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INDUCTION OF HYPOCHROMIC MACROCYTIC ANAEMIA IN *OREOCHROMIS* HYBRID (CICHLIDAE) EXPOSED TO 100mg/L (SUBLETHAL DOSE) OF ALUMINIUM

by

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ABSTRACT

The haematology of *Oreochromis* hybrid locally cultured for human consumption was investigated after exposing the fishes for 8 weeks to a sublethal dose of 100mg/L aluminium. The haematological changes based on the examination of blood variables during the 2, 4, 6 and 8 weeks of exposures have been reported. The physiological changes brought by the sublethal dose of aluminium in *Oreochromis* hybrid direct towards a normochromic microcytic anaemia which gradually progresses upon prolonged exposure to a normochromic macrocytic one, eventually becoming hypochromic macrocytic after 8 weeks (long term) exposure. In this instance, these changes have been attributed to the swelling of the red blood cells, haemodilution and impaired haemoglobin synthesis as a result of persistent low haematocrit and haemoglobin concentration recorded.

Keywords: *Oreochromis* hybrid, fish, anaemia, aluminium, sublethal, haematology.

INTRODUCTION

During recent years considerable attention has been focussed on the fates of metals and their derivatives in the aquatic environment. The weathering of rocks, soil forms, human activities and increased use of metal containing fertilizers in agriculture could lead to a continued rise of the concentration of metal pollutants in fresh water reservoirs as a result of water run off, thereby representing the greatest hazard to human consumers of fish (Marr & Creaser, 1983; Gutenmann *et al*. 1988). Metal metabolism and metal toxicology are gaining ever increasing importance and trace element levels have been measured extensively in individual food items and integrated human diets. Furthermore, bioaccumulation and biomagnification are capable of leading to toxic levels of metals in fish, even when the exposure is low.

Fishes are known for their ability to concentrate heavy metals in their muscles and various organs (Varshney, 1991). However, in recent years haematological variables were used more often when clinical diagnosis of fish physiology was applied to determine the sublethal concentrations of pollutants (Wedemeyer & Yasutake, 1977). Various workers have shown that the use of haematological parameters as indicators to metal toxicity can provide information on the physiological response of fish due to the close association of the circulatory system with the external environment (Casillas & Smith, 1977; Cyriac *et al*. 1989; Wepener *et al*. 1992; Srivastava & Narain, 1985). Haematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc. (Blaxhall, 1972; Duthie & Tort, 1985).

Although a number of studies have been carried out to determine the mode of toxicity of potentially dangerous metals in aquatic environment, little information is available concerning the effect of aluminium on aquatic life (Goldberg, 1962; Stapleton, 1968; Mc Dermott *et al*. 1976). Aluminium may enter natural waters via coal strip mining activities (as a by-product of some oil shale mining processes), water treatment facilities using aluminium sulphate (alum) as a coagulant for suspended solid particles, industrial wastes and acid rainfall. However, when aluminium becomes available to organisms through acidification of surface waters, it is toxic to fish (Driscoll *et al*. 1980). Its toxicity to humans was first clearly recognized in renal medicine, when the element was recognized as a causal agent in neurological diseases like Alzheimer's disease and bone disorders in patients dialysed with aluminium- containing water (Kerr *et al*. 1992; Klatzo *et al*. 1965; Perl & Good, 1992).
Oreochromis species is one of the mostly popular fresh water fish consumed in several countries. The ease with which it can be cultured has made *Oreochromis* an ideal model for culture in treated wastewater. The problem however, is that aluminium

and iron salts are used for flocculation of suspended solids during wastewater treatment.

Considering the significance of haematological parameters as indicators of fish health, this present work, which is part of our continuing studies on the use of aluminium and iron salts in monitoring water treatment, aims at studying the effect of aluminium on the haematological indices of *Oreochromis* fishes (locally cultured for human consumption) at different exposure periods.

MATERIALS AND METHODS

Fishes

Healthy male *Oreochromis* hybrid (average weight, 23.54g and length, 11.20cm) were obtained from La Ferme Experimental Fish Farm, Ministry of Fisheries, Mauritius and kept in 3500l polyethylene fiber tanks housed in the Wet Laboratory of the Department. The tank system had a domestic tap water flow-through system with air compressor to maintain constant aeration. The fishes were fed twice a day on a commercial floating feed during the experimental period, but food was withheld for the first 24 hr of each experiment and for 24 hr prior to sacrifice in order to reduce the risk of any external contamination of Al^{3+} from external sources. For instance, food which might well absorb some of the aluminium salt from water and also to prevent the formation of metal conjugates which might hinder other biochemical parameters under investigation. Faeces and food debris were siphoned out every 48hr.

Test animals and acclimation

During the experimental periods fifteen fish were placed in each of the 12 test glass aquaria, (90x30x50 cm) of 100l capacity, fitted with translucent covers as per the design of the experiment. Aeration of dechlorinated water was constantly maintained. The fishes were acclimated for 7 days in the following conditions: water temperature 18-22^{0}C; 12-h light-dark cycle; pH 7.6-8.2; hardness of water, 221-246ppm $CaCO_3$, 27.5-30.7ppm Ca^{2+} 193.5-215.8ppm Mg^{2+}; Electrical conductivity 0.25-0.29mS/cm. The fishes were then exposed to Al^{3+} ($Al_2(SO_4)_3.16H_2O$ salt was used) so that the total concentrations were 100mg/l of Al^{3+} per liter of water for the 80% of the 96-h median lethal concentration (LC_{50}) value and a pH 5.6. To calculate the 96-h LC_{50} and its 95% confidence limits, percentages of fish dead after a 96-h exposure and the different concentrations of aluminium sulphate in this flow-through toxicity test were recorded. The probit method was used to determine the LC_{50} which was 125 (80, 100)mg/L. To be certain the concentration of Al^{3+} was sublethal in the acute exposures, fish were exposed to 100mg/L Al^{3+} (80% of the determined 96-h LC_{50} value) for 2, 4, 6 and 8 weeks, respectively.

Forty-five fishes were used for each treatment-schedule and subdivided into three groups of fifteen each. One group received dechlorinated water only without Al^{3+} and was kept as control (untreated) group. The experimental fish did not exhibit any significant alterations in behaviour when compared with their control (untreated) counterparts.

Fishes were sacrificed by giving a blow on the head and immediately blood was drawn from the heart by means of disposable sterile syringe fitted with an insulin needle (0.5 diameter). Owing to insufficient amount of blood, the haematocrit determination for each experimental schedule was done on pooled samples in triplicate (from 5 fishes each) in sterile heparanized vials. Each triplicate was duplicated thus obtaining a total of six samples per treatment. Blood-filled heparanized microhaematocrit capillary tubes were centrifuged at 12000 for 5 min using a microhaematocrit centrifuge (Hermle model Z320) and the haematocrit (Hct) values were read directly. The haemoglobin concentration was measured by the cyanmethaemoglobin method (Blaxhall & Daisley, 1973) at a wavelength of 540nm. Concurrently, the Total Red Blood Cell (RBC) and Mean Corpuscular Volume (MCV) were obtained by employing a Coulter–model T540 cell Counter. The Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using the methods described by Dacie & Lewis (1963). MCH was calculated in picograms/cell = Hb/RBC x 10 and MCHC = (Hb in 100mg blood/Hct) x 100. Similar procedures were adopted for the control (untreated) groups belonging to the respective treatment periods. Blood sampling was completed in less than 2 minutes; the entire autopsy procedure was performed in less than 4 minutes to minimize the risk of stressful condition.

STATISTICAL ANALYSIS

Data obtained were analysed by a two-way ANOVA appropriate to each experiment and any statistical significance of difference between means was tested at 95% confidence level by the Student's *t* test. Analyses were performed using the Statistical Package for the Social Sciences (SPSS Ò, version 7.5.1, 1996).

RESULTS

During the course of the experiments no mortalities were recorded in the fishes exposed to Al^{3+}, the temperature had no apparent effect on feeding behaviour of *O.*hybrid. Table 1 shows the mean values of the haematological indices recorded from exposing the *Oreochromis* hybrid to 100mg/L aluminium for 2,4,6 and 8 weeks. Differences were measured against the control values determined under controlled laboratory conditions.

After the 2^{nd} week (short term exposure), the number of erythrocytes (RBC) underwent a significant decrease ($p<0.01$) which persisted till the end of the experiment.

Table 1: Mean Haematological Values Recorded at Different Periods in *Oreochromis* hybrid exposed to 100mg/L (sublethal) dose of Aluminium.

Haematological Indices	EXPOSURE PERIODS							
Sample Size (n)=6	Week 2		Week 4		Week 6		Week 8	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
RBC x 10^6 (mm^3)	1.47 ± 0.03 (3.57)	1.39 ± 0.02 ** (3.52)	1.54 ± 0.02 (3.08)	1.32 ± 0.03 ** (3.86)	1.56 ± 0.03 (3.57)	1.29 ± 0.03 ** (3.90)	1.61 ± 0.03 (3.52)	1.25 ± 0.03 ** (3.60)
Hb (g/dl)	6.79 ± 0.06 (2.16)	6.31 ± 0.20 * (7.76)	7.25 ± 0.06 (0.94)	6.15 ± 0.08 ** (5.19)	7.53 ± 0.08 (2.96)	5.68 ± 0.20 ** (8.62)	7.72 ± 0.06 (1.90)	5.13 ± 0.10 ** (4.77)
PCV (%)	21.68 ± 0.18 (3.38)	18.88 ± 0.37 ** (4.88)	22.88 ± 0.16 (1.78)	19.76 ± 0.13 ** (1.96)	23.08 ± 0.06 (0.64)	19.42 ± 0.17 ** (2.14)	23.18 ± 0.14 (1.48)	19.06 ± 0.50 ** (6.14)
MCV (µ)	148.26 ± 1.80 (1.66)	135.95 ± 1.15 ** (1.68)	143.51 ± 4.18 (7.08)	149.64 ± 1.67 * (2.73)	148.62 ± 0.58 (0.64)	150.18 ± 1.95 * (3.21)	142.68 ± 1.81 (1.74)	150.83 ± 1.89 ** (3.05)
MCH (µµg)	46.28 ± 0.51 (2.70)	45.51 ± 2.15 (11.62)	47.80 ± 0.56 (2.92)	46.25 ± 1.08 (5.72)	48.09 ± 0.32 (1.64)	45.98 ± 1.64 * (8.15)	47.86 ± 0.36 (1.85)	41.13 ± 0.62 ** (3.69)
MCHC (%)	31.23 ± 0.47 (3.67)	33.58 ± 1.36 (11.48)	33.75 ± 0.23 (1.87)	31.14 ± 0.48 ** (3.62)	32.62 ± 0.32 (2.38)	29.33 ± 1.05 ** (8.62)	33.57 ± 0.28 (1.46)	29.57 ± 1.02 ** (8.53)

Values are means + Standard Error. Figures in parentheses are the Coefficient of Variability (%).
* Significantly different from respective control ($p<0.05$)
** Highly Significant ($p<0.01$)

Moreover, fish belonging to week 8 (long term exposure) had a significantly lesser number of red blood cells ($P<0.01$) in contrast to those of weeks 2 & 4 (short term exposures). The haematocrit (PCV) data show a lower value for the treated groups ($p<0.01$), compared with the controls, after 2 weeks. This difference is maintained up to 8 weeks during exposure to aluminium. On the other hand, a slight decrease ($p<0.05$) was noted in the haemoglobin concentration (Hb) as from the 2nd week of exposure when compared to control and became more pronounced ($p<0.01$) on weeks 4, 6 and 8, respectively. The haemoglobin content, like the haematocrit values in aluminium exposed fish, showed a significant decrease thereby, resulting in an anaemic condition. Interestingly, the time plot of this variable and erythrocyte counts in the present investigation showed that the magnitude of stress to the fish was proportional to the concentration of aluminium to which the fish were exposed (unpublished data). After 2nd week of exposure, the MCV showed a slight decrease ($p<0.05$) thereby, indicating slight microcytosis. This variable underwent a reverse, increasing ($p<0.05$) on week 4 and becoming more marked ($p<0.01$) on subsequent exposures. Consequently, no significant change ($p>0.05$) was observed in the MCH in weeks 2 and 4 exposed fish, which nonetheless, showed a considerable decrease ($p<0.01$) of this variable on weeks 6 and 8, respectively. On the other hand, the MCHC underwent a significant decrease ($p<0.01$) as from week 4 up to the end of the experiment. Of particular interest is the fluctuation observed in both MCV and MCHC variables in exposed fish for different periods resulting in a microcytic hypochromic anaemia which progresses to a macrocytic hypochromic type after 8th week of exposure to 100 mg/L Al^{3+}.

DISCUSSION

The results presented above have revealed an interesting pattern of response on the haematological variables in aluminium-dosed fish. In addition, the duration of exposure (2-8 weeks) to 100 mg/L of aluminium has resulted in an anaemic condition in *Oreochromis* hybrid. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van Vuren, 1986). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology, since they live in close association with their environment and are sensitive to slight fluctuation that may occur within their internal milieu (Cassilas & Smith, 1977). In the light of the present study, the significant decrease in the total red blood cell count observed in *Oreochromis* hybrid exposed to 100mg/L of aluminium could be attributed to the destruction of the erythrocytes, thereby, limiting their synthesis. Similar trends in RBC in fishes exposed to various toxicants have been observed by other workers (Mc Leay, 1973; Smit *et al.* 1979; Koyama & Ozaki, 1984; Srivastava & Narain, 1985; Van der Merwe, 1992). At present, the distinct decrease in the level of haemoglobin (Hb) and increase in the mean corpuscular volume (MCV) observed

after the 2nd week of exposure clearly suggests that a haemodilution mechanism being operational. The mean corpuscular volume gives an indication of the status or size of the red blood cells and reflects an abnormal/normal cell division during erythropoiesis. The increase in MCV may be attributed to the swelling of the erythrocytes resulting in a macrocytic anaemia. Larsson *et al*. (1985) attributed the increase in MCV to the swelling of the RBC as a result of hypoxic condition or impaired water balance (osmotic stress) or macrocytic anaemia in fishes exposed to metal pollution. Haemodilution has been interpreted as a mechanism that reduces the concentration of an irritating factor in the circulatory system (Smit *et al*. 1979). Haemodilution has been observed in *Colisa fasciatus* exposed to zinc by Mishra & Srivastava (1979). Tort *et al*. (1987) observed a process of erythrocyte swelling in the dog fish, *Scyliorhinus canicula* exposed to copper. Such an increase of erythrocyte size is generally considered as a response against stress and would be a consequence of several factors like high PCO_2, high lactate concentration or low PO_2 in the blood, leading to a low ATP concentration, which would increase the oxygen affinity of blood (Soivo & Nikinmaa, 1981). In this instance, it is difficult to ascribe the swollen red blood cells to one of these factors owing to the nature of the experiment and, therefore, necessitates further elucidation. Since metals have been found to produce changes on blood gases and lactate, the swelling of red blood cells could be involved in the response of fish against heavy metal pollution (Tort *et al*. 1987). On the other hand, the decrease in MCV after short term (2 weeks) exposure coupled with a low haemoglobin content to aluminium indicates that the red blood cells have shrunk, either due to hypoxia or a microcytic anaemia. At this stage, microcytosis may be due to the decrease in the haematocrit during exposure. Similar pattern has been detected in *Labeo umbratus* after exposure to various pollutants (Van Vuren, 1986).

The fluctuation in the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in the present study, clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control fish, thereby, depicting an anaemic condition. Anaemia can be caused by a number of pathological conditions. In this instance, the change from a normochromic, microcytic condition after short-term (2 weeks) exposure to a normochromic, macrocytic one, eventually leading to hypochromic, macrocytic type is recorded for the first time in aluminium-dosed fishes. The macrocytosis is probably an adaptive response through the influx of immature erythrocytes from the haematopoietic tissues to the peripheral blood to make up the reduced RBC number and decreased haemoglobin concentration. These findings further support the hypothesis that haemodilution is a probable cause for decrease in Hb content in aluminium-dosed fishes. The MCHC is a good indicator of red blood cell swelling (Wepener *et al*. 1992). The MCHC, which is the ratio of blood haemoglobin

concentration as opposed to the haematocrit, is not influenced by the blood volume nor by the number of cells in the blood but can be interpreted incorrectly only when new cells, with a different haemoglobin concentration, are released into blood circulation (Soivo & Nikinmaa, 1981). The significant decrease in the MCHC after long-term (6-8 weeks) exposure, is probably an indication of red blood cell swelling and/or to a decrease in haemoglobin synthesis. Buckley *et al*. (1976) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants. Moreover, studies on aluminium toxicity in moribund fishes have revealed microscopic damage to gill tissues; ion-regulatory disturbances of plasma electrolytes (e.g. NaCl and Ca^{2+}); respiratory dysfunction characterized by plasma acidosis and concomitant hypoxia and hypercapnia and, osmoregulatory breakdown resulting in a net flux of water (Driscoll *et al*. 1980; Baker & Schofield, 1982; Birchall *et al*. 1989). Additional observations due to aluminium toxicity include both the apical and intracellular accumulation of aluminium at the gill epithelium (Jensen & Weber, 1987; Youson & Neville, 1987). However, aluminium effects on gill membrane permeability resulting in accelerated cell death may be considered as the general feature of aluminium toxicity in cells (Exley *et al*. 1991).

In the light of the present investigations, the conclusion is that the sublethal dose of aluminium exerts a profound influence on the haematology of *Oreochromis* hybrid after short and long term exposures by inducing a normochromic, microcytic condition to a normochromic, macrocytic one, eventually leading to hypochromic, macrocytic anaemic condition attributable to the swelling of the red blood cells, haemodilution and impaired haemoglobin synthesis. However, the mechanism underlying the effects of aluminium on the haematopoietic system of *Oreochromis* hybrid, merits further elucidation.

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