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Effect of Aluminum on Hemoglobin Content of Erythrocytes in the *Tilapia Zillii* Fish

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Abstract

The effects of aluminum chloride were investigated on the hemoglobin content in adult *Tilapia* Zillii that exposed to different concentration of 25µg, 50µg and 100µg aluminum per liter of acidic soft water PH 6.0 . This effect has been examined in the treated groups for three progressive time periods 24, 48 and 96 hours from beginning of exposure to aluminum. Results showed significant increase in hemoglobin (Hb) concentration, Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC).

Key-Words: Aluminum, Hemoglobin content, Tilapia, Fresh water Fish

Introduction

Aluminum is found everywhere in nature and it moves from soil to water and air via different nature powers. It inters nutrition chain of all creatures. In some cases aluminum found in food, air and water in quantity that can be measured, hence it impossible to avoid exposure to aluminum (Skalsky and Carchman, 1983). The main source can human being gets exposd to aluminum and its components from air water, food and medical drugs (ATSDR, 2006).

The medical importance of aluminum in the recent years has encouraged increasing studies that tackle toxic effects of this element on both man and animal (Epstein, 1990). Aluminum toxic effects have been thoroughly noted in plants and water creatures in different clinical circumstances (Wills and Savory, 1988).

Metals considered being the most important pollution factors in the study of water environmental pollution because of their toxicity and accumulation in creatures bodies (Turkmen *et al.*, 2005). Aluminum contaminated creatures as plants and invertebrates are highly suggestive to be an important means that transfer aluminum to ground animal food chain. Studies have indicated that such metal accumulation in living creatures that become on top of food for both big creatures and man in a way that threat life when eaten (Bishop , 2000).

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The importances of food and economical fish among other water creatures have won the huge study area of researchers. Aluminum toxic features in fish are clearly appearing in decrease of natural breeding process in different stages (Nakamoto and Hassler, 1992). Aluminum causes severe functional disturbances in fresh water fish when found in high concentration of acido – alkaline media (Brodeur et al., 2001). It really influences both food consumption and fish swimming activity that got exposed to this element, whereas it influences ionic and osmotic equilibrium.

As for rare researches that reviewed aluminum influence on fresh water fish and some studies have reviewed aluminum element effects on Tilapia zillii fish which known with their economical and nutritional importance. Therefore, the present study was designed to examine aluminum effects on hemoglobin content in the blood cells of *Tilapia Zillii*.

Material and Methods

Adult freshwater fish Tilapia Zillii, Gervais, 1848 (Family: Cichlidae), were obtained from the commercial catches of Umhfein lake (Umalruzam town) on the eastern coast of Libya. On arrival in the laboratory, the fish were placed in large tanks with aerated tap water and were fed with commercially pellets. Fish were acclimatized for 2 weeks under a natural photoperiod and an average temperature of 25 °c. The tap water used for the experiment had a PH value of 7± 0.1 and a total hardness of 20 mg $CaC0_3$ / L and was replaced every 4 days. A total of 60 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 15 – 17 cm. and 106 g respectively. The fish were batch distributed in to rectangular glass tanks (120 × 40 × 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 a nominal concentration of aluminum chloride ($ALCL_3$; $6H_2O$. Riedel – de Hāēn). In the case of the dissolved aluminum exposure, 3 treatments were established having aluminum concentration of 25, 50 and 100 µg / L in acidic water (PH 6.0) . The last tank that contains nonacidified water was left untreated as control group. The fish were exposed to metal separately and 4 replicate tanks were used and divided in to 3 subgroups. For each treatment 5 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, by taking 1 ml of blood samples from the caudal vessels using heparinized syringes with a 1.10 × 40 mm injection needle (pentaferte S.P.A. campli TE). Blood samples transferred to tubes containing ethylenediamine tetracetic acid – potassium (EDTA – K_2) as an anticoagulant .

Erythrocytes (RBC) were counted immediately after blood collection in hemocytometer (improved Neubaur, Weber Scientific Ltd.) according to Wintrobe (1934). To measure hematocrit (Hct), ammonium heparinized hematocrit capillary tubes (Fisher Scientific CO.) were filled with blood and centrifuged for 5 – min at 5000 × g in a microcapillary centrifuge (Haematokrit 24 , Hettich). Hemoglobin (Hb) concentration was estimated as cyanmethemoglobin (Blaxhall and Daisley , 1973) by adding 20 µL of whole blood to 5 ml of Drakins solution using spectrophotometer (Biosystems BTS – 302) , at a wavethength 546 nm . Mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated using the formulae mentioned by Dacie and Lewis (2001).

MCHC (%) = Hb / Hct × 100

MCH (pg) = Hb / RBC × 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 where 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 significant 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 effect of aluminum on hemoglobin concentration (Hbc) are presented in table (1) . Result showes that the level of aluminum 100 µg / L for 24 and 48 hours exposure in Hbc were highly significantly ($P < 0.001$) different from the control values . Also results showed significant ($P < 0.05$) increased in concentration of 25 µg / L treatment . while results showed exposure after 96 hours cause highly significant ($P < 0.001$) increased in Hbc for 50 µg / L treatment , and also significantly ($P < 0.01$) increased for 100 µg / L treatment .

The present study showed a progressive increasing in content of exposed fish groups than control. Significant increases in the Hbc were found in juvenile grayling , *Thymallus Thymallus* , were exposed for 6 days to acidified water containing a mixture of aluminum and iron at two test temperatures , 13 and 3 °c (Peuranen *et al*. 2003) . We suggested that the elevated Hb can be the result of increasing red blood cells numbers (Alwan, 2013).

The effect of aluminum treatment on mean corpuscular hemoglobin of *T.zillii* adults are presented in table (2). The results showed a significant increase of MCH. Similar result was also obtained by Wahbi *et al*. , 2004) for striped seabream, *Lithognathus Mormyrus* exposed to industrial wastes . Our results suggest that the increasing in mean cell hemoglobin could be due to increase in the numbers of red blood cells and Hb content.

Data presented in table (3) indicated a significant differences in their values by increasing aluminum concentration , accept that of the 50 µg / L concentration were reduced after 48 hours period when compared to control groups .

Mean corpuscular hemoglobin concentration measure was to assess the amount of red cell swelling (decreased MCHC) or shrinkage (increased MCHC) presents (Milligan and Wood, 1982). The present study revealed that administration of aluminum induced marked red cell shrinkage (increased MCHC). This result was not agreed with finding of Goss and Wood (1988). Which noted swelling in the red blood cells for rainbow trout, *salmon gaivdneri*, which exposed to 112 µg / L aluminum at PH 4.8 after 72 hours period. These differences may be due to different fish species and level of acidic water.

Conclusion

The measuring of hemoglobin content, which is used in this study, has provided valuable information. The employment of hemoglobin techniques has provided

valuable knowledge for fishery biologists in the assessment of fish health and in monitoring stress responses. We assume that variation in values of blood indices may be a defensive mechanism against aluminum toxicity through stimulastion of erythropoesis. We believe that further researches is needed to protect the aquatic organisms especially Tilapia, an African teleost fish of worldwide importance for aquaculture.

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Table 1. Mean hemoglobin concentration (g/ 100 mL)for the treatment after different period

Treatment groups	Time (hours)		
	24	48	96
Control	3.47± 0.25	3.30 ± 0.25	3.42 ± 0.24
25 µg AL/L	3.71 ± 0.02	4.08 ± 0.05 *	3.85 ± 0.12
50 µg AL/L	3.67 ± 0.17	3.5 ± 0.28	6.06 ± 0.28 ***
100 µg AL/L	4.92 ± 0.10 ***	5.46 ± 0.02 ***	4.59 ± 0.31 **

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 corpusculal hemoglobin (Pg)for the treatment after different period

Treatment groups	Time (hours)		
	24	48	96
Control	0.28±14.64	14.34 ± 0.57	14.01 ± 1.00
25 µg AL/L	15.72 ± 0.61	15.39 ± 0.39	15.77 ± 0.30
50 µg AL/L	15.42 ± 0.28	13.77 ± 0.30	20.54 ± 1.52 **
100 µg AL/L	17.50 ± 0.57 **	17.72 ± 0.50 ***	16.87 ± 0.93

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 percentage hemoglobin concentration for the treatment after different period

Treatment groups	Time (hours)		
	24	48	96
Control	17.35 ± 0.23	16.50 ± 0.28	16.28 ± 28
25 µg AL/L	18.55 ± 0.31 **	17.73 ± 0.05	18.33 ± 0.57 **
50 µg AL/L	18.35 ± 0.27 *	15.90 ± 0.57	24.24 ± 0.16 ***
100 µg AL/L	21.39 ± 0.22 ***	21.00 ± 0.28 ***	19.95 ± 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 *** $p < 0.001$

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