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Toxic Effect of Aluminium on Haematological and Immunological Profile of African Cat Fish, *Clarias gariepinus*

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Abstract: In the present investigation, *Clarias gariepinus* were exposed to different sublethal concentrations (50, 100, 150 & 200 ppm) of Aluminium Chloride ($AlCl_3$) for 96 hrs to investigate the changes in the haematological and immunological profile. Blood samples were collected from the fishes exposed to $AlCl_3$ for assessing a few haematological and immunological changes after 96 hrs. The results showed swelling of the red blood cells, haemodilution and hypochromic macrocytic anaemia. Leucocytosis and Lymphopenia were also observed in these $AlCl_3$ exposed fishes. Lymphopenia may be considered as a suggestive of immunosuppressive conditions. The results of the present study indicates that the treatment of *C. gariepinus* with sublethal concentrations of $AlCl_3$ for 96 hrs disturbs the normal functioning of haematopoietic system, resulting in reduced count of RBC, Hb, PCV and WBC as a dose-dependent effect.

Key words: Aluminium · Sublethal effect · Haemodilution · Macrocytic Anaemia · Lymphopenia

INTRODUCTION

Metals that contaminate the environment arise from natural sources and industrial activities besides the contribution from air [1]. Accumulation of heavy metals in aquatic bodies, soil and air also occurs mainly due to anthropogenic activities [2]. Aluminium (Al) is found as the third most abundant metal on earth which occurs as its oxides and silicates in nature [3]. Acidic soil liberates Al into the surface waters, where it acidifies the water and becomes toxic to fishes [4]. Inorganic monomeric form of Al is found to be most injurious to various fish species [5]. Neville and Campbell [6] reported Al as a gill toxicant to fish, causing ionoregulatory and respiratory disturbances in fishes. Thus, Aluminium remains as a major factor contributing for the killing of fishes in acidified aquatic bodies [7].

Cat fishes are bottom-dwelling carnivores, hence there is more chance for them to get exposed to metals that accumulate in the sediment and other prey fishes [8, 9]. In general, metals can enter into the fish through the skin, gill or orally through the water and food consumed by them. After absorption, metals are transported via blood stream to the liver for storage and also accumulate in various organs of the fish [10].

Haematology is used as an index to study the health status of various fish species by detecting their physiological changes under different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc [11]. The most common haematological variables measured during stress are blood cell count (RBC and WBC), hemoglobin content, Haematocrit/PCV and derived erythrocytic indices such as MCV, MCH and MCHC. Therefore, haematological variables can be used to determine the toxic effect of sublethal concentrations of pollutants [12, 13].

According to Das *et al.* [14] most of the toxicological studies are limited to the effects of lethal or acute doses of the pollutants. But, scanning of literature reveals that most physiological disorders occur only due to the exposure of sublethal concentrations of the toxicants [15]. The toxic stress and haematological effects of various metals such as mercury, copper and nickel on the haematology of *Clarias* sp have been reported earlier [13, 16, 17]. Red blood cell (RBC) system of most of the fish species react to heavy metal intoxication with anaemia but in some fishes, after short exposures, blood parameters (Haematocrit, RBC count, Mean corpuscular volume, hemoglobin content) may be increased [18, 19].

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The present study was undertaken to evaluate the haematological effects resulting from the exposure of the fresh water fish, *Clarias gariepinus* to sublethal concentrations of Aluminium Chloride.

MATERIALS AND METHODS

One hundred and fifty juveniles of *C. gariepinus* (67g x 25 cm) were purchased from a local fish farm and acclimatized in de-chlorinated tap water (60 L) for a week in plastic troughs. Fifty fishes were randomly collected for the experiment and further divided into five groups, each containing 10 fishes. The first group was kept as control and the other groups were exposed to sublethal concentrations of $AlCl_3$ namely 50, 100, 150 & 200 ppm (mg/L) for 96 hrs. For each treatment three replicates were maintained. Troughs were covered with mosquito net. The experimental set up was monitored closely to observe changes in the behaviour of the fish and also to remove the dead fish.

Collection of Blood Samples: At the end of 96 hrs exposure, fishes were randomly sampled from each group in 3 replicates. Blood samples were collected by heart puncture using disposable, sterile syringe fitted with an insulin needle and stored in sterile EDTA coated blood collection tubes to study the Erythrogram, total and differential leukocytic count.

Determination of Haematological Parameters: The total RBC counts were enumerated under compound microscope using Neubaeur ruled counting chamber with Hendrick [20] diluting fluid. Enumeration of WBC was done using Neubaeur chamber with Shaw [21] solution as per the methodology described by Hesser [22]. Hemoglobin (Hb) concentration was measured with Hb kit using cyanmethemoglobin method [23]. PCV/Haematocrit value was determined by the standard microhematocrit method. Blood samples were loaded into standard heparinized capillary tubes, spun in a microhematocrit centrifuge at 12, 000 rpm for 5 min and measured on a microcapillary reader.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formula described by Jain [24] and Abalaka [25].

$$\text{MCV (In femtoliters, fl)} = \frac{\text{PCV}}{\text{RBC}} \times 10$$

$$\text{MCH (In picograms, pg)} = \frac{\text{Hb}}{\text{RBC}} \times 10$$

$$\text{MCHC (g/dl)} = \frac{\text{Hb}}{\text{PCV}} \times 100$$

Statistical Analysis: The mean values of exposed fishes were compared statistically with control by student's t-test [13, 26] using the SPSS (16.0) programme.

RESULTS

The mean RBC, Hb, PCV / H.Ct and the derived erythrocytic values such as MCV, MCH and MCHC of the fishes exposed to Aluminium Chloride are presented in Table 1. There is a progressive decline in the haematological parameters like RBC (Figure 1(a)), Hemoglobin (Figure 1(b)) and PCV (Figure 1(c)). Significant increase is noted in MCV value (Figure 1(d)). The mean total WBC count (TLC) and differential WBC count values are presented in Table.2.There seems to be a reduction in total WBC count (Figure 2(a)) and Lymphocyte count (Figure 2(b)).

Table 1: Erythrogram (Mean ± SD) in African Catfish, *C. gariepinus* exposed to Aluminium Chloride

Haematological Parameters	Control	50 ppm	100 ppm	150 ppm	200 ppm
RBC (million/cu. m)	2.03 ± 0.14	2.23** ± 0.17	2.19** ± 0.12	1.99* ± 0.15	1.30** ± 0.08
Hb (g/dL)	9.9 ± 0.04	11.2** ± 0.87	10.9* ± 0.83	9.5** ± 0.56	6.3** ± 0.50
PCV (%)	22.5 ± 1.62	24.8** ± 1.89	23.4** ± 1.25	21.5* ± 1.70	14.9** ± 1.12
MCV (fL)	110.8 ± 8.62	111.4** ± 8.09	106.9** ± 8.49	108** ± 8.46	114.6** ± 8.86
MCH (pg)	48.6 ± 3.46	50.2** ± 3.91	50.1** ± 4.01	47.7** ±3.80	48.46* ± 3.82
MCHC (g/dL)	44 ± 3.02	45.0* ± 3.10	46.58* ± 3.48	44.18* ± 3.50	42.28** ± 3.34

* Significantly different from respective control (p<0.05)

** Significantly different from respective control (p<0.01)

Table 2: Leukogram (Mean ± SD) in African Catfish, *C. gariepinus* exposed to Aluminium Chloride

Immunological Parameters	Control	50 ppm	100 ppm	150 ppm	200 ppm
TLC (cells/cu.mm)	15470 ±548.6	18700** ± 495.0	14630** ± 664.8	14570** ± 570.2	9720** ± 673.6
Lymphocyte (%)	99.3 ± 6.91	98.9* ± 7.92	98.0** ± 7.83	96.3** ±7.67	90.9** ± 7.17
Neutrophil (%)	0.5 ± 0.01	0.4* ± 0.03	0.8* ± 0.05	1.1** ± 0.08	3.3** ± 0.18
Monocyte (%)	-	-	-	-	0.2 ± 0.01
Esinophil (%)	0.2 ± 0.02	0.7** ± 0.05	1.2** ± 0.08	2.6** ± 0.24	5.3** ± 0.42
Basophil (%)	-	-	-	-	0.3 ± 0.02

* Significantly different from respective control ($p<0.05$)
** Significantly different from respective control ($p<0.01$)

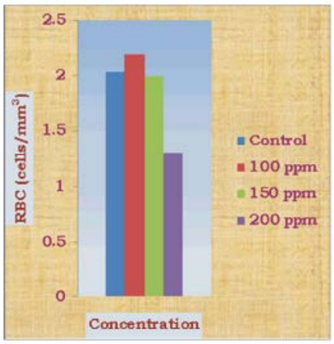

Fig. 1(a)

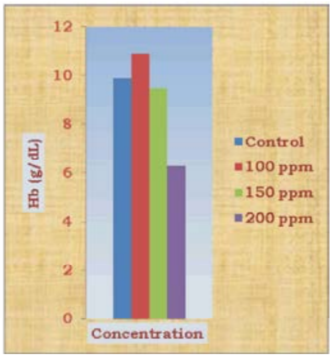

Fig. 1(b)

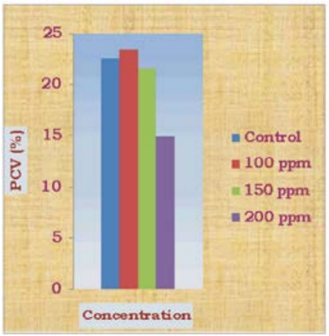

Fig. 1(c)

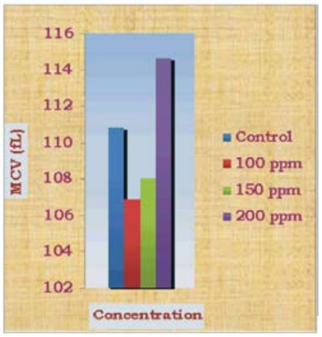

Fig. 1(d)

Fig. 1: Erythrogram in African Cat fish, *C. gariepinus* exposed to Aluminium Chloride

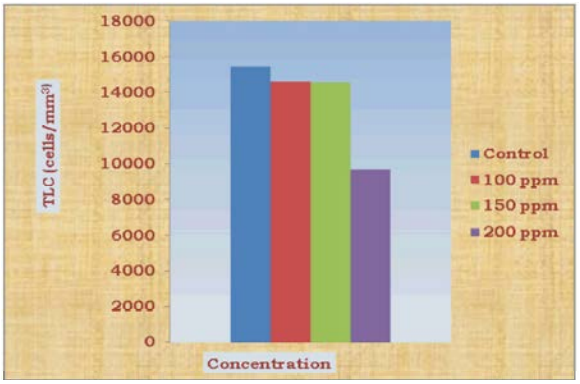

Fig. 2 (a)

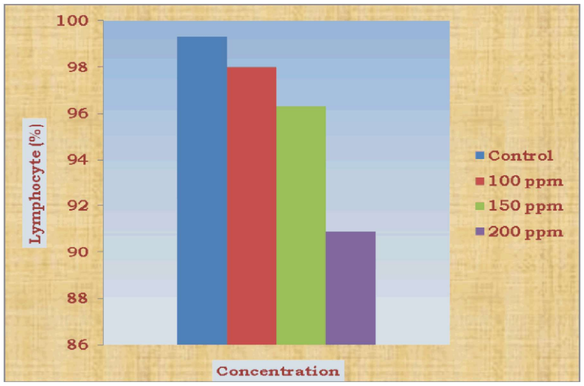

Fig. 2 (b)

Fig. 2: Leukogram in African Catfish, *C.gariepinus* exposed to Aluminium Chloride

DISCUSSION

The haematological report of the present experiment reveals that the 96 hrs exposure of *C. gariepinus* to sublethal concentrations of $AlCl_3$ shows decrease in the haematological parameters such as total RBC, Hemoglobin and PCV (Haematocrit). Allin and Wilson [27] reported that juvenile rainbow trout, *Oncorhynchus mykiss*, exposed to Aluminium for 34 days in acidic soft water had significantly fewer RBC and lower PCV than the controls. Similar trends in RBC count, Hb and PCV in fresh water fishes exposed to various metal toxicants such as nickel, chromium, cadmium, lead and metal industry effluent have been observed by other researchers [17, 26, 28-30].

Bhagwant and Bhikajee [31] observed a significant decrease in the total red blood cell count, Hb and PCV in *Oreochromis* hybrid exposed to 100 mg/L of Aluminium which could be due to the destruction of the erythrocytes. Hence, a decrease in haematocrit and hemoglobin values may be due to the lysis of erythrocytes. Joshi *et al*. [32] suggested that heavy metal exposure may result in the impairment of intestinal absorption of iron and there by reduce the rate of red blood cell production and results in anaemia. According to Maheswaran *et al*. [13] a decrease in the production of RBC or an increase in the destruction of RBC can be the cause for the occurrence of anaemia. Generally, anaemia is considered as an early manifestation of acute and chronic toxic effect of metals [32].

An increase in the mean corpuscular volume (MCV) and fluctuation in the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also observed in the present study. The higher MCV value, suggests that the anaemic condition may be due to the destruction of red cells as suggested by Sakthivel [33]. An increased MCV may be considered as an index of RBC destruction leading to anaemia [34]. The increase in the MCV values can be considered as an indicator of the size or state of the red blood cells [17]. Larsson *et al*. [12] hypothesized that an increase in MCV and the swelling of RBC may be due to hypoxic condition or impaired osmoregulation or macrocytic anaemia in fishes exposed to metal pollution. Hence, an increase in MCV value may be due to the swelling of the erythrocytes which results in macrocytic anaemia. Tort and Torres [35] observed erythrocyte swelling in the dog fish, *Scyliorhinus caniculata* exposed to copper.

Allin and Wilson [27] reported that juvenile rainbow trout, *O. mykiss*, exposed to Aluminium had significantly fewer RBC and lower PCV value than the control, indicating the sign of haemodilution. They also suggested that in fishes exposed to Aluminium, haemodilution may reduce the blood oxygen-carrying capacity and the aerobic capacity of the fish [27]. Smit *et al*. [36] suggested that haemodilution may be an adaptive mechanism to reduce the concentration of any irritating toxicant in the circulatory system. Haemodilution had been observed earlier in *Colisa fasciatus* exposed to zinc [37] and *C. gariepinus* exposed to copper [16].

Our current study on Leukogram of *C. gariepinus,* reveals that there is a reduction in the total WBC count. Svoboda [38] suggested that a fish under stress releases epinephrine, which results in the contraction of spleen and a decrease in leucocyte count, thus weaken the immune system. Ololade and Oginni [17] suggested the decreased number of white blood cells (Leucopenia) may be due to the higher concentration of the exposed metal in the kidney and liver. There is also a progressive decline in the lymphocyte count (Lymphopenia) of the experimental fishes. Garcia-Medina *et al*. [39] reported that Aluminium can induce oxidative stress and exert genotoxic damage in lymphocytes of common carp, *Cyprinus carpio*. Lymphopenia may be considered as suggestive of immunosuppressive conditions [40]. Similar kind of Leucocytosis and decreased percentage of lymphocytes was observed in *Anabas testudineus* (Bloch) exposed to Titanium dioxide effluent by Nair *et al*. [41] and in fresh water teleosts exposed to cobalt by Srivastava and Agrawal [42]. An increase in Neutrophil count is usually considered as an index of tissue damage or the entry of foreign bodies into the blood stream as reported by Sakthivel [33].

CONCLUSION

Damaged metabolism in animals has been reported to repress their immune system, hence harming the blood cells [43]. From the findings of the present investigation, it can be concluded that the sublethal concentration of Aluminium Chloride exerts a profound influence on the haematology of *C. gariepinus* after 96 hrs of exposure by inducing hypochromic, macrocytic anaemia condition attributable to the swelling of the red blood cells, impaired haemoglobin synthesis and haemodilution. The fish exposed to sublethal concentration of Aluminium Chloride also shows leucocytosis and decreased percentage of lymphocyte (Lymphopenia). However, the mechanism underlying the effects of Aluminium on the haematopoietic system of *Clarias gariepinus* need to be elucidated further.

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