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THE HAEMATOLOGICAL CHANGES OF FRESH WATER TELEOST FISH *CYPRINUS CARPIO VAR. COMMUNIS* EXPOSED TO ACUTE ALUMINIUM SULPHATE TREATMENT.

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ABSTRACT

The present study discusses the haematological changes of the fish *cyprinus carpio* that exposed to aluminium sulphate for 96 hrs. The haematological changes based on the examination of some blood variables during 96 hrs of exposure have been reported. The blood parameters were: haemoglobin, RBC, WBC, Hematocrit, MCHC, MCH and MCV. Results showed haemoglobin WBC and MCH were significantly higher when compared with control groups, while there was significant reduction in RBC, PCV, MCHC and MCV. This result indicates that acute level of aluminum can alter the blood parameters level of fish.

Keywords: Aluminium,Cyprinus carpio, Acute study , Blood parameters

INTRODUCTION

Environmental pollution represents a major problem in both developed and undeveloped countries (Kazi *et al*., 2009; Ozden, 2010). In spite of their natural occurrence in the aquatic ecosystem, metals represent a major environmental problem of increasing concern, and their monitoring has received significant attention in the field (Pandey *et al*., 2003; Barnhoorn and van Vuren, 2004) and under laboratory conditions (Long *et al*., 2003; Osman *et al*., 2007). Aluminum Al is a harmful metal to the aquatic ecosystem, being responsible for events of toxicity with serious ecological consequences (Correia *et al*.,2010). It is also found in the

atmospheric air of the big cities and industrialized areas (Casarini *et al*.,2001), and is used as a flocculation agent in water treatment (Silva *et al*., 2007; Camargo *et al*., 2009).

Haematological examinations have been used as indicator of the physiological stress response to endogenous or exogenous changes and product systematic relationships and physiological adaptations of animals (Lermena et al., 2004; Kocabotmaz and Ekingen, 2000). This work aims to test the haematological changes manifestations associated with the aluminium toxicity of the fish *Cyprinus carpio*.

MATERIALS AND METHODOLOGY

Fish were acclimatized to laboratory conditions for about 15 days, before the commencement of the experiment. During this period, fish were fed, **ad libitum** with rice bran and groundnut oil cake once in a day. After feeding, water was changed daily in order to maintain clear environment and [to avoid any accumulated metabolic waste] and aerated to ensure sufficient, oxygen supply. The median lethal concentration [LC50] of Aluminium Sulphate was determined for 96 hours by probit analysis method of Finney [1978]. The LC50 value of Aluminium sulphate in fish *Cyprinus carpio* for 96 hrs was 22.64 ppm. Fish were exposed to acute concentration for a period of 96 h and common control was maintained for test. After acute treatment, the fish from acute and Control group were sacrificed for blood parameters test.

Blood was taken by cardiac puncture using plastic disposable syringe. The collected blood was rinsed with heparin [anticoagulant] in eppendorf tubes. The blood was used to determine the number of erythrocytes by means of a Neubauer hemocytometer slide at a magnification of ×400. The blood was diluted to 1:50 in 0.9% (w/v) saline. Count the erythrocytes occurring in five small squares at the centre of the grid, a total area of 0.02 mm^3 (1/50 of 1 mm^3). The total area counted here (0.02mm^3, at a dilution of 1:50) should be sufficient for an accurate count to be obtained. The dilution is 1:50, therefore the number of cells occurring per mm^3 may be calculated as follows:

Number of cells occurring per mm^3 = Number of cells counted in 0.02 mm^3 × 50 (area counted) × 50 (dilution) [Russia and Sood, 1992].

The blood was used to determine the number of leucocyte by means of a Neubauer hemocytometer slide at a magnification of ×400. The blood was diluted to 1:50 in Dacies fluid [Abdelhamid *et al.*, 2009]. Count the leucocytes occurring in the four corner squares marked on the grid, a total area of 0.1 mm^3. The total area counted here (0.1 mm^3, at a dilution of 1:50), should be sufficient for an accurate count to be obtained. The dilution is 1:50, therefore the number of cells occurring per mm^3 may be calculated as follows:

Number of cells occurring per mm^3 = Number of cells counted in 0.1 mm^3 × 10 (area counted) × 50 (dilution) [Russia and Sood, 1992].

The cyanohemoglobin method has been the standard method used in hematological studies for a number of decades. Add 20 µl of blood and 5 ml of Drabkin's solution in a test tube were mixed thoroughly. It stands for 10 minutes and optical density of the solution was measured at 540 nm using spectrophotometer [Drabkin, 1964].

Hematocrit capillary tubes were two-third filled with the whole blood and centrifuged in a hematocrit centrifuge for 5 min at 13500 rpm and the percentage of the packed cell-volume was determined by the hematocrit tube reader [Nelson and Morris, 1989]. Red blood cell indices are that provide information about the content and size of [Benfey and Sutterlin, 1984]. Mean corpuscular volume (MCV) is the average size of a red blood cell and is calculated by dividing the by the red blood cell count.

$$MCV = \frac{Hct}{RBC}$$

Mean corpuscular hemoglobin (MCH) is the average amount of (Hb) per red blood cell and is calculated by dividing the hemoglobin by the red blood cell count.

$$MCH = \frac{Hb}{RBC}$$

Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin per red blood cell and is calculated by dividing the hemoglobin by the hematocrit.

$$MCHC = \frac{Hb}{Hct}$$

The Statistical analysis was performed by using the student 't' test to compare the significant values between the control and treatment

RESULT AND DISCUSSION

The present study was analysis the effect of aluminiumsulphate on haemotological parameters [hemoglobin, crythrocytes, white blood cell count, Hematocrit, mean cellular Haemoglobin concentration, mean cell valume and mean cell Hemoglobin], of Fish *Cyprinus*, *Carpio*Var. *Communis* was investigated during acute treatment.

During the study period, the physio – chemical features were maintained at constant level. The observed result was presented in table-1. In acute treatment, haemoglobin, WBC and MCH were found an increase level of 61.01%, 52.46% and 70.47% at the end of 96 hrs respectively. However, RBC, PCV, MCHC and MCV was noted a decreased level of +.18%, 26.38%, 26.30 and 20.69% during acute treatment statistical analysis revealed the significant changes in hemoglobin, RBC, WBC, PCV, MCHC, MCH and MCV level were analyzed by control.

Table 1 : Changes in the blood Parameters of *Cyprinus Carpio* var. *Communis* during acute [LC50 96 h] aluminium sulphate treatment

Parameters	Control	Acute Treatment	't' Test Value
Haemoglobin [g/dl]	3.3450± 0.112	5.386 ± 0.207 [+61.02]	8.64**
Erythrocytes [Million/cu.mm]	1.899 ± 0.0006	1.763 ± 0.018 [-1.18]	8.55**
WBC [thousand/cu.mm]	15.287 ±0.073	23.307 ±0.122 [+52.46]	56.24***
Hematocrit	4.238 ± 0.031	3.120 ±0.032	25.09***

[%]		[-26.38]	
MCHL [g/dl]	78.912 ± 2.558	58.155 ±2.882 [-26.30]	5.38**
MCH [pictograms]	17.608 ± 0.599	30.108± 2.015 [70.47]	5.90**
MCV [cubic micra)	22.312 ± 0.168	17.695± 0.070 [-20.69]	25.32***

Values are mean ± S.E of 3 individual observations
'+' - % increase over control; '-', % decrease over control
Significant at * P < 0.05; ** P < 0.01; and *** P <0.001; ns – Not significant

Haematological parameters are routinely used for the evaluation of physiological environmental and husbandry stressors in fishes (Fanouraki, *et al*., 2007). Several factors have been reported to affect haematological responses in fish. These include sex, age, size, environmental and physiological conditions (Sowunmi, 2003). However, in the present study, the decreases in blood parameters of *Tinca tinca* were attributed to: (i) impairment in gill tissues, (ii) stress-mediated hormonal imbalance, (iii) rupturing of erythrocytes in skin lesions by direct action of the parasite, and (iv) haemodilution caused by haemorrhage. The mortality was caused by the breakdown in osmotic balance when the tissues (skin and muscular layer) were destroyed by the penetration of the hyphae and the lethargy that resulted from excessive energy exerted to overcome infection stress. The increase in mucus thickness on the gills observed in the present study may increase the diffusion distance between water and blood haemoglobin, which may rapidly impair O2 and CO2 exchange. The impairment in gas exchange triggers erythropoiesis to increase the number of erythrocytes to maintain haemoglobin at normal levels. However, it is possible that prolonged or continuous stimulation of a system may cause suppression or exhaustion of this capacity, resulting in decreased erythrocytes, as reported for leucocytes in fish [Gill and Pant, 1985].

It is concluded that the fish hematological parameters, such as HB, RBC and PCV are decreased showing anemia conditions, where as the WBC count is increased. Toxicants caused hyperproteinaemia, hyperlipaemia, hypoglycemia and hypochalesterolemia [Sudha Summarwar, 2012].

The mean corpuscular values are concerned with the volume of the average erythrocyte and the amount of haemoglobin in the average erythrocyte and the three types are Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC), which measures the Volume, Weight and the Concentration of Haemoglobin respectively (Wedemeyer *et al.*, 1983). The values of this indices recorded in this study agrees with the findings of Anyanwu *et al.*, (2007) who observed same in black jaw tilapia transfer directly from brackish water to fresh water. The decreased value of MCHC and the increase values of MCH and MCV indicates the extent of the shrinking cell size of erythrocytes stress induced by acclimation.

Finally the present study suggests providing the valuable information for fish biologist in the assessment of fish health and monitoring stress response we assume that the variation of blood indices may be a defensive mechanism against stress induced by metal toxicity of fish.

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