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Changes in the Haematological Parameters of *Clarias gariepinus* Exposed to Lead Poisoning

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Abstract: *Clarias gariepinus* juveniles were exposed to sublethal lead poisoning in the laboratory for 28 days in a static renewable bioassay system. Ninety *Clarias gariepinus* juveniles (mean length 35.0±2.50 cm and mean weight 150±5.20 g) divided into 3 groups of thirty fish each were used for the study. The fish were exposed to 0sublethal concentrations (0.0, 0.1 and 0.4 mg L^{-1}) of lead as lead chloride. The changes in the haematological parameters of the fish were assayed every seven days. When compared with the control, the haemoglobin concentration decreased significantly ($p<0.05$) with increasing lead concentration and with duration. Erythropoiesis increased significantly ($p<0.05$) with lead concentration. The erythrocyte count differed significantly ($p<0.05$) in the between the treatment groups. There was significant leucocytosis as the test concentration increased and with duration. The morphological indices MCV, MCH and MCHC decreased as the test concentration increased except on day 28 when the MCHC increased with concentration ($p<0.05$).

Key words: Clarias, lead, anaemia, erythrocytosis, haemoglobin, leucocytosis, Nigeria

INTRODUCTION

Intense activities in both the industrial and agricultural sectors have led to increased the levels of heavy metals in natural waters (Nimmo *et al*., 1998; Jordao *et al*., 2002). Heavy metals are serious pollutants of the aquatic environment because of their persistence and ability to bioaccumulate in aquatic organisms (Veena and Radhkrishnan, 2007). The contamination of the water bodies with heavy metals has become a source of concern not only because of their threat to aquatic life especially fishes (Al-Masri *et al*., 2002; Karbassi *et al*., 2006) but also due to the public health implications of such contaminations (Farombi *et al*., 2007).

The Miamata bay disease and the recent loss of hundreds of lives in Zamfara state, Nigeria due to lead contamination of water bodies are good examples of the dangers of metal pollution to human health. In animals, heavy metal may produce histological changes in the kidneys, liver, gastrointestinal tract, testes, heart, blood vessels, blood cells, bone marrow and pancreas (Selypes *et al*., 1992; Domingo, 1994; Waalkes *et al*., 1994). Lead causes early mortality of mature red blood cells and inhibits of haemoglobin formation through the inhibition of erythrocyte alpha-amino levulinic acid dehydratase (Johansson-Sjobeck and Larsson, 1978). Several studies (Hilmy *et al*., 1980; Van Vuren, 1986, Allen, 1993; Nussey *et al*., 1995; Luskova, 1997) have shown that changes in water quality by metal contaminants will induce physiological alteration in fish including changes in the values of some of the haematological parameters. Blood cell responses are important indicators of changes in the internal and/or external environment of the fish.

In fish, exposure to chemical pollutants induces either increase or decrease in the haematological and some biochemical parameters (Hilmy *et al*., 1980; Van Vuren, 1986, Allen, 1993; Nussey *et al*., 1995; Luskova, 1997; Annune and Ahuma, 1998). Thus, the changes in the haematological parameters are good indicators of changes in the water quality. This study was undertaken with a view to determining the effect of sublethal lead chloride on the haematological parameters of *Clarias gariepinus*.

MATERIALS AND METHODS

Collection and acclimatization of the fish: The fish was collected from Mrs. Oparaku's fish farm, University of Nigeria Nsukka, Enugu state and transported to the laboratory in a plastic container. The fish was acclimatized for 3 weeks in the laboratory before the commencement of the study. The fish was fed with 35% crude protein diet throughout the study and the water was continuously changed every 2 days throughout the period of acclimatization.

Experimental design: A total of ninety juvenile *Clarias gariepinus* (mean length of 35.00±2.50 cm and average weight of 150±5.20 g) were used for the experiment. They

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were divided into 3 groups of thirty fish each. Each group was further randomized into three replicate experiments that contained ten fish each. The fish in treatments 1 and 2 were treated with 0.1 and 0.4 mg L^{-1} of lead chloride, respectively. The 3rd group was exposed to tap water only and it served as the control. The water in the replicate experiments was changed everyday to maintain the toxicant concentrations. One fish from each replicate experiment was killed every seven days for 28 days. The blood was collected for haematological assay as earlier described (Oluah, 2001).

Haematological assay methods: The haemoglobin was determined by the cyanmethanemoglobin method (Blaxhall and Daisley, 1973) while the haematocrit was determined by the microhaematocrit method (Allen, 1994). The Neubauer's improved microscopic counter was used in counting the erythrocyte after the blood was diluted with Dacie's fluid. Similarly, the leucocyte count was done using the Neubauer microscopic counter after diluting the blood with Turk's fluid. The haematological indices were calculated following Tort and Torres (1988) method.

Statistical analysis: One-way Analysis of Variance (ANOVA) was used to analyze the data followed by FLSD post hoc test.

RESULTS

The mean changes in the haematological parameters of *Clarias gariepinus* exposed to lead chloride are shown in Table 1-4. After 7 days, the haemoglobin concentration decreased from the control value of 9.35±0.45 to 7.55±0.21 and 7.10±0.42 in the groups exposed to 0.1 and 0.4 mg L^{-1}, respectively after 7 days (Table 1). It further decreased to 6.7±2.26 and 6.95±0.07 on day 14 in the fish exposed to 0.1 and 0.4 mg L^{-1} lead, respectively after 2 weeks (Table 2). It slightly increased to 7.0 g dL^{-1} on day 21. After 28 days, the haemoglobin concentrations were 6.15±0.21 and 5.25±0.35 in the groups treated with 0.1 and 0.4 mg L^{-1} lead, respectively. Generally, the haemoglobin concentration decreased ($p<0.05$) in the treatment groups with duration of the study and test concentrations.

The haemoglobin concentration in the treatment groups differed significantly ($p<0.05$) throughout the study except on the day 21 of the study. The haematocrit of *Clarias gariepinus* exposed to the sublethal lead (II) ions were generally higher in the lead-exposed fish than in the control (27.0±1.41) within the 1st 7 days of the study (Table 1). After the 1st week, the haematocrit of *C. gariepinus* in the treatment group were lower than control ($p<0.05$). The haematocrit values in the treatment experiments did not vary on day 21 ($p<0.05$). Thereafter, the haematocrit of the fish treated with lead decreased with test concentration.

The erythrocyte counts in the groups were significantly different ($p<0.05$). The red blood cell showed a considerable increase in the treatment groups than in the control ($p<0.05$) and this was more pronounced in the fish exposed to 0.04 mg L^{-1} lead. The result of the study showed that lead had significant effect on the erythrocyte count in *C. gariepinus*.

The morphological indices of the blood of the fish were affected by lead (II) ions. The MCV decreased with increasing lead concentration except in the 1st week when the MCV value was higher in the fish exposed to 0.1 mg L^{-1} lead than in the fish treated with 0.4 mg L^{-1}. The MCV generally decreased with increasing lead concentration and with duration of exposure. The MCH values were in the treatment groups were lower ($p<0.05$) than the control and it also decreased with increasing lead concentration. The MCHC values also decreased with increasing test concentrations except by the last week of the study. The MCHC values were significantly different in the treatment groups ($p<0.05$).

DISCUSSION

The result of the study showed that lead chloride caused significant changes in the blood parameters of *Clarias gariepinus*. There was a significant decrease in the haemoglobin concentration in the treatment groups when compared with the control. Similar significant decrease in haemoglobin level was reported by Christensen *et al.* (1977) in brook trout (*Salvelinus fontinalis*) exposed to lead for 6-8 weeks and in *Oreochromis mossambicus* exposed to 0.16 mg L^{-1} copper (Nussey *et al.*, 1995). However, Larsson *et al.* (1985) reported no reduction in the haemoglobin concentration in the white fish (*Coreganus* sp.) poisoned with lead nitrate when compared with the control.

The erythocytosis observed in this study was also reported by Nussey *et al.* (1995) in *Oreochromis mossambicus* after short term exposure to 0.16 mg L^{-1} copper. It is also in agreement with the reported of increase in erythrocyte count in carp and rainbow trout exposed to copper and in *Heteropnestes fossilis* treated with copper (Mishra and Srivastava, 1980; Singh and Reddy, 1990). Similarly, Tort and Torres (1988) reported increased erythrocyte count and haemaoglobin but decreased haematocrit in the dogfish after 24 h exposure to 25 μg L^{-1} cadmium. Also, Gill and Pant (1987) observed that there were increased values of erythrocyte and

Table 1: Changes in the haematological parameters of *Clarias gariepinus* after 7 days of exposure lead (II) chloride

Concentration (mg L^{-1})	Hb (g dL^{-1})	Hct (%)	RBC×10^6 mm^{-3}	WBC×10^4 mm^{-3}	MCV (μm^3)	MCH (pg)	MCHC (%)
0.00	9.35±0.64	27.00±1.41	2.32±0.08	2.47±0.08	116.80±10.03	40.37±1.39	34.74±4.17
0.10	7.55±0.21	36.00±1.41	2.64±0.04	2.54±0.04	136.34±3.160	28.59±0.34	20.98±0.23
0.40	7.10±0.42	29.00±1.41	2.75±0.04	2.94±0.10	105.51±6.770	25.84±1.94	24.48±0.27

Table 2: Changes in the haematological parameters of *Clarias gariepinus* after 14 days of exposure to lead (II) chloride

Concentration (mg L^{-1})	Hb (g dL^{-1})	Hct (%)	RBCC×10^6 mm^{-3}	WBC×10^4 mm^{-3}	MCV (μm^3)	MCH (pg)	MCHC (%)
0.00	9.20±1.56	28.00±1.41	2.80±0.27	2.80±0.02	100.71±14.71	33.28±8.75	32.76±3.90
0.10	6.70±2.26	21.00±4.24	2.72±0.16	3.38±0.60	76.89±11.21	24.43±6.92	31.46±4.42
0.40	6.95±0.07	23.00±2.83	3.41±0.59	3.96±0.06	67.45±20.35	20.71±3.81	30.43±3.42

Table 3: Changes in the haematological parameters of *Clarias gariepinus* after 21 days of exposure to lead (II) chloride

Concentration (mg L^{-1})	Hb (g dL^{-1})	Hct (%)	RBC×10^6 mm^{-3}	WBC×10^4 mm^{-3}	MCV (μm^3)	MCH (pg)	MCHC (%)
0.00	8.15±0.49	28.50±0.71	2.54±0.23	3.09±0.16	107.86±6.42	30.80±0.81	28.63±2.45
0.10	7.00±0.28	25.00±1.41	2.68±0.01	4.28±0.11	94.77±6.90	26.12±0.92	28.01±0.45
0.40	7.00±0.14	25.50±0.71	2.78±0.06	4.55±0.19	92.39±1.14	25.23±0.06	27.46±0.21

Table 4: Changes in the haematological parameters of *Clarias gariepinus* after 28 days of exposure to lead (II) chloride

Concentration (mg L^{-1})	Hb (g dL^{-1})	Hct (%)	RBC×10^6 mm^{-3}	WBC×10^4 mm^{-3}	MCV (μm^3)	MCH (pg)	MCHC (%)
0.00	7.10±0.14	29.50±0.71	2.24±0.04	4.70±0.04	131.98±1.07	31.77±0.13	24.07±0.10
0.10	6.15±0.21	22.50±2.12	2.27±0.04	4.84±0.01	99.28±7.81	27.15±0.51	27.41±1.64
0.40	5.25±0.35	20.50±2.12	3.68±0.10	5.55±0.04	55.65±4.26	14.26±0.58	25.66±0.93

haematocrit counts in *Barbus conchonius* during chronic exposure to chromium. Also, Nussey *et al.* (1995) observed marginal erythrocytosis in *Oreochromis mossambicus* after exposure to 0.16 mg L^{-1} copper at 19 and 29°C but not at 29°C when the tilapia was exposed to 0.40 mg L^{-1} copper. However, anaemia was reported in *Salmo gairdneri* exposed to 0.3 ppm lead (Johansson-Sjobeck and Larsson, 1978). Also Allen (1993) reported decreased erythrocyte count in *Oreochromis aureus* after exposure to 10 ppm lead.

Erythrocytopenia was also reported in *Colisa fasciatus* exposed to zinc (Mishra and Srivastava, 1980) and in *Clarias gariepinus* treated with copper and lead (Annune and Ahuma, 1998). In their study with *Anguilla anguilla*, Santos and Hull did not observe changes in the blood parameters after exposure to 0.3 ppm lead for 1 month.

The significant erythrocytosis coupled with decreased haemoglobin and haematocrit values and concomitant reduction in MCV, MCH and MCHC in this study are relevant bioindicators of stress and microcytic anaemia. Earlier reports (Nilsson and Grove, 1974; Cyriac *et al.*, 1989; Wepener *et al.*, 1992; Nussey *et al.*, 1995) noted that the observed erythrocythosis could be triggered by shortage of oxygen during the exposure period. This according to these researchers would impose oxygen debt in the fish, thereby promoting anaerobic respiration in the fish with the attendant high carbon dioxide level in the blood. Under this prevailing circumstance, the fish would begin to produce immature erythrocytes as a compensatory and adaptive response to cope with the challenge in an attempt to deliver more oxygen to the tissues. Also, the decreases in both the haematocrit and haemoglobin values in *C. gariepinus* during the study are indications of anaemia and reduced haemoglobin synthesis. Witeska and Kosciuk (2003) further noted that heavy metals might alter the properties of hemoglobin by decreasing their affinity towards oxygen and reducing their binding capacity thereby rendering the erythrocytes more fragile and permeable which probably results in cell damage.

The data on MCV values indicated erythrocyte shrinkage due lead exposure aand this may possibly be due to impaired water balance as a result exposure to lead or stress. Since, mature erythrocytes are large in size, the decreased MCV further shows that the erythrocytosis observed resulted in the production of immature cells for the purpose of extracting oxygen from the environment and transporting same to the tissues. Similar, decrease in MCV was recorded by Nussey *et al.* (1995) in *O. mossambicus* treated with copper and in *Labeo umbratus* exposed to pollutants (Van Vuren, 1986). Significant reduction in MCV was also reported in *Clarias albopunctatus* exposed to actellic (Mgbenka *et al.*, 2005) and cadmium (Oluah, 2001).

Also, MCV was reduced in stripped bass exposed to mercury (Dawson, 1982) and in tilapia *O. aureus* treated with 0.10 ppm mercury after 1 week (Allen, 1994). Since, the MCH and MCHC are the red blood cell morphological indices reflecting the haemoglobin concentration, the observed decrease in these parameters may indicate that the haemoglobin concentration in the control fish was higher than in lead-exposed fish. It may further suggest impaired haemoglobin synthesis in the treated fish. Reduced MCH and MCHC in fish were also reported by Van Vuren (1986) in *Labeo umbratus* exposed to

detergents and fertilizers. The observed leucocytosis in this study is similar to the report of Nussey *et al*. (1995) when the Mozambique tilapia was treated with 0.16 and 0.40 mg L^{-1} copper. Gill and Pant (1987) observed that the stimulation of the immune system causes an increase in leucocyte in *Oreochromis aureus* after mercury exposure. On the hand, leucopenia was observed in dogfish *Scyliorhinus canicula* after 96 h exposure to 0.25 μg L^{-1} cadmium and in *O. massabicus* treated with 0.1-10.0 μg L^{-1} cadmium (Ruparelia *et al*., 1990).

CONCLUSION

The result of this study showed that sublethal concentrations of lead chloride affect the haematological parameters of *Clarias gariepinus* and suggests that the evaluation of these parameters and other biochemical impacts in the fish could be a useful tool in monitoring subtle effects of metal contaminants in fish.

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