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International Journal of Biotechnology and Bioengineering

ISSN 2475-3432

Research Article Open Access

Alterations in the Leucocytes and Serum Biochemistry in grey mullet (Mugil cephalus L,) fingerlings Exposed to Sub lethal doses of Lead for different exposure periods

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Citation: Hussien. M. EL-Shafei.(2017) Alterations in the Leucocytes and Serum Biochemistry in grey mullet (Mugil cephalus L,) fingerlings Exposed to Sub lethal doses of Lead for different exposure periods. Int J biotech & bioeng 3.6, 159-164.

Received April 20, 2017; Accepted May 03, 2017; Published July 05, 2017.

Abstract

Grey Mullet Mugil cephalus L, fingerlings were chronically exposed to sub lethal concentrations of lead(Pb) (0.1 and 0.4 mg/L for twenty eight. The changes in the leucocytes and serum glucose, protein, and total cholesterol of the fish were determined every seven days in a renewable static bioassay system. At the end of the study, these parameters were significantly ($p < 0.05$) elevated in the Pb - exposed groups when compared with the control group. Showing a pronounced leuocytosis in the Pb-exposed fish. The magnitude of increase was influenced by increasing of exposure period and Pb concentration. The Pb exposed fish were significantly ($p<0.05$) hyperglycemic and hyperbcholesteremic. The serum glucose levels on the 7th day were 26.50 ± 2.12 g/dl and 30.50 ± 0.70 g/dl in the fish exposed to 0.1 and 0.4 mg Pb /L respectively. On the 28th day , the serum glucose concentrations were 52.50 ± 2.12 and 70.00 ± 2.83 g Pb /dl in the groups exposed to 0.1 and 0.4 mg Pb/L , respectively. The cholesterol concentration increased from 113.5±3.53 mg Pb /dl on day 7 to 208.0 ± 1.80 mg/dl on day 28 in the group exposed to 0.1mg Pb /L. When the fish was exposed to 0.4mg Pb /L lead acetate, the cholesterol concentration increased from 131.5 ±3.54 in the first week to 288± 5.19mg Pb /dl on 28th day of the study. The serum protein concentration was also significantly ($p< 0.05$) increased in the Pb – exposed groups when compared with the control group, it increased from 4.04±0.06mg Pb /dl on day 7 to 5.30±0.05 mg/dl on day 28 in the fish exposed to 0.1mgPb /L. When the fish treated with 0.4mg Pb /L , the serum protein increased from 4.45±0.37 mg/dl on day 7 to 6.18±0.19 mg Pb /dl on day 28, respectively. These changes are indicative of stress imposed on the fish by lead and could be used as indices of lead poisoning.

Keywords: lead, hyperglycemia, cholesterol, protein, leukocyte

Introduction

Lead(Pb) is one of the most widely used heavy metals that has wide applications in such products as storage batteries (lead accumulator), electric cable sheaths, alloys, pesticides, paints, petrol and rubber products among other uses [1] . In water bodies, Pb forms complexes with sediments or organic materials [2] . and in the process enters the food chain. The problem posed by Pb in the aquatic system is complex due to its non-degradability and interactions with other materials to form complexes that may potentiate their toxic effects[3] Lead deposits in various fish organs: liver, kidneys and spleen, but also digestive tract and gills [4]. Accumulation of lead in different fish species has been determined in several works[5], leading to disorders in fish body. When C. batrachus exposed to 5 ppm of lead nitrate for 150 days, it exhibited marked inhibition of gonadal growth and showed decrease in cholesterol and lipid levels in brain, testis and ovary whereas the liver showed an elevation of both [6]. [7] observed a very high number of red let cells (RCs) in the epidermis of common carp and rainbow trout kept in lead polluted water. Hepatocyte vacuolization, hepatic cirrhosis, necrosis, shrinkage, parenchyma degeneration, nuclear psychosis and increase of sinusoidal spaces were the distinct changes observed in the liver of lead-exposed fish [8]. The characteristic symptoms of chronic lead toxicity include changes in the blood parameters with severe damage to erythrocytes and leucocytes and damage in the nervous system [9]. Low levels of Pb pollution could cause some adverse effects on fish health and reproduction [10]. Also, lead was found to impair the embryonic and larval development of fish species [11]. [12] monitored the effect of lead on the Chinese sturgeon, Acipenser sinensis. They

observed deformities as body (spinal) curvatures. The authors also reported reduced ability of locomotion and foraging by deformed juveniles. The purpose of the study was therefore to investigate the effect of sub lethal lead on the leucocyte and some biochemical parameters in grey mullet Mugil cephalus,L- with a view to using them as biomarkers of lead toxicity.

MATERIALS AND METHODS

Collection and handling of experimental of fish.

The fish fingerlings were bought from Fish Farms at Manzalla lake Damietta , and transported to a wet laboratory in two 50 L plastic fish transport containers during march 2015 . Once in the wet laboratory, fish was introduced into 200 L plastic container for acclimatization for two week . The water was continually aerated to ensure that the dissolved oxygen level remained above 6 mg/L and fish were fed on a 32% crude protein diet at 3% body weight daily at 10.0 h. The laboratory water was analyzed for different physico-chemical parameters (APHA, 2010) and lead. No fish mortality was recorded during acclimatization. The LC50 value for lead was statistically determined [13]

Experimental design and in vivo studies

One hundred and twenty fingerlings of Mugil Cephalus L, (mean length of 12.00±2.25 cm and average weight of 35±3.20g) were used for this study. They were divided into three groups of forty fingerlings per each. Each group was further divided into two replicates, twenty fish per replicate. Fish were exposed to 0.1 mg Pb /L and the second group was exposed to 0.4 mg Pb /L. The third group was exposed to tap water only and it served as control group. The water in the experiment was changed every day to maintain the same Pb concentrations. Three fish from each replicate experiment was killed every seven days for twenty eight days for the analysis.

Blood Collection

The blood of the fish was collected every seven days through both cardiac puncture [14] . The blood was collected into three different vials . One of the vials containing fluoride oxalate was use to collect blood used for glucose determination. The other two vials were without anticoagulant and were used to collect the blood for the biochemical test, and thin blood smear.

White Blood Cell Count (WBCC)

The leucocyte count was done using the Neubauer microscopic counter after diluting the blood with Turk's dilution fluid. The differential white blood cell count was done by preparing a thin blood smear and staining same with Geimsa Romanosky stain [15]. The stained blood was left for 25 minutes for the Geimsa stain to act on it. Thereafter the stained blood was flooded with distilled water and rocked gently to evenly mix the distilled water and stain. The stained blood was washed with water and allowed to dry. The slide was viewed with a binocular microscope to identify the leukocyte species which was calculated as a percentage.

Biochemical analyses

The blood serum was obtained by centrifuging the blood sample at 5000 rpm for five minutes and the protein in the samples was spectrophotometrically determined using Biuret method [16] at 540 nm. The blood glucose was determined by [17] method and the cholesterol concentration was determined by the method of [18].

Statistical Analysis

The data was statistically analyzed using two way analysis of variance (ANOVA) followed by LSD post hoc test at 95% confidence interval [19].

Result

Water quality parameters, cations , anions, and background metals in acclimation. were presented in table(1)

Leukocyte Response

The changes in the total leucocytes and differential white blood cell count Pb- exposed to Fish are presented in (Table 2) . The leucocytes count in the control group did not vary ($p > 0.05$) significantly during the study. The leucocytes counts increased significantly with increasing Pb concentrations and exposure period. Five different subspecies of leucocytes (lymphocytes, monocytes, neutrophils, eosinophils and basophils) were identified in the fish during the study. The lymphocyte and the monocytes constituted the a granulocytes identified while neutrophil, eosinophils and basophils were the granulocytes recorded due to the presence of granules in their cytoplasm. The lymphocytes were the most abundant leucocytes group identified in the blood of the fish exposed to lead acetate . Both small and large lymphocytes were found during the study and they accounted for more than 80 % of the white blood cells. The lymphocytes increased significantly ($p < 0.05$) in the lead-exposed fish when compared with the control group . The lymphocytes were significantly different ($p> 0.05$) in the treatment groups and the lymphocytosis was both concentration and duration dependent. The monocytes decreased in the lead-exposed fish on the 7th day and thereafter, it increased significantly in the treatment groups ($p <0.05$) when compared with the control group . The basophiles were the most abundant granular leucocytes in the peripheral blood of Mugil cephalus,L- exposed to lead acetate . The proportion was highest in the first week and on day 21 of the study. The neutrophils are the second largest granular white blood cell in the fish while the eosinophils are the least abundant subpopulation.

Effect on serum glucose

The glucose concentration in the control group did not vary throughout the study (Table 3). The serum glucose level on day 7 were 26.50 ± 2.12 g/dl and 30.50 ± 0.70 g/dl in the fish exposed to 0.1 and 0.4 mg Pb /L, respectively. On day 28, the serum glucose concentrations were 52.50 ± 2.12 and 70.00 ± 2.83 g/dl in the groups exposed to 0.1 and 0.4 mg Pb /l lead, respectively.

There was concentration and duration significant increase ($p < 0.05$) in the treatment groups when compared with the control and the values differed also in the treatment groups ($p < 0.05$) at each sampling period

Effect on serum protein

The results showed that the protein level in the control did not vary ($p > 0.05$) throughout the study while the serum protein in the Pb – exposed fish did not differ ($p > 0.05$) from the control value during the first 14 days (Table 3). Generally, there was progressive concentration and duration dependent increases in the serum protein in the Pb–exposed fish as it increased from 4.04±0.06mg/dl on day 7 to 5.30±0.05 mg/dl on day 28 in the fish exposed to 0.1mg Pb /L. When the fish treated with 0.4mg Pb /L, the serum protein increased from 4.45±0.37 mg/dl on day 7 to 6.18±0.19 mg/dl on day 28, respectively. Statistical analysis showed that serum protein levels in the treatment groups differed significantly ($p <0.05$) at the end of the study.

Effect on serum cholesterol

The serum cholesterol level in the control group did not vary ($p < 0.05$) throughout the study (Table 3). When compared with the control group , the cholesterol concentration was significantly higher ($P < 0.05$) in the Pb -exposed fish. Also, the cholesterol level differed significantly ($p < 0.05$) in the treatment groups throughout the study. The cholesterol concentration increased significantly from 113.5±3.53 mg/dl on day 7 to 208.0 ± 1.80 mg/dl on day 28 in the group exposed to 0.1mgPb/L. When the fish was exposed to 0.4mgPb/L, the cholesterol concentration increased significantly from 131.5 ±3.54 mg/dl in the first week to 288± 5.19 mg/dl on 28th day of the study.

Table (1): Water quality parameters, cations , anions, and background metals in acclimation.

Parameter	Acclimation water
Temperature (°C)	25±1°C
pH	8.1±0.2
Dissolved oxygen (mg/l)	8.2-8.9
Total Hardness (mg/l as CaCO3)	106.2
Total alkalinity(mg/l as CaCO3)	42.8
Total dissolved solids(mg/l)	173
Sodium (mg/l)	5.0
Calcium (mg/l)	31.0
Potasuim(mg/l)	0.6
Magnesium (mg/l)	6.1
Cl- (mg/l)	10.5
NH3(mg/l)	0.034
SO4 (mg/l)	13.0
PO4 (mg/l)	0.03
Copper(µg/l)	0.63
lead (µg/l)	0.015
Zinc (µg/l)	0..35

Table (2) : Effect of different lead concentration on the differential white blood cell count of grey mullet Mugil cephalus L.

Leucocyte species	Concentration Pb(mg/L)	Duration(days)			
		7	14	21	28
Lymphocyte	**Control**	81.00±1.41a1	80.50±3.54 a1 1	80.00±1.41a1	85.50±0.71 a1
	0.10	83.50±2.12b1	84.50±0.71 b2	85.50±2.12b3	3 87.50±3.54 b4
	0.40	83.50±2.12 b1	86.00±2.83 b2	87.00±1.41c3	87.00±1.41 b4
Monocyte (%)	**Control**	2.50±0.71a 1	2.30±1.41 a 1	2.40±0.71 a 1	2.50±0.71 a 1
	0.10	1.00±1.41b1 2	5.50±1.41 b 2	6.00±1.41 b 2	4.00±0.00 b 2
	0.40	0.50±0.71c 1 3	6.00±2.83 c 2	5.00±1.41 c 3	5.50±1.41 c 3

Neutrophil (%)	**Control**	1.50±0.71 a 1	1.50±0.71 a 1	1.50±0.71 a 1	1.30±0.71 a 1
	0.10	1.00±1.41b 1	2.00±1.41 b 2	1.50±0.71a 3	1.50±0.71a 3
	0.40	0.50±0.71c 1 3	1.50±0.71c 2	0.50±0.71b 1	2.00±1.41b 3
Eosinphil (%)	**Control**	0.50±0.71 a 1	0.50±0.71 a 1	0.60±0.71 a 1	0.50±0.71 a 1
	0.10	0.50±0.71 a 1	1.00±1.41b 2	0.50±0.71 a 1	-
	0.40	-	0.50±0.71 a 1	0.50±0.71 a 1	0.50±0.71 a 1
Basophil (%)	**Control**	10.50±0.71 a 1	10.50±2.12 a 1	9.80±2.12 a 1	10.50±2.12 a 1
	0.10	15.50±0.71 a 1	5.50±0.71b 2	8.50±0.71b 2	7.00±2.83b 3
	0.40	15.50±0.71 a 1	6.00±2.83c 2 3	7.00±4.24c 3	7.00±1.41b 3
Leuco-cyte(x104/mm	**Control**	2.47±0.08a 1	2.50±0.02a 1	2.49±0.16 a 1	2.47±0.04a 1
	0.10	2.54±0.04b1	3.38±0.60b 2	4.28±0.11b3	4.84±0.01b4
	0.40	2.94±0.10 b1	3.96±0.06c2	4.55±0.19c3	5.55±0.04 c4

Table (3) : change in some Biochemical parameters in grey mulle Mugil cephalus L exposed to different Pb doses for different exposure periods.days.

Parameters	**Concentration(mg/L)**	**Duration(days)**			
		7	**14**	**21**	**28**
Glucose(mg/dl)	**Control**	17.25± 2.08	17.6±18	17.4±18	
	0.10	26.50 ± 2.12	32.45± 3.12	41.52± 08.12	52.50 ± 2.12
	0.40	30.50 ± 0.70	44. 60± 4.12	56.40± 3.12	70.00 ± 2.83
Cholesterol(m/dl)	**Control**	54.06±4.50	55.06±6.40	56.06±3.52	57.05±5.40
	0.10	122.5±3.53	156.5±4.63	187.4±2.50	214.0 ± 1.80
	0.40	152.5 ±3.54	197.5±2.51	243.5±3.53	308± 5.19
Total Protein(g/dl)	**Control**	3.9±05	4.1±03	4.1±0.03	4.1±01
	0.10	4.06±0.06	17.6±18	4.72±0.06	5.28±0.05
	0.40	4.54±0.37	32.45± 3.12	5.14±0.06	38±0.19

Value in the same column with the same superscript (lower case) are not significantly different (p = 0.05)between different concentrations within the same exposure duration. Values with different numeric superscripts differ significantly (P=0.05) between different exposure periods within the same concentration.

The results of the study show that lead has significant effects on the leucocyte count of Mugil cephalus L- and the small lymphocytes decreased in number while the neutrophils increased. On the contrary, [20] found decreased leucocyte counts in Clarias gariepinus exposed to 0.45mg Pb /L for 4 to 5 days. The general leucocytosis reported in this study is consistent with the observation of [21] in the dogfish exposed to 50µg/L Cd for 4 days.

Changes of blood glucose are a good indicator of metal stress in fish [22] and alterations in the glucose level might be related to renal injury, liver damage, and lack of nutrition [23]. This study showed a dose- dependent increase of glucose level after first 15-days. The observed increase in the serum glucose level in Mugil Cephalus L, exposed to lead in this study is in accord with report of some earlier workers. Similar increase in plasma glucose was also reported in Prochidolus lineatus exposed to lead [24] and in Oreochromis niloticus exposed to copper [25]. It has been widely reported that hyperglycaemia in fish arises due to the stimulation of catecholamines and corticosteroids [26] .

The increased serum glucose level in this study is an evidence of stress due to lead exposure as [27] argued that coping with such stress is an energy demanding process that requires the fish to mobilize metabolically energy substrates through intense gluconeogenesis. Glucose being one of such known substrates is mobilized through gluconeogenesis to meet this challenge [28] . Heavy metals have been reported to act antagonistically with glucocorticoids by inhibiting the receptors thereby disrupting the osmotic and mineral regulatory mechanisms [29]. Serum increase in glucose due to toxicants has have been associated with hypothalamus-sympathetic-chromaffin cells [30] instead of the hypothalamus-pituitary-internal axis [31] that is known to have

tremendous influence on carbohydrate metabolism.

The results of this study showed that the serum total protein increased significantly in Mugil cephalus, L ,exposed to lead. Similar increases in the serum protein level were reported in Oreochromis niloticus exposed to metals [27]. Also increased plasma protein was reported in Mugil exposed to 0.5ppm copper and cadmium [32] . On the contrary, decreased tissue protein was reported in Oreochromis niloticus treated with cadmium [33] and in Cyprinus carpio when exposed to heavy metals [34] .

The enhanced serum cholesterol in this study is an indication of hypercholesteremia in the fish due to the stimulatory effect on the cholesterol biosynthetic pathway. Reduced serum cholesterol level has been reported in Oreochromis niloticus exposed to some heavy metals [35], [27] and in Lepomis macrochirus exposed to methyl mercuric chloride [36].Cholesterol concentration in the serum of cadmium exposed fish also showed a different pattern. Another study showed a reduction in cholesterol within 15-days, possibly due to tissue damage in the kidney[37]. On the contrary, in Oreochromis niloticus, an increase in cholesterol was seen during a 21 day period due to cadmium [38].

This alteration in cholesterol concentration could be due to hazardous effects of metals on cell membrane. The observed elevated cholesterol in this study could have resulted in part to the adverse effect of lead on the liver leading to altered cholesterol metabolism resulting in increased serum cholesterol. This according to [38] could be due to liver and kidney failure that resulted in the release of cholesterol into the blood stream. Thus, increase in cholesterol levels are good indicators of environmental stress in fishes.

Conclusion

Generally, the leucocytosis observed in this study gives indication that exposing the fish to lead predisposes it to secondary infections. Also, the reported hyperglycemia, increased serum protein and cholesterol levels are indications of altered carbohydrate, lipid and protein metabolism in the fish due to lead exposure. Serum parameters which provide information as to state of the internal environment of the fish are known to respond quickly to changes in the water quality. The changes in these biomarkers are a reflection of organ dysfunction in the fish due to metal exposure and these biomarkers could be used in ecotoxicological assessment and as early warning indicators of pollution.

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