

Assessment of the Hazardous Effect of Lead Pollution on *Oreochromis niloticus*, Including Hematological, Biochemical and Immunological Parameters

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Abstract: Heavy metals are persistent contaminants in the environment causing serious illness in fish, animals and human. Lead represents the main toxic element in nature. Lead has a tendency to accumulate in tissue and organs of exposed fish. The present study aimed to investigate the effect of lead pollution on fish with special reference to the hematological, immunological, serum biochemical parameters, where fifty healthy Nile tilapia fish were divided into 3 groups. Fish of gp1 served as a control. Fish of gp. 2 & 3 were used for the determination of acute lethal concentration dose and the pathological effect of lead on the exposed Nile tilapia. Blood samples were collected to obtain serum for biochemical studies and heparinized blood for hematological investigations. RBCs, Hb, Hct and MCHC showed significant elevations, the serum GPT and GOT were increased significantly. L.D.H, glucose and cortisol were elevated, while serum cholesterol concentration was reduced significantly.

Key words: Lead pollution · *Oreochromis niloticus* · Biochemical changes

INTRODUCTION

Lead occurs naturally in the environment. However, most lead concentrations that are found in the environment are a result of human activities. Due to the application of lead in gasoline, an unnatural lead-cycle has consisted, in car engines lead is burned, so that lead salts (chlorides, bromides, oxides) will originate, these lead salts enter the environment through the exhausts of cars. The larger particles will drop to the ground immediately and pollute soils or surface waters, the smaller particles will travel long distances through air and remain in the atmosphere. Part of this lead will fall back on earth when it is raining. This lead-cycle caused by human production is much more extended than the natural lead-cycle and has caused lead pollution to be a worldwide issue [1].

The high level of lead could be also due to the industrial discharges from superphosphate factories, traffics of high way or motor vehicles as well as the extensive use of agrochemicals such as fertilizers, pesticides and growth promoters [2].

Lead can enter the human body through uptake of food (65%), Water (20%) and air (15%) and cause several

unwanted effects, such as: disruption the biosynthesis of haemoglobin and anaemia, a rise in blood pressure, kidney damage, miscarriages and subtle abortions, disruption of nervous systems, brain damage, declined fertility of men through sperm damage and diminished learning abilities of children and behavioural disruptions of children, such as aggression, impulsive behavior and hyperactivity. Lead can enter a foetus through the placenta of the mother, because of this, it can cause serious damage to the nervous system and the brains of unborn children [3].

Industrial and agricultural discharges are considered the primary source of metal poisoning to fish in Egypt [4]. Lead has a tendency to accumulate in tissue and organs of exposed fish resulting in hepatic and renal dysfunction with growth retardation [5]. Consequently, it could induce alterations in hematological and serum biochemical parameters [6] as well as pathological changes in most body organs [7].

The present study aimed to investigate the effect of lead pollution on fish with special reference to the hematological, immunological, serum biochemical parameters.

MATERIALS AND METHODS

1- Fish: Fifty healthy tilapia fish of both sexes and 150±50 gm body weight, were obtained alive and transported immediately to the laboratory. They were kept in 5 glass aquaria (100 X 30 X 50 cm) that provided daily with a tap water and continuously with filtered air. The water temperature was adjusted at 18-22°C along the period of experiment using thermostatic heater. The fish were fed a balanced ration daily using the formula suggested by Ahmed and Matty [8]. Fish were kept under observation for 2 weeks.

Fish were divided into 3 groups (gps). Fish of gp1 (10) served as a control with no treatment. Fish of gp. 2 & 3 (20, each) were used for the determination of acute lethal concentration dose (LD₅₀ /72 hr, gp2) and to investigate the pathological effect of lead on the exposed fish (gp3).

2-Experiments

A- Determination of acute lethal concentration dose:

To determine lethal concentration dose, fish of gp. 2 were subdivided into 5 equal subgroups. Subgroup 1 served as a control. Other 4 subgroups exposed to 35, 75, 150 and 300 mg/L of lead acetate; respectively. Each dose was dissolved in the distal water of each aquarium. The number of dead fish was recorded within 72 hrs post-exposure and the acute lethal concentration dose was calculated according to the formula of Brown [9].

B- Long term exposure: Fish of gp3 were exposed to 1/100 of LD₅₀ /72 hr (1.5 mg /L) of lead acetate for 2 weeks according to Taylor *et al.* [10]. The excreta were removed regularly and the water was replaced within 4 days interval. Fish were kept under observation along the 14 days of exposure.

3- Sampling: Blood sample were collected from the caudal vein after 3, 7, 14 days of exposure, part of blood was left to clot and then centrifuged at 3000 r.p.m. to obtain serum for biochemical studies, the other part was heparinized for hematological investigations using the methods of Drabkin [11].

4-Hematological examinations: The erythrocytic indices (RBCs, Hb, Hct & MCHC) were estimated according to Schalm [12].

5-Serum biochemical analysis: Kits Biomerieux France were used for the determination of serum glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), serum glucose, serum cholesterol and total protein.

Serum cortisol hormone was analyzed by means of a gemmacoat 125-cortisol radio-immunassay kit (Diagnostic corporation, USA). Serum Ig M was also measured according to Fuda, *et al.* [13].

6-Statistical analysis: The obtained data were statistically analysed as T test according to Snedecor and Cochran [14].

RESULTS

A-Determination of acute lethal concentration dose:

Experiment 1 revealed that, the acute lethal concentration dose was 150 mg/L during the 1st 72 hrs post-exposure.

B- Long term exposure: The effect of lead acetate exposure on RBC's count, Hb level, Hct and MCHC values of exposed tilapia fish were recorded in Table (1). Polycythemia was observed on the 14 day (p<0.01). Blood

Table 1: Effect of lead on some hematological parameters of tilapia fish along the period of experiment (Mean±S.E.)

Parameter	3 days		7 days		14 days	
	Control	Exp.	Control	Exp.	Control	Exp.
RBCs 10 ⁶ /mm ³	3.30±0.23	3.53± 0.61	3.20 ±0.40	4.0 ± 0.84	3.60± 0.69	4.90± 0.78*
HB g/dl	7.20±0.54	8.00±0.20	7.00±0.16	8.80±0.70	7.20±0.23	9.30±0.64**
H.Ct%	19.70±1.20	22.80±1.24	19.94±0.29	27.30±1.54	21.60±1.64	29.69±1.94**
MCHc%	33.60±1.90	34.70±1.40	32.42±0.94	37.42±1.26	31.24±1.42	41.57±1.17**

Exp: experimental* Significant at p<0.01. ** Non-significant

Table 2: Effect of lead on the serum biochemical parameters of tilapia fish along the period of experiment (Mean±S.E.)

Parameter	3 days		7 days		14 days	
	Control	Exp.	Control	Exp.	Control	Exp.
GPT (I.U/L)	29.12±1.3	52.00±0.44	31.20±0.64	44.40±1.29	33.00±1.11	49.90±2.68*
GOT (I.U/L)	39.20±2.0	41.68±3.00	41.70±1.20	48.80±2.48	39.32±1.00	55.40±3.74**
L.D.H (I.U/L)	191.00±3.23	191.00±3.94	191.00±4.28	203.00±5.23	195.00±3.40	150.00±5.28*
A.L.P (K.A U/L)	3.80±1.20	3.00±1.64	3.78±1.084	4.97±1.90	2.82±1.60	5.94±2.25**
Glucose (mg / dl)	29.24±2.00	33.68±1.20	29.82±1.20	42.20±2.80	30.64±1.80	62.23±2.78**
Total protein (g/dl)	2.27±0.32	2.42±0.12	2.60±0.64	3.00±0.29	2.25±0.55	4.88±0.94*
Cholesterol (ng/dl)	121.40±3.20	127.60±2.90	127.00±3.00	173.00±3.23	121.90±2.40	99.39±4.30*
Ig. M (ng/ml)	1.90±0.13	1.75±0.24	1.91±0.42	1.53±0.92	1.84±0.12	0.24±0.075*
Cortisol (ng/ml)	0.85±0.23	1.43±0.07	0.99±1.23	1.87±1.20	0.84±0.72	1.93±1.64*

Exp: experimental* Significant at $p < 0.01$. ** Non-significant

Hb, Hct and MCHC showed a significant elevation by 14 days of experiment.

Table 2 revealed the changes of some biochemical constituents in the blood of tilapia fish due to lead acetate exposure. The obtained data revealed that serum GPT activity increased significantly by 14 days of exposure. A significant elevation in serum GOT activity was also observed on the 14th day ($p < 0.01$). L.D.H serum activity was elevated along the whole period of experiment especially on the day 14th. Hyperglycemia was constant findings from the beginning of the experiment until the end of the experiment. Serum cholesterol concentration was increased, on the 3rd day and the 7th day and was reduced significantly, on the day 14th day. Cortisol hormone was elevated along the whole period of experiment especially on day 14th.

DISCUSSION

Regarding the impact of lead on the hematological profile of Nile tilapia, polycythemia accompanied by elevated hemoglobin level, Hct value and MCHC were observed. Similar findings were reported by Mckim *et al.* [15], Hilmy *et al.* [16] and Taylor *et al.* [10] recorded polycythemia in rasy barb. But in contrary to our finding Hb level and MCHC were reduced in *Clarias lazera* exposed to copper [17]. The increased RBCs count may be due to stimulation of erythropoietin by elevated demands for O₂ or Co₂ transport as a result of increased metabolic activity or destruction of gill membranes causing faulty gaseous exchange. The increase Hb content could be explained as a process where the body tries to replace the oxidized denatured Hb [18]. The increase of Hct value and MCHC may be attributed to swelling of RBCs due to increased Co₂ in blood, hypoxia or stressful procedures [19, 20].

Exposure of Nile tilapia to sublethal concentration (1.5 mg/L) of lead acetate for 14 days resulted in a marked increase in the activities of serum GPT, GOT, LDH and ALP. The present findings agree with our microscopic findings, which revealed a marked degeneration and necrosis of hepatocytes as the elevation in transaminases activities may be attributed to the liver injury [21].

Serum cholesterol level, in the present study, showed a significant reduction that could be due to greater level of utilization of cholesterol during corticosteroidogenesis, as it is the precursor for steroid hormones [22]. In addition, they reported a rise in the blood protein resulted in a high density of lipoprotein in the serum and was suggested to be the cause of hypocholesterolemia in exposed fish.

Our results showed similar findings as that of Gill *et al.* [6] and Snieszko [23], who reported that, exposure of fish to lead had no significant increase on blood glucose of *salmo gairnei*. The blood glucose level reflected the changes in carbohydrate metabolism under hypoxia and stress conditions. Rise of glucose level indicated the presence of stressful stimuli eliciting rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue and accompanied by cortisol elevation [24]. Concerning serum protein level, a significant increase was noted 14 days postexposure to lead. The elevated protein concentration may be due to the induction of protein synthesis in liver.

The serum Ig. M was determined to find out information about fish immune system which was previously investigated in different species by many authors as Fuda *et al.* [13] O'Neill [25], in this work, the purified Ig. M was revealed a single perception against specific polyvalent antiserum to tilapia fish Ig, similar results was obtained by Bagee *et al.* [26] who found that, *Coho salmon* Ig was detected by specific anti Ig 14. Our

study revealed a significant decrease in Ig. M level in fish exposed to lead pollution if compared within control groups. Anderson *et al.* [27] found a relation between cortisol and IgM as when cortisol increased IgM decrease. The significant increase in cortisol level in fish exposed to lead could be attributed to stress factors and the intoxication of fish [28].

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