RBC as suggested by Larson (1975) [9] in winter flounders, *Pleuronectus flexus*, exposed to cadmium. And later, an increase in RBC at day 16 and 32 is due to restoration near to

normalcy in the organs and regained the erythropoiesis in the organs.

In hexavalent chromium exposed fishes, a decrease was observed in RBC count at day 1 may be due to immediate toxic stress is responsible for reduction in the rate of production and / or increase in the loss or destruction of RBC and was progressively increased in number of RBC at days 8, 16 and 32 is due to increase in erythropoiesis regained the erythropoietic capacity of the organs such as liver and kidney are reasons for reaching near to normalcy in RBC number. Erythrocyte count in the blood of Heavy metal model mixture exposed to rainbow trout was significantly lower as compared to control, even in fish exposed to the lowest concentration of Heavy metal model mixture (S.S. Chandanshive *et al.*, 2012) [4].

The WBC number in trivalent chromium exposed fishes was increased at day 1 and 16 and a slight decrease at day 8 and 32. In hexavalent chromium exposed fishes there was a decrease at day 1 and increased gradually from day 8, 16 and 32. The WBC increase may be an adaptive response to the new environment and as a potent defense mechanism of the fish against the toxic entities (Sabitha Borah, 2006) [12].

Table 4: RBC and WBC count (million/mm3) in the blood of *Catla catla* at different periods of exposure to sublethal concentration of trivalent and hexavalent chromium. Each value is a mean of six replicants. Percent change over the respective control is given in parentheses. S.D. ± : Standard Deviation P : Level of Significance P : Level of Significanc

Organ		No of days exposed								
			Trivalent Chromium				Hexavalent Chromium			
		Control	1	8	16	32	1	8	16	32
RBC million/ cumm	Mean S.D. %	2.34 ±0.084	2.48 ±0.027 (+5.98)	1.94 ±0.021 (-17.09)	2.11 ±0.041 (-9.82)	2.21 ±0.047 (-5.55)	1.52 ±0.024 (-35.04)	1.26 ±0.041 (-46.15)	1.85 ±0.047 (-20.94)	2.21 ±0.023 (-5.55)
WBC million/ cumm	Mean S.D. %	6.91 ±0.042	7.42 ±0.036 (+7.38)	6.81 ±0.043 (-1.44)	7.51 ±0.038 (+8.68)	6.11 ±0.014 (-11.57)	5.04 ±0.041 -27.06	6.39 ±0.024 (-7.52)	7.78 ±0.034 (+12.59)	7.98 ±0.052 (+15.48)

*Denotes not significant with control (P>0.005)

The differences between control and experimental are statistically significant (P<0.005). All are statistically significant.

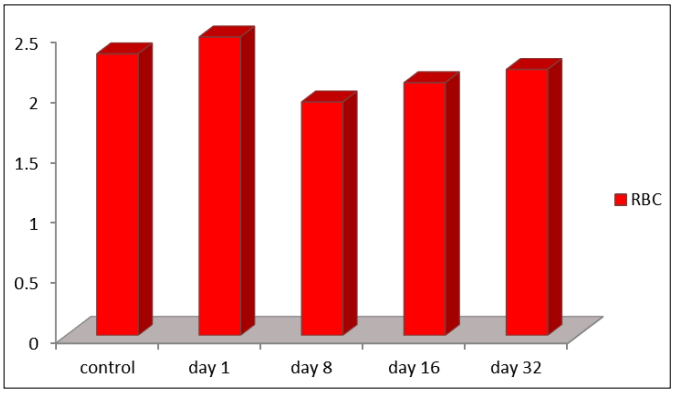

Fig 3: RBC Count (millions/mm3) in trivalent Cr exposed fishes

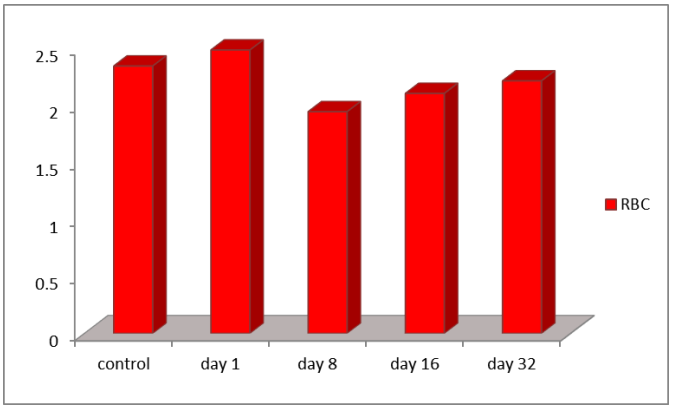

Fig 5: RBC Count (millions/mm3) in hexavalent Cr exposed fishes

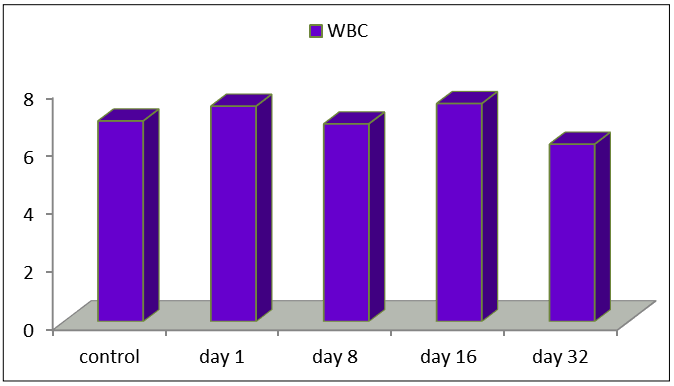

Fig 5: WBC Count (millions/mm3) in trivalent Cr exposed fishes

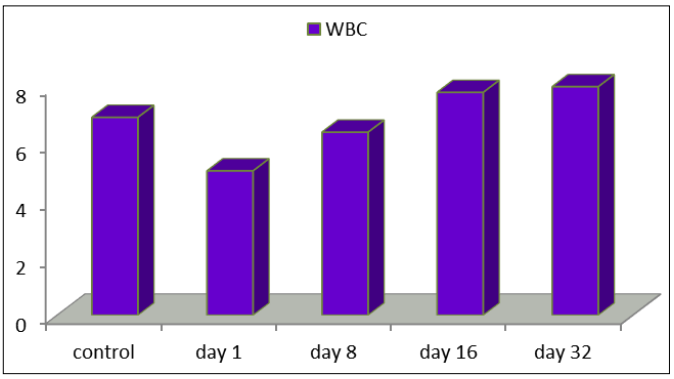

Fig 6: WBC Count (millions/mm3) in hexavalent Cr exposed fishes

References

1. Alkinson J, Judd FW. Comparative haematology of *Lepomis microlophus* and *Chicchlasoma cyanoguttaum*. Copeia. 1978; 12:1230-1237.

2. Alkinson J, Judd FW. Comparative haematology of *Lepomis microlophus* and *Chichlasoma cyanoguttatum*, Copeia. 1987; 12:230-237.
3. Bensal SK, Verma SR, Gupta AK, Daleia RC. Physiological dysfunction of haemopoietic system in a fresh water teleost, *Labeo rohita* following chronic chlordane exposure part-I alterations in certain haematological parameters. Buill. Environ. Contam. Toxicol. 1979; 22:666-673.
4. Chandanshive SS, Sarwade PP, Atul Humbe1, Mohekar AD. Effect of heavy metal model mixture on haematological parameters of *Labeo rohita* from Gharni Dam Nalegaon, Latur International Multidisciplinary Research Journal. 2012; 2(4):10-12. ISSN: 2231-6302.
5. Folmar JC. Effects of chemical contaminants on blood chemistry of teleost fish: a bibliography and synopsis of selected effects. Environ. Toxicol. Chem. 1993; 12:337-375.
6. Gill TS, Pant JC. Cadmium toxicity inducement of changes in blood and tissue metabolites in fish. Toxicol. Lett. 1983; 18:195-200.
7. Golovina NA. Morpho functional characteristics of the blood of fish as objects of aquaculture. Doctoral thesis. Moscow. 1996; 53. (in Russian).
8. Golovina NA, Trombicky ID. Haematology of pond fish. Kishinev. Shtiinca. 1989, 158. (in Russian).
9. Larsson A. Some biochemical effects of cadmium on fish. In: Koeman J.H., Stirk J.J.T.W.A. (eds). Sublethal effects of toxic chemicals on aquatic animals. Elsevier, Amsterdam. 1975, 3-13.
10. Lagler KF, Bardach JE, Miller RR, May Passino DR. Blood and circulation. In: Icthyology, 2nd edition, John Wiley and Sons, New York. 1977, 213.
11. Luskova V. Annual cycles and normal values of hematological parameters in fishes. Acta Sc. Nat. Brno. 1997; 31(5):70.
12. Sabita Borah. Haematological and respiratory anomalies in the fresh water fish *Heteropneuster fossilis* exposed to crude oil. J. Ecotoxicol. Environ. Monit. 2006; 16(3):285-289.
13. Saravanan TS, Harikrishna R. Effect of sublethal concentrations of copperand endosulfan on haenmatological parameters of fresh water fish, Saro therrodon mossambicus (Trewaves). J. Ecobiol. 1999; 11(1):13-18.
14. Saravanan TS, Harikrishna R. Effect of sublethal concentration of copper and endosulfan on haematological parameters of fresh water fish, *Sarotherrodon mossambicus* (Trewaves). J. Ecobiol. 1999; 11(1):13-18.
15. Srivastava AK, Mishra J. Effects of Lindane on blood chloride in the Indian Cat fish, *Heteropneustes fossils*, Acta. Hydrobilo. 1982; 24:175-192.
16. Srivastava AK, Singh NH. Effect of acute exposure of methyl parathion on carbohydrate metabolism of Indian catfish, *Heteropneustes fossilis*. 1981; 48:26.
17. Vosylienë MZ. The effect of heavy metal mixture on haematological parameters of rainbow trout. Heavy metals in environment. An integrated approach. Ed. D.A. Lovejoy. 1999, 295-298.
18. Zhiteneva LD, Poltavceva TG, Rudnickaja OA. Atlas of normal and pathological cells in the blood of fish. Rostov-on-Don. 1989, 112.