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Changes in Haematological Parameters of the Fish, *Oreochromis niloticus* Treated with Sublethal Concentration of Cadmium

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Abstract: The sub-chronic and chronic exposure of Tilapia, *Oreochromis niloticus* to sublethal concentration of 5.5 ppm cadmium (0.3 of 96 h LC₅₀) for 1, 3 and 5 weeks was studied. Exposure of Tilapia to cadmium resulted into a number of haematological alterations. Erythropenia, a marked decrease in values of haemoglobin content, haematocrit and mean corpuscular haemoglobin were observed at all experimental periods of cadmium exposure. The values of mean cell volume were decreased at the second and last periods. The levels of mean corpuscular haemoglobin concentration decreased significantly at first period compared to the control value.

Key words: Cadmium, toxicity, blood, *Oreochromis niloticus*

INTRODUCTION

Aquatic organisms are in direct contact with the environment and are susceptible to any change that may occur. The introduction of many of the relatively toxic heavy metal cations in small amounts into an aquatic environment causes multiple changes in the internal dynamic of aquatic organisms, even at sublethal levels. By measuring specific physiological and biochemical alterations in the blood of fish exposed to short periods of sublethal stressors may provide a sensitive method for predicting the effects of chronic exposure and survival, reproduction and growth^[1]. The effects of heavy metals have been reviewed extensively by various researchers in the field of fish biology^[2-5]. The range of their stressful effects is quite broad and encompasses behavioural modification, alterations in feeding strategies, swimming activity, schooling, predator-prey avoidance, reproduction, learning^[6], disturbed osmoregulation^[7,8], respiratory stress^[9] as well as tissue damage^[10-12].

Haematology is often used to detect physiological changes following different stress conditions, or for example exposure to metals. Thus haematology can be considered as an essential index to the general health status. The most common haematological variables measured during stress included red and white blood cells count, haemoglobin content, haematocrit value and red blood cell indices. Fish haematological parameters are often determined as an index of their health status^[13]. When the values of haematological parameters are obtained under abnormal condition it should be possible to monitor the change in the physical and chemical properties of the water^[14]. Thus fish could be used as a warning system to indicate the presence of pollutants in natural water^[1].

Haematological variables were used more often when clinical diagnosis of fish physiology was applied to determine the sublethal concentrations of pollutants^[15]. It has been illustrated that the use of haematological variables as indicators of stress^[16,17], Toxic substances^[18-20] as well as metals^[21-25], can provide information of the physiological response fish make to a changing external environment. This a result of the close association of the circulatory system with the external environment^[16]. Cadmium is a well-known cumulative poison in animals and enters surface water with the discharge of industrial wastes or by leaching of soil, to which sewage sludge is added. It is biologically very reactive and therefore gives rise to both acute and chronic poisoning. Cadmium is a widespread environmental pollutant that is highly toxic and is considered as non-essential element and have no biological function^[26,27].

The objective of this study was to investigate the haematological parameters of adult *Oreochromis niloticus* exposed to a sublethal concentration of cadmium for 1, 3 and 5 weeks.

MATERIALS AND METHODS

Live specimens of *Oreochromis niloticus* (189±8.6 g weight) were collected from a local hatchery. Fish were acclimated in the laboratory for 10 days. The water used in the experiment had the following mean values for the water characteristics: temperature 23±1°C, pH 7.2±0.1, CaCO₃ hardness 96±1 ppm and oxygen concentration 9.3 ppm. During this time and exposure to cadmium they were fed daily on commercial fish pellets (35% protein).

To investigate the effects of cadmium on blood, 5 groups of 10 fish each were exposed to 5.5 ppm of

cadmium; 0.3 of the calculated 96h LC₅₀. Fish were sampled for the haematological investigations at 1, 3 and 5 weeks following exposure to cadmium. Five groups of control fish in tap water were sampled for each specified parameters. During the experimental periods the test medium was renewed every three days to sustain nominal concentration.

For blood analyses, the caudal peduncle was cut, blood was collected in heparinized tubes. Total red blood cells count (RBC) was determined according to the classical method using the Neubauer haemocytometer. Haemoglobin content (Hb) was estimated colourmetrically according to the method of Miale^[28]. Haematocrit value (Hct) was measured using a microcapillary reader. Mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated using the following equations^[29]:

$$\begin{aligned} \text{MCV in } \mu^3\text{m} &= \text{Hct} / \text{RBC} \times 10 \\ \text{MCH in Pg/cell} &= \text{Hb} / \text{RBC} \times 10 \\ \text{MCHC in g } 100 \text{ mL}^{-1} &= \text{Hb} / \text{Hct} \times 100 \end{aligned}$$

The statistical significance between the treated and control groups was calculated by using Student's t-test. The level of significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Notable declines in red blood cells count (RBC) were observed at all periods (Table 1). The maximum decrease of 30.7% RBC was observed after 5 weeks. The values of haemoglobin content (Hb) were decreased significantly with greatest rate of 38.8% at the last period of cadmium exposure. The fish showed a significant decline in the values of haematocrit (Hct) through all periods. In comparison with control, the maximum decrease of 36.2% haematocrit was noted at the last period of exposure. Also, the levels of mean cell volume (MCV) were declined at second and last periods. The mean corpuscular haemoglobin (MCH) were statistically decreased at all periods of cadmium administration. The maximum decreases of 13.8% MCV and 17.2% MCH were observed at third week of cadmium exposure. On the other hand, mean corpuscular haemoglobin concentration (MCHC) decreased significantly at the first period of experiment (Table 1).

In the present investigation, the administration of cadmium induced declines in the number of red blood cells, haemoglobin content and haematocrit values. The observed decrease in erythrocytes, haemoglobin and haematocrit is consistent with the previous studies of anaemia in fish, rats and rabbits exposed to cadmium, mercury, lead, nickel, copper and zinc^[4,30,35,36].

Table 1: Haematological changes (Mean±SD) in the fish, *Oreochromis niloticus* exposed to 5.5 ppm cadmium (n=10)

Parameters	Treatment	Exposure periods (Week)		
		1	3	5
Red blood cell (10 ⁶ mm ⁻³)	C	1.494±0.06	1.346±0.05	1.555±0.09
	T	1.232±0.08* (-17.5%)	1.144±0.11* (-15.0%)	1.077±0.10* (-30.7%)
Haemoglobin (g 100 mL ⁻¹)	C	8.59±0.21	8.09±0.07	8.47±0.30
	T	6.25±0.37* (-27.2%)	5.67±0.33* (-29.9%)	5.18±0.33* (-38.8%)
Haematocrit (%)	C	38.34±1.50	37.27±3.05	40.16±2.70
	T	30.91±1.17* (-19.4%)	27.27±2.74* (-26.8%)	25.63±1.23* (-36.2%)
MCV (μm)	C	257.08±15.74	276.78±18.82	258.46±13.49
	T	251.48±13.50 (-2.2%)	238.67±11.45* (-13.8%)	238.05±19.52* (-7.9%)
MCH (Pg/cell)	C	57.61±3.21	60.16±1.94	54.61±3.33
	T	50.83±3.64* (-11.8%)	49.83±3.63* (-17.2%)	48.46±5.35* (-11.3%)
MCHC (g 100 mL ⁻¹)	C	22.43±0.81	21.84±1.86	21.19±1.72
	T	20.24±1.21* (-9.8%)	20.92±1.68 (-4.2%)	20.26±1.71 (-4.4%)

- C indicates controls, T indicates exposed, + or - indicates increase or decrease percentage, * Statistically significant at $p \leq 0.05$; Student's t-test

Lowe-Jinde and Niimi^[31] stated that the anaemia was probably not due to increased destruction of erythrocytes since the spleen size was unchanged but might be due to a decrease in the synthesis or release of erythrocytes into the circulation. Houston and Keen^[37] and Lowe-Jinde and Niimi^[31] observed a reduction in the number of erythroblasts-proerythrocytes in goldfish, *Carassius auratus* and rainbow trout, *Salmo gairdneri* exposed to cadmium. They suggested that cadmium caused a reduction in erythropoiesis and impeded the formation of red blood cells. Additionally, the accumulation of cadmium in various organs particularly, the kidney and liver has been observed^[38-41] and it is conceivable that the activity of these haematopoietic tissues may be suppressed. Moreover, cadmium induced anaemia appears to be associated with a defect in iron metabolism caused by a deficiency in intestinal absorption^[42,43]. Additionally, Thomas *et al.*^[44] stated that the presence of cadmium in the organism causes a decrease of blood iron level which may be the cause of decreased concentration of haemoglobin. However, it can not be excluded that the present erythropenia and declines of haemoglobin and haematocrit in the experimental fish, *Oreochromis niloticus* could be due to a decrease in the synthesis or release of erythrocytes into circulation or/and an increase in the rate of erythrocytes destruction. Beena and Viswaranjan^[32] reported that the number of immature red blood cells was significantly increased in the fish, *Cyprinus carpio*, exposed to cadmium or mercury. The decreased in the mean cell volume caused by cadmium in *Oreochromis niloticus* is the result of the release of immature red blood cells from haematopoietic tissues. Immature cells are released to compensate for the loss of the red blood cells. The decreased in mean

corpuscular haemoglobin is the result of the increased number of immature red blood cells with lower haemoglobin content that were added to the circulation. Wepener^[45] proposed a similar suggestion to the decrease of mean corpuscular haemoglobin in the fish, *Tilapia sparmanii*, exposed to some heavy metals. The observed decreases of the both haemoglobin content and haematocrit value reflect the decreased value of the mean corpuscular haemoglobin concentration. Finally, the present investigation records that cadmium severely impairs various haematological parameters and hence poses a threat to the biological functions of experimental animals.

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