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Effect of aluminum exposure on some blood parameters in the *Tilapia zillii* fish

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Abstract

The present study discusses the effect of aluminum chloride of the freshwater fish *Tilapia Zillii* that exposed to different concentrations of 25, 50, and 100 mg aluminum per liter of acidic soft water (PH 6.0). The hematological changes based on the examination of some blood variables during the 24, 48, and 96 hours of exposures have been reported. The hematological parameters determined were: total erythrocytes count (RBC), hematocrit (HCT), and mean corpuscular volume (MCV). Results showed statistically differences in RBC count and HCT were significantly higher when compared with control groups, while there was significant reduction in mean corpuscular count. Changes increased with increase in concentration of aluminum and time of exposure. Observation of blood parameters allows the most rapid detection of changes in fish after the exposure to xenobiotics.

Key-Words: Aluminum, Hematological parameters, *Tilapia*, Fresh water fish

Introduction

Human population growth and industrial development have been the major causes of coastal contamination around the world during recent years (Caussy , *et al.* 2003) . Discharges of metal effluents in to rivers may cause deleterious effects to the health (Tavares & Carvalho , 1992) . Fish contaminants can reach man through the food chain (Pfeiffer,*et.at*.,1985).

Aluminum is released to the environment by both natural processes and anthropogenic sources (ATSOR,2006). Aluminum may enter natural water via coal strip mining activities, water treatment facilities using aluminum sulphate (alum) as a coagulant for suspended solid particles , industrial wastes and acid rainfall . however , when aluminum becomes available to organisms through acidification of surface waters , it is toxic to fish (Driscoll,*et.at*.,1980) . The main effects of aluminum exposure in fish are respiratory and ion regulatory disturbances (Gensemer & Playle, 1999) .

Hematological parameters 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. Therefore, hematological techniques are the most common method to determine the sub-lethal effects of the pollutants (Larsson, Haux, & Sjöbeck, 1985).

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Tilapia species is one of the mostly popular fresh water fish consumed in several countries . They are mainly lacustrine fish that are well adapted to enclosed water. Therefore , *Tilapia zillii* was chosen in this experiment. The present study was conducted in order to evaluated the sub-lethal effects of aluminum on the hematological indices of tilapia fishes under laboratory condition.

Material and Methods

Random fish samples of adult tropical fresh water fish *Tilapia Zillii* , Gervais , 1848 (Family : Cichlidae) , were obtained from the commercial catches of umhfein lake (umalruzam town) on the eastern coast of Libya and transferred to the laboratory where the experiments were conducted . The animals were put in large containers with continuous aeration. The experimental room was air conditioned (25 °c) and the photoperiod during the experiment was natural . The tap water used for the experiment had a PH value of 7± 0.1 and a total hardness of 20 mg $CaCO_3$ / L and was replaced every 4 days. The fish were fed once a day with an artificial fish pellets. Fish were acclimated in the water for 2 weeks immediately before the exposure experiment.

A total of 20 adult tilapia (*T. Zillii*) of both sexes were used . The average body length and weight of the fish at the beginning of the experiment were 17 cm. and 106 g respectively. The fish were batch distributed in to rectangular glass tanks (120 x 40 x 30 cm) each filed with 100 liters of dechlorinated water ; and allowing one hour for acclimation to laboratory conditions .

The tanks were aerated with air stones attached to an air compressor for oxygen saturation . The fish were exposed to the metal separately, each containing 5 fish. The fish were exposed to a nominal concentration of aluminum chloride ($AlCl_3$; $6H_2O$ .Riedel – de Haën). In the case of the dissolved aluminum exposure , 3 treatments were established having aluminum concentrations of 25 , 50 and 100 µg/ L in acidic water (PH 6.0) . The last tank was left untreated as control

by taking 1 ml of blood samples from the caudal vessels using heparin zed syringes with a 1.10 x 40 mm injection needle (pentaferte S.P.A. Campli TE) . Blood samples transferred to group. The fish were exposed to the aluminum for a period of 24, 48 and 96 hours (acute short – term exposure).

At the end of the 24, 48 and 96 hours exposure periods, variations in blood parameters were monitored throughout the exposure tubes containing ethylenediamine tetra acetic acid – potassium (EDTA-K_2) as an anticoagulant.

Erythrocytes (RBC) were counted immediately after blood collection in hemocytometer (Improved Neubauer .Weber Scientific Ltd.) according to Wintrobe (1934) . To measure hematocrit (HCT) , ammonium heparin zed hematocrit capillary tubes (Fisher Scientific com.) were filled with blood and centrifuged for 5 min. at 5000 x g in a micro capillary centrifuge (Haematokrit 24 . Hettich). The percentage of hematocrit was determined by the use of a micro capillary reader. Mean cell volume (MCV) was calculated using the formulae mentioned by Dacie and Lewis (2001). MCV (µ3) = HCT / RBC X 10 .

Data collected from the experiment were subjected to one way analysis of variance (ANOVA) test using the statistical package for the social sciences (SPSS) , and were significant difference were indicated , means were tested using Least Significant Difference (LSD) test to compare the means of treated groups against that of the corresponding control . In all cases, P< 0.05 was the accepted significance level. Significant values are indicated with an asterisk. Statistically significant differences are indicated as follows: * P < 0.05 , ** P < 0.01 , and *** P < 0.001 .

Results and Discussion

The alterations in the number of the red blood cells count are presented table 1 . Aluminum concentration showed no increase on the mean of blood cell count after 24 hours , accept that of the 100 µg / L concentration were increased significantly (P < 0.05) different from the control values . Results showed an increase in the range of mean No. of RBC count with increasing the exposure period and concentration giving , there was a significant increase (P < 0.001) in the mean No. of (RBC) count after 48 hours exposure to the three levels of aluminum treatments , and also the concentration of 50 µg / L significantly (P < 0.05) increased in the No. of (RBC) count after 96 hours exposure . Comparable to that reported by Alwan *et.al*., (2009) .

Aluminum becomes more soluble and hence, potentially more toxic to fresh water biota as PH decreases below 6.0 (Gensemer & Playle, 1999). In the PH range 4.5 to 6.0 aluminum in respiratory problems associated with precipitation of aluminum on the gill mucus (IPCS, 1997). Aluminum accumulation could have been accompanied by mucus formation increasing the distance between water and the blood in gills. This increase in mucus thickness causes the diffusion distance between the water and the blood hemoglobin to increase. Since diffusion is inversely proportional to the square of the distance, a slight increase in diffusion distance rapidly impairs O_2 and Co_2 exchange (Goss & Wood , 1988).

Significant elevated in No. of (RBC) count was observed in this study. Similarly, the RBC count of dog fish was increased when the fish was exposed to cadmium for a period of 24 – 96 hours (Tort & Torres, 1998). On the other hand, Allin & Wilson (1999) reported that juvenile rainbow trout, exposed to aluminum for 34 days in acidic soft water had significantly fewer RBC than the controls, indicating hemodilution.

Erythropoiesis , whereby the number of red blood cells in the circulation is increased is in fact a first mechanism through which fish might compensate for poor oxygen up take in prevailing hypoxic conditions (Wepenr , van vuren & Du preez , 1992) . A second mechanism by which fish might compensate for poor oxygen up take during hypoxic conditions is via the release of large number of mature red blood cells in the general circulation. This is thought to be stimulated by B – adrenergic action on the hemopoietic tissues, which contract and release stored mature red cells (Wepener, *et. al*., 1992). This mechanism might, however, compensate for short – term oxygen concentration variations in blood or water (Nespolo & Rosen man, 2002).

The effects of aluminum treatment on blood hematocrit (HCT) of *T. Zillii* adults are presented in table (2). The results show that the blood HCT value was marked significantly (P < o.o5) increased for 50 µg / L concentration after 48 and 96 hours from aluminum exposed , also there was highly significant (P < 0.001) for 25 and 100 µg / L concentrations after 48 hours from aluminum exposed . The increase in HCT values was previously recorded by Poleo & Hytterod (2003). for Atlantic Salmon exposed to aluminum – rich water for 3 weeks at PH 9.5 . An increase in the blood HCT value has often been shown to be a good indicator of aluminum toxicity. The high HCT value in aluminum – exposed fish has been explained on one hand by an increased RBC volume caused by osmotic changes due to ion losses from the blood plasma and on the other hand by increased numbers of BRC as a result of adrenergic – splinic contraction in hypoxic conditions (Witters , et. al., 1991).

The present study show that the level of 100 µg / L of aluminum induced a highly significantly (P < 0.01) reduction in mean cell volume (MCV) for a period of 24 hours and there was also a highly significantly reduction in MCV for 48 hours relative to the control (table 3). In spite of the increase in the RBC count, a decrease in MCV may show the extent of the shrinking cell size due to aluminum intoxication (Alwan, *et. al*., 2009) . The MCV gives an indication of the status 0r size of the red blood cells and reflects an abnormal or normal cell division during erythropoiesis. The decrease in MCV indicates that RBC have shrunk , therefore , we suggest that the increase in RBC value but the reduced erythrocyte size indicate a high percentage of immature red blood cells in circulation after short – term exposure to aluminum . These different erythrocyte parameters suggest an increased release of microcytic cells in the general circulation of the fish.

The present study suggests that the perturbations in these blood indices attributed to a defense reaction against toxicity of aluminum through the stimulation of erythropiosis or may be due to the disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sub lethal concentrations of aluminum .

Conclusion

In conclusions, result indicates that some hematological parameters, which are used in this study, has provided valuable information. The employment of hematological techniques has provided valuable knowledge for fishery biologists in the assessment of fish health and in monitoring stress responses. Assume that variation in values of blood indices may be a defensive mechanism against aluminum toxicity through stimulation of erythropoesis. It remains for further studies needed to protect the aquatic organisms especially Tilapia, an African teleost fish of worldwide importance for aquaculture.

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Table 1: Mean number of the red blood cells count (10^6 / mm^3) for the treatment after different periods

Treatment groups	Time (hours)		
	24	48	96
Control	2.37 ± 0.00	2.30 ± 0.02	2.44 ± 0.01
25 µg AL / L	2. 36 ± 0.05	2.65 ± 0.00 ***	2.44 ± 0.44
50 µg AL / L	2.38 ± 0.24	2.55 ± 0.02 ***	2.95 ± 0.02 ***
100 µg AL/ L	2.81 ± 0.01 *	3.08 ±0.02 ***	2.72 ± 0.01

Data are presented as the mean ± standard error
Significant differences with the control groups are indicated with asterisks: * P < 0.05, ** P < 0.01, and *** P < 0.001.

Table 2: Mean percentage hematocrit for the treatment after different periods

Treatment groups	Time (hours)		
	24	48	96
Control	20 ± 0. 86	20 ± 0.28	21 ± 0.57
25 µg AL / L	20 ± 0.57	23 ± 0.57 ***	21 ± 0.28
50 µg AL / L	20 ± 1.73	22 ± 0.57 *	25 ± 1.52 *
100 µg AL/ L	23 ± 0.57 *	26 ± 0.50 ***	23 ± 0.57

Data are presented as the mean ± standard error
Significant differences with the control groups are indicated with asterisks: * P < 0.05, ** P < 0.01, and *** P < 0.001.

Table 3: Mean corpuscular volume (μ^3) for the treatment after different periods

Treatment groups	Time (hours)		
	24	48	96
Control	84.38 ± 0.03	86.95 ± 0.01	86.06 ± 0.03
25 µg AL / L	84.74 ± 0.42	86.79 ± 0.02	86.06 ± 0.57
50 µg AL/L	84.03 ± 0.57	86.61 ± 0.46	84.74 ± 0.05
100 µg AL/ L	81.85 ± 0.06 **	84.41 ± 0.03 ***	84.55 ± 0.58

Data are presented as the mean ± standard error
Significant differences with the control groups are indicated with asterisks: * P < 0.05, ** P < 0.01, and *** P < 0.001.