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RESEARCH ARTICLE

HAEMATOLOGICAL CHANGES IN A FRESH WATER FISH, *ANABAS TESTUDINEUS* BLOCH ON EXPOSURE TO HEAVY METAL TOXICANT CADMIUM CHLORIDE

***Mini, V. S.**

Department of Zoology, University of Kerala, Thiruvananthapuram, India

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ABSTRACT

The study mainly focused on the effect of different concentrations of $CdCl_2$ on haematological parameters of freshwater fish, *Anabas testudineus*. Serum glucose content, haematocrit, total RBC and WBC count were measured after 7, 14 and 28 days of exposure to different concentrations of $CdCl_2$ (2ppm, 3ppm and 4ppm). 96-h LC_{50} of $CdCl_2$ was 7ppm. Levels of serum glucose content were increased after 14 and 28 days of exposure to different concentrations of $CdCl_2$ (2ppm, 3ppm and 4ppm). The haematocrit value and RBC count were significantly reduced after 14 days of exposure to the three concentrations of $CdCl_2$ while the leucocyte count was significantly increased with in 7 days of exposure to the middle and higher concentrations (3ppm and 4ppm).

INTRODUCTION

Endocrine disruptors are environmental chemicals that when absorbed in to the body either mimics or block hormones and disrupts the body's normal physiological functions (**Takatsuki and Yamaguchi, 2001; Binitha and Francis Sunny, 2009**). They may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. Out of the several heavy metals in the industrial waste streams, cadmium is reported to be associated with the effluents of battery, electroplating and metal finishing, mining and metallurgy and paints and dye industries (**Forstner and Prosi, 1979**). Common compounds of cadmium include cadmium chloride [$CdCl_2$], cadmium oxide, and cadmium sulphide and cadmium acetate. The two oxidation states of cadmium are the metallic [Cd^0] and divalent [Cd^{2+}]. Cadmium is a non-essential element with no known biological function (**Martinez *et al.*, 1999**) naturally found at low concentrations in natural waters (**Allen, 1994; Bennet-Chambers *et al.*, 1999**). The target organs for Cd^{2+} toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones in higher vertebrates (**Roberts, 2003**). If the laboratory testing procedures indicate blood levels of Cd^{2+} above 5mcg/dL and creatinine levels in urine above 10mcg/dL, then it can be considered to be suggestive of Cd^{2+} toxicity. The occurrence of Cd^{2+} in considerably toxic amounts was reported by earlier workers in various aquatic ecosystems (**Kiran *et al.*, 2006**). Cd^{2+} was found to be teratogenic, embryo toxic, carcinogenic, nephrotoxic in humans and the risk is greater among smokers (**Luo *et al.*, 1993**). The outbreak of "itai – itai – byo" or "ouch – ouch disease", in Japan was the historical event that drew the world's attention to the environmental hazards of Cd^{2+} poisoning for the first time (**Sarunya *et al.*, 2006**). The shrimp [*M.bahia*] was the most sensitive species tested with 96h LC_{50}s ranging from 14.7 to 16µg/L (**Voyer and Modica, 1990**). Bio-enhancement of Cd^{2+} transfer along a food chain was studied by **Seebaugh *et al.*, (2005)** and fish are reported to be used as biological indicators to assess water pollution (**Rashed, 2001**).

****Corresponding author: Mini, V. S.,***
Department of Zoology, University of Kerala, Thiruvananthapuram, India.

Blood physiology is currently considered as an essential index to the general health status in a number of fish species (**Abou El-Naga *et al.*, 2001**). Changes in haematological variables are now in use when clinical diagnosis of fish physiology is applied to determine the effects of external stressors. Freshwater cichlids *O. mossambicus* exposed to 10µg Cd/L in ambient water for 2, 4, 14 and 35 days elicited significant hyperglycemia and elevation of plasma cortisol levels and Cd^{2+} induced typical stress response on 2, 4, and 14 days. The plasma cortisol and glucose level returned to control values on 35 days exposure. Exposure to Cd^{2+} (0.5 and 1.0mg/L) caused a dose dependent decrease in haemoglobin, haematocrit and erythrocyte counts and plasma protein level but an increase glucose level on a fresh water fish Heteroclarias (**Sobha *et al.*,** 2007). The study on toxicity of Cd^{2+} and its impact on biochemical constituents like glucose, glycogen, total proteins, lipids and free aminoacids in the freshwater edible carp *Catla catla* showed significant fall in all the biochemical constituents except glucose in all the tissues (**Kori-Siakpere *et al*., 2006**). The present work was aimed to determine the haematological effects of sublethal concentrations of $CdCl_2$ in freshwater Teleost, *Anabas testudineus*. Fish are the group of animals most at threat from aquatic pollution and with their physiological similarity to mammals together with their long term exposure in natural habitats, provides a suitable biomonitor for environmental pollution. Evidences are now accumulating that even low levels of pollutants can disrupt the functioning of the endocrine system of fish (**Kime, 1999**).

MATERIALS AND METHODS

Experimental model

Prior to experiment the animal model *Anabas testudineus* Bloch collected from local suppliers were acclimatized for two weeks in a glass aquaria filled with dechlorinated tap water under laboratory conditions [natural photoperiod and temperature 26$\pm$2^{0}C]. The fish were fed with protein rich feed on alternate days.

Test chemical

Cadmium chloride [$CdCl_2.H_2O$ RM – 469 – 100g purchased from HiMedia, Mumbai] was used as the test chemical and sublethal doses [80, 120 and 160ppm] of cadmium chloride were finalised after determining the LC_{50} of Cd^{2+}. The laboratory acclimatized fish were divided in to ten groups of eight each in separate aquarium tanks. The first group of fish was served as control. Fish in group II, III and IV were exposed to 2mg $CdCl_2$/L of water, groups V, VI and VII to 3mg $CdCl_2$/L and groups of VIII, IX and X 4mg $CdCl_2$/L for a period of 7, 14 and 28 days respectively. After stipulated periods of exposure, fish were sacrificed. Then liver was excised straight away and frozen immediately at $^-$80^{0}C (NBS, USA) for enzymes assay. The blood was collected from caudal artery and centrifuged at 10,000 r.p.m for 10 minutes in a high speed refrigerated centrifuge [Eppendorf, Germany]. The supernatant was collected and kept in an ultra low freezer at $^-$80^{0}C until biochemical analysis.

Serum glucose measurement

Serum glucose values were determined spectrophotometrically using diagnostic kit manufactured by Span diagnostic Ltd. Surat and as per the standard protocol supplied by the manufacturers (**Tietz, 1976**).

Haematological profile

The total RBC count was performed normally on an improved Neubauer haemocytometer using Hayem's solution diluting fluid and total leucocytes count was determined according to and using Truck's solution (**Lucky, 1977; Schalm, 1986**). Haematocrit (Hct) was determined by centrifugation of whole blood at 1,200 rpm for 5 min. in microhaematocrit capillary tubes (**Stoskopf and Saunders, 1993**).

Statistics

Data were collected from six animals in each group. Statistical analysis was done by SPSS statistical package. Data were analyzed by one – way analysis of variance, which helps to understand whether or not there were differences between groups of means. Groups that were not significantly different in **Duncan's (1955)** multiple range tests were considered homogenous. Difference was considered significant when P < 0.05.

RESULTS

Exposure of fish to different concentrations of $CdCl_2$ revealed significant changes in the haematological parameters studied at different periods of time in *Anabas testudineus* Bloch.

Serum glucose content

Cadmium chloride caused effect in the form of hyperglycemia on exposure to all the three sublethal doses of CdC_{l2} (2, 3 and 4mg/L) Table 1 and Fig 1.

[Results are expressed as mean $\pm$ SEM of 6 animals (n=6)]

Dose of Exposure of $CdCl_2$ (mg/L)

Table 1. Effect of sublethal doses of $CdCl_2$ (2, 3 and 4mg/L) on blood glucose (Glu) content and haematocrit (Hct) value in *Anabas testudineus*

		2	3	4
Control	Glu	65.52±0.41^a	65.52±0.41^a	65.52±0.41^a
	Hct	60.54±0.53^a	60.54±0.53^a	60.54±0.53^a
7 days	Glu	65.53±0.41^a	66.08±0.62^a	66.81±0.40^a
	Hct	59.19±0.71^a	59.40±0.76^a	49.67±0.50^b
14 days	Glu	76.49±0.55^b	75.11±0.31^b	76.64±0.61^b
	Hct	50.37±0.56^b	49.98±0.48^b	40.95±0.74^c
28 days	Glu	82.51±0.51^c	82.70±0.73^c	84.01±0.41^c
	Hct	41.88±0.57^c	39.63±0.97^c	39.75±0.87^c

Glucose content expressed as mg/dL.
Haematocrit value expressed as percentage (%).

The significant difference between the groups was analysed by one-way analysis of variance, mean values of groups with different superscripts letters in a given row are significantly different (P<0.05) as determined by Duncan's multiple range test.

Haematological profiles

Fish exposed to the higher $CdCl_2$ concentrations (3 and 4mg/L) showed significantly increased total leucocytes count Table 2 and Fig 4 but the erythrocytes count Table 2 and Fig 3 and haematocrit values Table 1 and Fig 2 were decreased.

[Results are expressed as mean $\pm$ SEM of 6 animals (n=6)]

Dose of Exposure of $CdCl_2$ (mg/L)

Total RBC Count expressed as 10^6 / mm^3. Total WBC Count expressed as 10^3 / mm^3. The significant difference between the groups was analysed by one-way analysis of variance, mean values of groups with different superscripts letters in a given row are significantly different (P<0.05) as determined by Duncan's multiple range test.

Table 2. Effect of sublethal doses of $CdCl_2$ (2, 3 and 4mg/L) on total RBC (TRBC) and total WBC (TWBC) count in *Anabas testudineus*

		2	3	4
Control	TRBC	2.76 ± 0.004^a	2.76 ± 0.003^a	2.76 ± 0.003^a
	TWBC	3.45 ± 0.07^a	3.45 ± 0.07^a	3.45 ± 0.07^a
7 days	TRBC	2.76 ± 0.003^a	2.75 ± 0.006^b	2.76 ± 0.005^a
	TWBC	3.51 ± 0.03^a	4.75 ± 0.05^b	4.10 ± 0.02^b
14 days	TRBC	2.74 ± 0.004^b	2.75 ± 0.006^b	2.75 ± 0.005^b
	TWBC	3.98 ± 0.02^b	5.12 ± 0.03^c	6.10 ± 0.40^c
28 days	TRBC	2.75 ± 0.006^a	2.74 ± 0.005^b	2.72 ± 0.002^c
	TWBC	4.15 ± 0.12^b	5.85 ± 0.05^d	7.05 ± 0.15^d

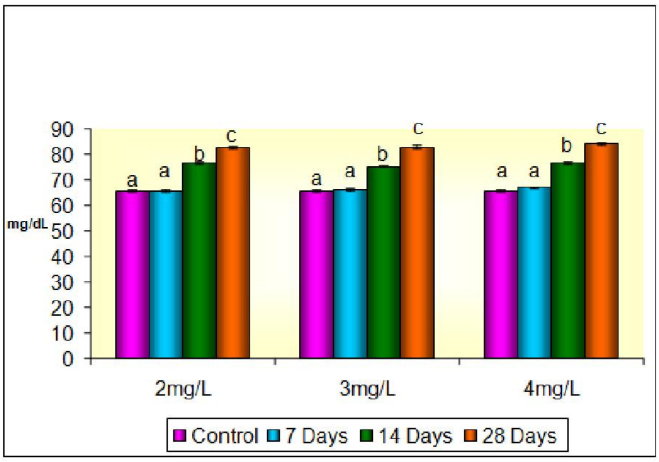

Fig.1. Effect of sublethal doses of $CdCl_2$ exposure on Glucose Content in *Anabas tesudineus*

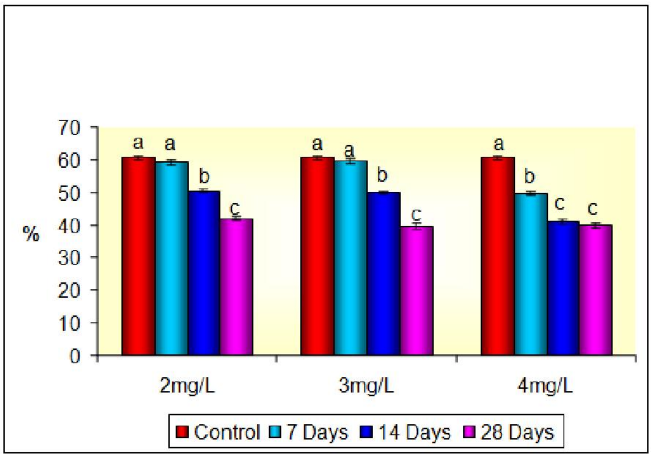

Fig. 2. Effect of sublethal doses of $CdCl_2$ exposure on Haematocrit in *Anabas tesudineus*

Each bar represents mean + SEM of six animals
Groups with different letters significantly different

DISCUSSION

The present study clearly reveals that exposure to sublethal doses of $CdCl_2$ has specific influence on haematological parameters in *Anabas testudineus.* Exposure to $CdCl_2$ significantly increased the blood glucose level. The information obtained indicated that $CdCl_2$ also significantly affected the total blood cell count and haematocrit value in fish. Fish are responding to various stressors by a series of biochemical and physiological stress reactions, so called secondary stress responses comparable to those of higher vertebrates. In many fish species, the blood / plasma glucose level has the tendency to increase due to experimental stress. Hyperglycemic response in Heteroclarias on exposure of Cd^{2+} indicates disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen mediated by adrenocortical hormones and reduced insulin secretory activity (**Almeidaa *et al*., 2001**). Increase in serum glucose levels in fish under stress was reported previously (**Vosyliene and Jankaite, 2006**). This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis (**Barnhart, 1969**). The present study illustrated that glucose recorded high values than control group level and was explained through gluconeogenesis, which mean formation of glucose from non-carbohydrate source. An increase in blood glucose and muscle glycogen values was observed in *Mugil seheli* due to toxicity of cadmium and copper (**Blaxhall, 1972**). Hyperglycemia was observed in Grey Mullet on exposure to $CdCl_2$ and in Rainbow truot and *Salmogaidneri* (**Swift and Lloyd, 1974**).

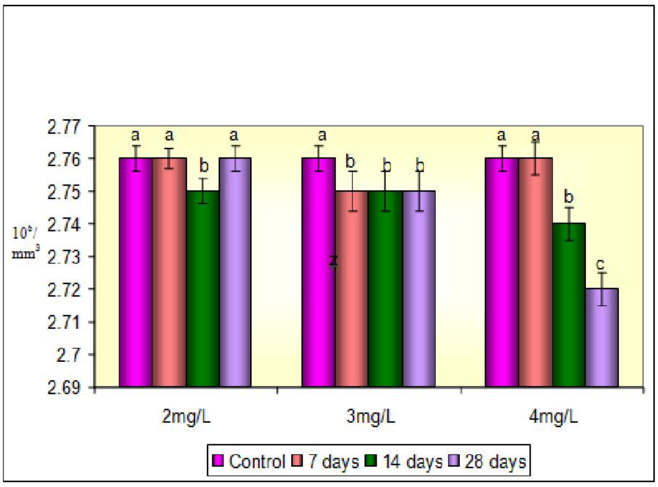

Fig. 3. Effect of $CdCl_2$ exposure (2, 3 and 4mg/L on Total RBC count in *Anabas testudineus*

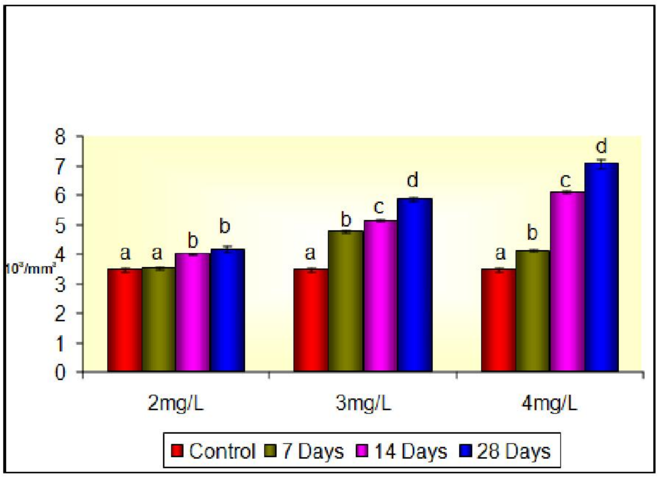

Fig. 4. Effect of sublethal doses of $CdCl_2$ Exposure on Total Leucocyte Count in *Anabas tesudineus*

Each bar represents mean + SEM of six animals.
Groups with different letters significantly different

Changes in haematological values occur in relation to physiological stress, disease and toxic environmental conditions (**Hoeger *et al*., 2005**). Haematocrit is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen carrying capacity of the blood. The forgoing result indicates that marked difference are seen in different haematological indices (Hct, TRBC, TLC) under stressful environments. The present finding agree with the previous results (**Larsson *et al*., 1985**) in which recorded a decrease in haematocrit level in brown trout after exposure to diclofanac for 7 days. In Grey Mullet the packed cell volume (PCV) values was decreased when exposed to $CdCl_2$ and the result is in agreement with previous findings (**Nomiyama, 1988**). Blood cells of teleost are produced from haemopoietic tissues of the kidney and the spleen (**Heath, 1982**). The red blood cells have the important function of haemoglobin transport which carries oxygen to all tissues in the body (**Hibiya, 1982**). The decreased red blood cell number following exposure to Cd^{2+} could be as a result of haemolysis or destruction of red blood cells.

Decreases in the red blood cells could also be as a result of internal bleeding caused by damaged kidney. Similar findings supporting the present study were recorded for Heteroclarias exposed to sub lethal concentrations of Cd^{2+} (**Kori-Siakpere *et al*., 2006**). In fish, the white blood cells respond to various stressors including infections and chemical irritants (**Christensen *et al*., 1978**). The significant increase of lymphocytes, total leucocytes was observed in fish exposed to $CdCl_2$. Increased TLC has been suggested due to stimulated lymphopoiesis and enhanced release of lymphocyte from lymphoid tissues. Such lymphocyte response in the presence of toxic substances perhaps associated with pollutant induced tissue damage and severe disturbance of non-specific immune system leading to increased production of leucocytes (**Das and Mukherjee, 2003**). Overall, the results of our study highlight the stress to which freshwater fish are exposed through the uncontrolled discharge of heavy metals in the aquatic environment. It could be concluded that cadmium chloride induced deleterious effects on the haematological parameters in fish.

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