INTERNATIONAL JOURNAL OF ENVIRONMENT
Volume-4, Issue-2, March-May 2015 ISSN 2091-2854
Received:5 January *Revised:15 February* *Accepted:5 May*

STUDIES OF HAEMATOLOGICAL PARAMETERS OF *Oreochromis niloticus* EXPOSED TO CADMIUM CHLORIDE ($CdCl_2$, $2H_2O$)

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Abstract

The effects of Cadmium Chloride on the specimen Nile tilapia (*Oreochromis niloticus*) were examined for evaluation as acute toxicity for 96 hours. From the recorded information using Probit Analysis-Finney Method [Log-normal Distribution] the results revealed the mean 96 hour LC_{50} value to be 3.5095 mg/L indicating very high potential toxicity. Also derived value for mean NOEC=0.9062 mg/L defined safe limits of cadmium. In order to assess the effect of prolonged exposure of low concentration of cadmium haematological assessment was made after 24 hour, 7 days and 15 days of exposure. The study of haematological parameters demonstrated significant reduction in Red Blood Cell count, Haemoglobin, Haematocrit and Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH); while elevation was reported in Mean Corpuscular Volume (MCV). Leading to conclusion that mature RBCs are destructed and the erythrocyte production is inhibited due to reduction of haemesynthesis. Stimulation of erythropoiesis is considered as one of the reasons for decrement in values of Haemoglobin, Haematocrit and RBC count.

Keywords: Cadmium, haematotoxicity, Nile Tilapia

Introduction

Cadmium is used for the manufacturing of batteries, pigments, coating and plating, stabilizers for plastic, nonferrous alloys, photovoltaic devices, fertilizers, automobiles etc. and is considered as one of the most toxic heavy metals. Rivers and lake shores are the areas primarily affected by cadmium waste from industries in big cities (Randi, 1996).

Fish being the inhabitants of water cannot escape from the effects of the aquatic pollutants (Olaifa et al., 2004; Clarkson, 1998). Fish are used widely to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for hostile effects and death in the aquatic systems (Farkas et al., 2002; Yousuf and El-Shahawi, 1999). The studies carried out on various fish have revealed that heavy metals can amend the physiological activities and biochemical parameters in tissues as well as in blood (Basha and Rani, 2003; Canli, 1995). Nile Tilapia (*Oreochromis niloticus*) offers a relatively high tolerance for temperature and pH variance and is able to easily adapt to laboratory conditions. Therefore, the study on the fish is appropriately justified.

Acute toxicity tests for evaluating the threat of chemical contamination to various organisms have been established and well documented (Sanders and Cope 1966: Macek and McAllister 1970). Static acute toxicity tests provide quicker and repeatable dose-response curves for estimation of toxic effects of chemicals on aquatic organisms. Usually acute toxicity tests are employed to estimate the exposure concentration causing 50% mortality (LC_{5O}) to test organisms for a specified exposure period. The LC_{5O} has gained acceptance among toxicologists and is one of the most highly rated test for assessing hostile effects of chemical contaminants to aquatic animals.

For many years, the importance of correct haematological assessment in toxicology studies has been recognized. Such parameters indicate abnormal environmental conditions apart from providing information about the health of test animal, (Elahee and Bhagwant, 2007). Haematological tests can rapidly yield information about the existence, status and degree of possible sickness in organisms (Blaxhall and Daisley, 1973).

Materials and Methods

Oreochromis niloticus as test organism

The fish are available throughout the year. The fish were obtained from a local breeder, four weeks prior to each experiment. They were acclimatized for two weeks in common tanks and then transferred to experiment aquariums and allowed to acclimatize for one more week.

LC_{50} Estimation

OECD guidelines for testing of chemicals (1992) were adopted for our experiment. We carried out a 96 hour acute test on the specimen. Seventy healthy, adult *Oreochromis niloticus* specimens were selected as test organisms. After an acclimatisation period of two weeks in a set of aquariums, the fish were transferred from the initial acclimation tank to six acute toxicity testing tanks (ten in each one), and allowed to acclimatise for one week before the exposures commenced. The fish were not fed in the 24 hour to the onset of exposure till the experiment time. Then the experimental exposures were executed. The toxicant was added by means of a stock solution prepared.

Out of the six groups one group was kept as control with reference to other groups. While rest exposure groups each of ten specimens, were exposed to the respective cadmium concentration of 1mg/L, 2mg/L, 3mg/L, 4mg/L and 5mg/L $CdCl_2$ respectively. Being healthy specimens no death was noted in control group. Lethality was recorded for each group at an interval of 1, 2, 4, 6, 12, 24, 36, 48, 72 and 96 hours. From the collected data LC_{50} value was calculated using Probit Analysis - Finney Method [Log-normal Distribution].

Estimation of Haematological effects

Chronic studies generally complement acute studies, assuming that the conditions are structured to enhance the results obtained in acute experiments. The specimens in group of 30 (10 X 3 to avoid oxygen depletion, high levels of waste production per tank and stress induced by crowding) were exposed to the decided concentration of cadmium after primary and secondary acclimation. The fish were fed on a daily basis for the entire period of exposure. Out of the four groups one group was exposed to no cadmium. i.e. was kept as control with reference to other groups. While rest exposure groups each of thirty specimens, were exposed to the respective cadmium concentration of 0.1mg/L, 0.2mg/L and 0.3mg/L respectively. Being lower concentration of cadmium than required to cause lethal effects no

death was observed in any of the groups. After exposure of 24 hours, 7 days and 15 days nine fishes were sacrificed to collect blood samples from them. The collected blood samples were analyzed for measurement of different haematological parameters like Hb, RBCs, Hct, MCV, MCH and MCHC. Total RBC count, determination of Hb% and other parameters of blood viz., MHC, MCV and MCHC were done.

Results

LC_{50} Estimation

During the exposure period irregularity in swimming pattern, vertical swimming and motionlessness was observed. Breathing problems, balance problems and sudden, jerky movements were observed. Lethality was recorded at intervals of 1, 2, 4, 6, 12, 24, 36, 48, 72 and 96 hours. The recorded observations of casualty are listed in table 1.

The results were calculated using Probit Analysis - Finney Method [Log-normal Distribution] (Finney, 1971). Mean values of the NOEC (concentration at which no mortality detected) was found to be 1.2017 mg/L. Using Probit Analysis - Finney Method [Log-normal Distribution] the LC_{50}, LC_{50} Lower confidence Limit and LC_{50} Upper confidence Limit were calculated to be 3.4312 mg/L, 2.7361 mg/L and 4.477 mg/L respectively. Detailed results calculated using Probit Analysis - Finney Method [Log-normal Distribution] for the acute tests is shown in table 2.

Estimation of Haematological effects

The present study reveals that the fish exposed to Cd demonstrated noteworthy decline in RBCs, Hb and Hct. The Mean Corpuscular Haemoglobin (MCH) inclined with concentrations of toxicant. Unlike the MCV, the MCH and MCHC values declined, with concentration of cadmium. The changes in each of the parameter are listed in table 3, while the same is depicted graphically in fig 1.

Discussion

Mucus accumulation was observed on the body surface and gill filament of dead fish. This might be attributed to increase in the activity of mucus cells due to subsequent exposure to pollutants. Reports of Ayuba and Ofojekwu (2005) and Omitoyin (2007) support this conclusion. In

freshwater fish, cadmium uptake occurs mainly via gills (Winner and Gause, 1986). This point toward the fact that, the gills have a large effective area serving as the main uptake site for cadmium.

Our study led us to LC_{50}values 3.5095 mg/L using Probit Analysis - Finney Method [Log-normal Distribution] **(**Finney D.J., 1978). Cadmium 96-h LC_{50} value for Nile Tilapia was reported 11.16 mg/L by Mohsen Abdel-Tawwab et al. (2008).

The present study revealed that the fish exposed to Cd showed significant reduction in RBCs, Hb and Hct. While Unlike MCH & MCHC values, the Mean Corpuscular Volume (MCV) increased with concentrations of toxicant in Nile tilapia, *Oreochromis niloticus* at sublethal levels of cadmium.

This may be as a result of the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemesynthesis affected by pollutants, (James and Sampath, 1999). Also, the decline in RBCs count may be attributed to acute haemolytic crisis that results in severe anaemia in most vertebrates including fish species exposed to different environmental pollutants. Karuppasamy et al. (2005) found a significant decrease in total erythrocyte count, haemoglobin content, haematocrit value and Mean Corpuscular Haemoglobin Concentration in air breathing fish, *Channa punctatus* after exposure to sublethal dose of Cd.

The increase of MCV and decrease of MCH and MCHC may be attributed to a defense against Cd toxicity through the stimulation of erythropoiesis or may be related to the decrease in RBCs, Hb and Hct due to the disturbances that occurred in both metabolic and haemopoietic activities of fish exposed to sublethal concentration of pollutants. The change in the MCH may be attributed to the reduction in oxygen carrying capacity of blood and eventually stimulating erythropoiesis (Hodson et al., 1978). The related decrease in haematological indices proved the toxic effect of heavy metals that affect both metabolic and haemopoietic activities (Moussa, 1999 ; Gill and Epple, 1993). Hussain et al. (2011) and Atef M. Al-Attar (2005) have reported in their studies that the RBC, Hb and Hct show significant decrease upon exposure to cadmium. Changes in MCV, MCH and MCHC are also showing similar nature in studies of Atef M. Al-Attar (2005), Ahmed E. Noor EL Deen et al. (2010) and Adel M.E. Shalaby (2007). Similar results are available from researchers working with

other heavy metals on Nile Tilapia Nilton et al. (2007) as well as cadmium on other fishes Ruparelia et al. (1990), Vinodhini et al. (2009) and Yamawaki et al . (1986).

The significant reduction in these parameters may be indicative of severe anaemia caused by destruction of erythrocytes (Omoniyi et al., 2002; Kori-Siakpere et al., 2009), Haemodilution (Adeyemo, 2005) resulting from impaired osmoregulation across the gill epithelium. Gaafar et al., (2010) reported that prolonged reduction in haemoglobin content can impair oxygen transport and degeneration of the erythrocytes may be due to pathological condition in fish exposed to toxicants.

Concentration of haemoglobin being the oxygen-carrying component in the blood of fish; can be used as a good indicator of anaemic condition (Blaxhall and Daisley, 1973). Decreased haemoglobin following metal exposure usually results in haemodilution; considered as a mechanism that reduces the concentration of the toxicant in the circulatory system (Smit et al., 1979). The observed reduction in the haemoglobin and haematocrit values in the fish could also be attributed to the lysing of erythrocytes. Samprath et al. (1993) and Musa and Omoregie (1999) reported similar results. Thus, the significant reduction in these parameters is an indication of severe anaemia.

Haematocrit is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen-carrying capacity of the blood (Larsson et al., 1985). Aggregation of red blood cells in damaged gills may cause of the reduction of circulating erythrocytes of stressed fish (Singh and Singh, 1982). The significant decrease in the Haematocrit in this study could be attributed to gill damage and/or impaired osmoregulation causing anaemia and haemodilution.

Table 1: Acute toxicity Experiments observations (figures show casualty at recording time)

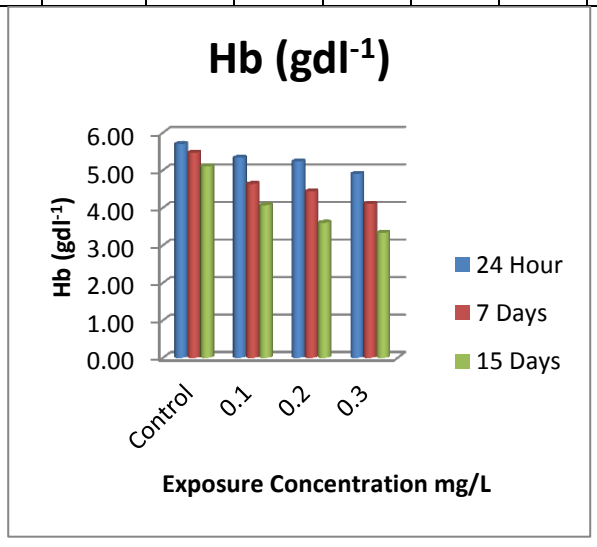
concentration (in mg/L)	Experiment 1										Experiment 2									
	Exposure Time (in Hours)										Exposure Time (in Hours)									
	1	2	4	6	12	24	36	48	72	96	1	2	4	6	12	24	36	48	72	96
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	1	1	2	2	0	0	0	0	0	0	0	1	1	1
3	0	0	0	0	0	0	1	2	3	4	0	0	0	0	0	0	1	2	3	3
4	0	0	0	0	1	1	2	4	5	6	0	0	0	0	1	2	3	5	6	7
5	0	0	0	1	1	2	3	4	6	7	0	0	0	1	1	2	4	5	8	9

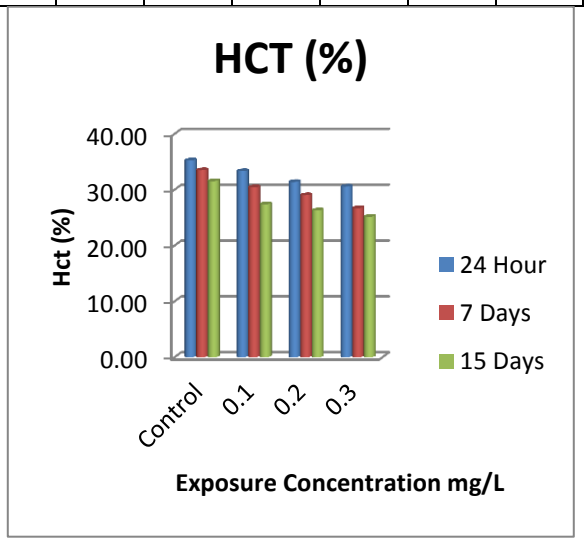
Table 2: Acute toxicity Experiment results

Parameter	Experiment 1	Experiment 2	Average
Mortality in control group	0	0	0
NOEC (concentration at which no mortality detected)	0.9062 mg/L	1.4972 mg/L	1.2017 mg/L
LC_{50}	3.5095 mg/L	3.3528 mg/L	3.4312 mg/L
LC_{50} Lower confidence Limit	2.7166 mg/L	2.7555 mg/L	2.7361 mg/L
LC_{50} Upper confidence Limit	4.9494 mg/L	4.0045 mg/L	4.477 mg/L

Table 3: Results of Haematological studies

	Conc.	Hb (g/dl)		Hct (%)		MCV (μm^3)		MCH (pg)		MCHC (%)		BC (10^6 mm^-	
24 Hr	Control	5.70	±0.10	35.26	±1.07	191.19	±11.34	30.91	±1.63	16.17	±0.42	1.85	±0.07
	0.1	5.33	±0.06	33.33	±1.03	201.80	±10.54	32.28	±1.32	16.01	±0.50	1.65	±0.05
	0.2	5.23	±0.06	31.40	±1.30	198.32	±15.59	33.04	±1.85	16.68	±0.56	1.59	±0.07
	0.3	4.90	±0.10	30.56	±1.32	204.16	±21.41	32.70	±2.62	16.04	±0.38	1.50	±0.09
7 Days	Control	5.47	±0.12	33.50	±1.03	198.61	±25.16	32.33	±2.86	16.33	±0.81	1.70	±0.16
	0.1	4.63	±0.15	30.44	±0.79	209.53	±6.50	31.93	±2.12	15.24	±0.87	1.45	±0.05
	0.2	4.43	±0.06	29.02	±1.36	207.87	±7.65	31.79	±1.78	15.30	±0.87	1.40	±0.06
	0.3	4.10	±0.10	26.71	±1.51	203.51	±17.65	31.29	±3.07	15.38	±0.83	1.32	±0.09
15 Days	Control	5.10	±0.10	31.55	±0.63	188.43	±10.02	30.46	±1.78	16.17	±0.33	1.68	±0.07
	0.1	4.07	±0.15	27.40	±1.03	207.72	±18.80	30.78	±1.81	14.86	±0.97	1.32	±0.07
	0.2	3.60	±0.10	26.31	±1.10	223.26	±15.51	30.54	±1.67	13.69	±0.20	1.18	±0.04
	0.3	3.33	±0.15	25.16	±0.19	225.59	±11.02	29.90	±2.22	13.25	±0.56	1.12	±0.05





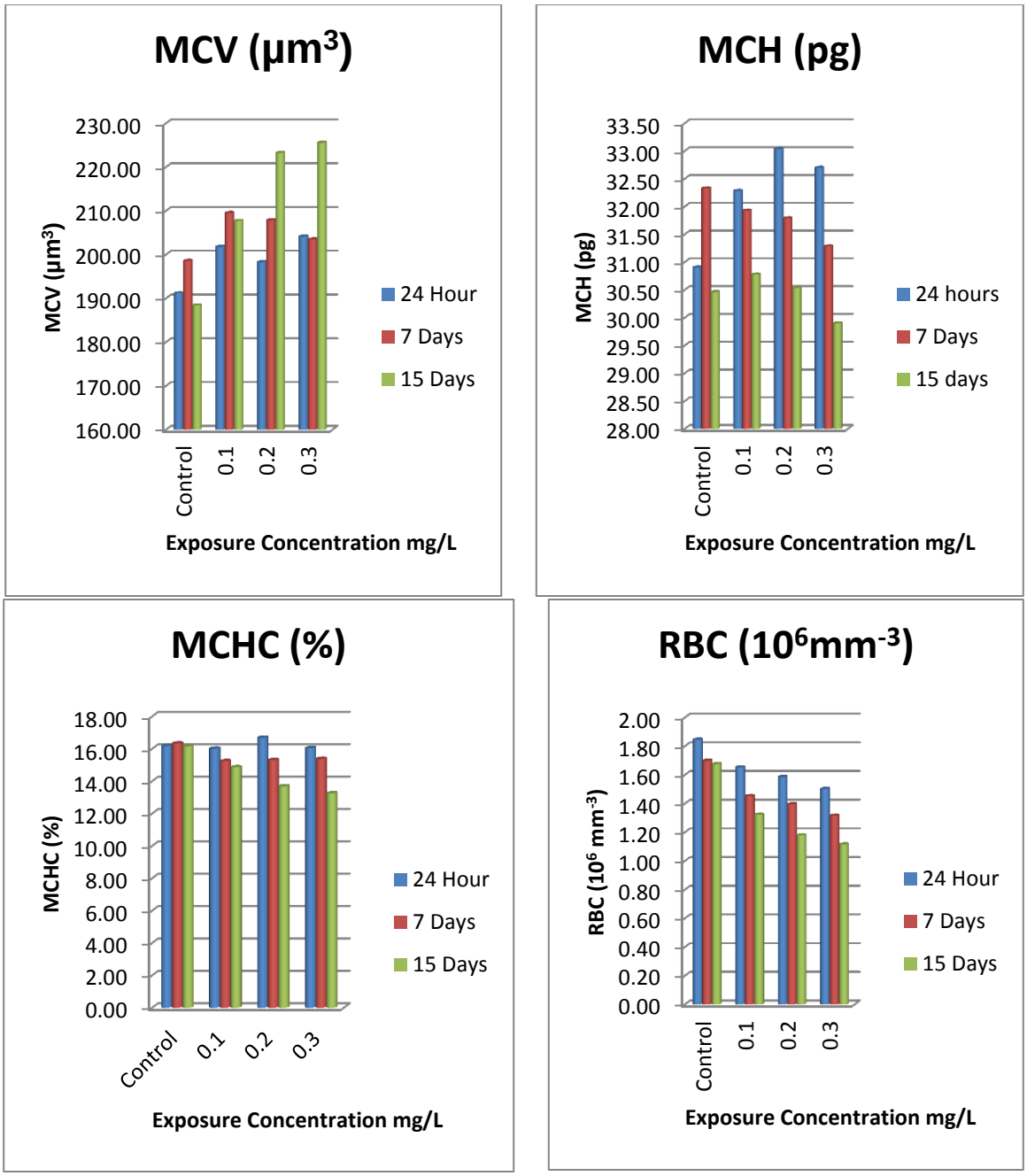


Fig 1. Deviation in Hb, Hct, MCV, MCH, MCHC and RBC count to chronic exposure of $CdCl_2$, $2H_2O$

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