Research Article

Effects of Sub-lethal Exposure to Lead Acetate on Haematological Indices and Growth Rate of Bunni *Mesopotamichthys sharpeyi*

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Abstract | The objective of the current study is to determine the effects of lead acetate (Pb $(CH_3COO_3)_2$) on haematological parameters, behavioural changes and body weight in the cyprinid fish Bunni *Mesopotamichthys sharpeyi.* For this purpose, a total of 120 fish were distributed randomly among four treatments in addition to a control group. Treatments included exposing to lead acetate at 0.2mg l^{-1} and 0.4 mg l^{-1} with replacement of aquarium water every two days and adding lead acetate continuously or for the first time only. After 15, 30 and 60 days blood parameters were measured. Body weight was measured at beginning and end of experimental period. Behavioural responses were also recorded during the course of experiment. Results revealed significant decrease in red and white blood cells count, hemoglobin concentration, packed cell volume compared to control group. Results showed a presence of improvement of clinical and blood picture in treatments with changing water aquarium continuously. Abnormal behaviour was observed in all treatments but the severity of signs increased with exposing to high concentration of lead acetate. Growth rate showed significant decrease in all treatments in comparison with control group. In conclusion, exposure to lead acetate could cause several changes in blood profiles, but changing of water aquarium without adding lead acetate led to improvement of health status of fish.

Keywords | Lead acetate, Haematological indices , *Mesopotamichthys sharpeyi, barbus*

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INTRODUCTION

The contamination of freshwater with a wide range of pollutants has become a matter of concern over the last few decades (Al-awady, 2011). Increased human activities especially with rapid development of agriculture and industry have resulted in a significant increase in levels of pollutant such as heavy metals (Abdel-Baki et al., 2011). Heavy metal pollution is one of the most important environmental problems today. Fish are exposed to unnaturally high levels of these metals including lead. Lead (Pb) Lead and its products are harmful pollutants in the environment as well as being produced by manufacturing and mining actions (Moulis, 2010). Furthermore, several researchers have reported that toxic and non-biodegradable heavy metals such as lead accumulate in many fish species, causing toxicological effects (Gordon et al., 2002; Khoshnood et al., 2011). Pb has been recognized as strong biological poisons because of their persistent nature, toxicity, tendency to accumulate in organisms and undergo food chain amplification (Olowu et al., 2010; Al-awady, 2011). In addition, Lead has an extremely high affinity for erythrocytes (Salman, 2014) and is a known inhibitor of dehydrogenase of delta amino levulinic acid (ALA-D), an enzyme participating in heme synthesis which may cause deformities of fish erythrocyte, membrane disruption and often induces anemia in fish (Olanike et al., 2008; Horiguchi et al., 2011). It has been noted that heavy metals had a negative impact on all haematological parameters in fish. Haematological parameters have been used as tool in order to determine the specific and non-specific effects of environmental and physical stress. Fish blood indices have been increasingly examined as valuable parameters for the presence of toxicants. Changes of the haematological profiles could be used as an important tool for the evaluation of pathological conditions of fish (Al-Rudainy et al., 2015).

However, there is a lack of information about the blood

response to stress in commercial fish species in Iraq such as Bunni *Mesopotamichthys sharpeyi* (Al-Rudainy et al., 2008). Hence, the present study aimed at studying the effect of lead acetate on haematological parameters including red blood cell (RBC), white blood Cell (WBC), packed cell volume (PCV%) and hemoglobin (Hb), also measuring the effect of exposure on clinical signs (behaviour of fish) and growth rate.

MATERIALS AND METHODS

Experimental Design

A total of 120 fingerlings of Bunni fish *Mesopotamichthys sharpeyi* weighting 10-15 g were purchased from the Al-Sewera hatchery, Wasit, Iraq. Fish were acclimated to laboratory condition for 15 days before initiation of the experiment. Fish were briefly bathed in NaCl (10%) for 5min to remove external parasites if present. After two weeks of acclimation, 100 fish were transferred to small aquaria tanks at the rate of 10 fish.tank^{-1} and randomly distributed to five treatments (the experiment was run in duplicates). First treatment (T1) exposed to lead acetate 0.4 mg l^{-1} with replacement of water aquarium entirely every two days and added lead acetate continuously, the second treatment (T2) exposed to lead acetate 0.4 mg l^{-1} in first time only with replacement water aquarium entirely every two days during experimental period, third treatment (T3) exposed to lead acetate 0.2mg l^{-1} with replacement water aquarium entirely per two days and adding lead acetate continuously, fourth treatment (T4) exposed to lead acetate 0.2 mg l^{-1} in first time only with replacement water aquarium entirely every two days during experimental period. Control set was also run for comparison. During the 60-days experimental period, fish were fed a commercial diet at a rate of 3% body weight mass daily.Water quality was recorded every three days of the experimental period (temperature 23±0.5, pH 7.3±0.3, DO 6.2±0.5). The observation of toxic symptoms such as stress, movement, respiration, swimming and responses to external stimuli were periodically recorded. Body weight was measured at the beginning and end of the experimental period.

Haematological Parameters

After 15, 30 and 60 days of exposure to lead acetate, blood samples were collected via cardiac puncture technique using a sterile disposable syringe and blood was transferred into a tube containing EDTA solution for haematological tests. WBC and RBC count were performed by diluting blood with Decies fluid and cells were counted using a haemocytometer Neubauer chamber under light microscope (Dacie and Lewis, 1984). PCV% were immediately determined after sampling by placing fresh blood in capillary tubes and centrifuged for 5 min at 10,000 rpm in a microhematocrit centrifuge then measuring PCV% according to the method mentioned by Archer (1985). Hemoglobin concentrations (Hb g dl^{-1}) were colorimetrically determined by measuring cynomethemoglobin according to Coles (1986).

Statistical Analysis

Statistical analysis was performed using Statgraphicsvs 5.1 software (StatSoft, USA). All data were expressed as M± S.D. Statistical analysis of data was performed on the basis of one- way analysis of variance (ANOVA) for experiment group differences were determined using least significant difference (LSD). The probability ≤0.05 was considered to be significant.

RESULTS AND DISCUSSION

Haematological Indices

Haematological parameters can be considered as biomarker of toxicity in fish studies (Al-Rudainy et al., 2015). There are few studies related blood responses in fish after chronic or sub chronic exposure to heavy metals. Salman (2014) reported that haematological parameters allow rapid detection of changes in fish. In the present study haematological indices varied significantly after exposure of Bunni to different levels of lead acetate for 60 days.

Results of haematological parameters are presented in Table 1. After 15 days exposure to lead acetate WBC count showed a significance increase (p≤0.05) in all treatments than control group and there were significant differences between treatments. T1 and T2 recorded the highest values followed by T3 and T4 respectively. A significant increase (P≤0.05) in WBC count was noticed in T1 and T3 compared to other treatments for various periods. After 30 and 60 days exposure to lead WBC count showed a significant increase (p≤0.05) in all treatments than control group. Such increase was significantly superior to that seen after 15 days exposure. The highest value of WBC count was recorded in T1 followed by T2, T3 and T4. respectively.

The significant increase in WBCs count could be due to an increase in antibody production which helps in survival and recovery of the fish exposed to heavy metals (Joshi and Deep, 2002). In the present study, the significant increase in leukocytes count indicates hypersensitivity of leucocytes to lead acetate and these changes may be due to immunological reactions to produce antibodies to cope up with stress induced by lead acetate. Fink and Salibian (2005) reported that leukocytes increase could be due to an induced proliferation as a result of the chemical toxicity, of pluripotential hematopoietic cells that, in turn, may be a consequence of a depletion circulating differentiated. These results are in line with Mustafa (2012) who reported an increase in leucocytes count when they exposed fishes to heavy metal, also agree with Salman (2014) who study haematological and histopathological effects of cadmium

chloride on *M.sharpeyi*.

Table 1: Results of hematological tests (WBCs count, RBCs count, Hb concentration and PCV%) of M. sharpeyi which exposed to different concentrations of Lead acetate during experiment period

	Blood Parameters			
Treatments	WBC $\times 10^3/mm^3$	RBC $\times 10^3/mm^3$	PCV%	Hb g/dl
Control	21.86±0.01 I	2.32±0.01 A	38.00±1.16 A	15.10±0.60 A
15 days				
T1	23.92±0.02 D a	2.16±0.01 A b	32.70±1.80 D c	11.90±1.30 B a
T2	23.61±0.01 E b	2.22±0.01 A a	34.00±1.73 C b	12.70±0.60 AB a
T3	22.25±0.03 H c	2.20±0.01 A a	35.30±1.80 C a	13.70±1.10 AB a
T4	22.21±0.01 H c	2.24±0.02 A 0a	35.30±0.70 C a	13.6±0.60 AB a
30 days				
T1	24.50±0.06 C a	1.92±0.03 B b	22.70±1.76 G b	4.90±1.17 C c
T2	23.23±0.03 F c	2.23±0.02 A a	32.70±0.7 D a	11.80±0.43 B a
T3	23.54±0.02 E b	2.15±0.02 A a	28.00±1.16 E b	8.68±2.30 C b
T4	22.55±0.02 H d	2.25±0.58 A a	33.3±0.70 D a	12.15±0.30 AB a
60 days				
T1	27.3±0.05 A a	1.51±0.04 B c	18±2.3 H c	4.53±1.39 D c
T2	22.75±0.04 G c	2.25±0.02 A a	36.60±1.33 B a	14.62±0.90 AB a
T3	25.61±0.02 B b	1.89±0.02 B b	24.70±1.76 F b	6.08±1.11 C b
T4	22.50±0.11 H d	2.28±0.01 A a	36.70±0.7 B a	14.48±2.31 AB a

Different vertically capital letters represent significant variation at p≤0.05 in different periods; Different vertically small letters represent significant variation at p≤0.05in same period.

Results of RBCs count showed a significant decrease (p≤0.05) in T1, compared with T2, T3, T4 and control group after 15 days, but no significant variations was noticed between T2,T3 and T4 during the same period. Same trend was also seen after 30 days, with little variation found after 60 days. After 30 and 60 days, RBC count revealed no significant differences in all treatments (p>0.05) compared to control group. There was significant difference (p≤0.05) in RBC count only between T1 and other treatments. Decreased in RBC count are in agreement with Mishra and Srivastava (1980) who had reported a decrease in RBC in *Colisa fasciatus* exposed to zink. Also, Allen (1994) registered reduction in RBC count in *Oreochromis aureus* after exposure to 10 ppm Pb. The reduction in RBC count could be due to impaired water balance as a result exposure to Pb. Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to gill damage and the nature of the changes show a release of erythrocytes from the blood depots (Salman, 2014).

It has been seen that Hb and PCV% decreased in all treatments throughout the duration of the study in comparison with control. They have recorded a significant decrease (p≤0.05) than control after 15 days exposure. Differences between all test exposures, however, were not significant. After 30 and 60 days exposure to lead, PCV% and Hb concentration showed significant decrease (p≤0.05) than control. The lowest value for Hb and PCV % was recorded in T1 which was significantly different compared to other treatments. The results of statistical analysis in different periods revealed significant variations (p≤0.05) in T1 and T3 compared with the other treatments, but T4 showed no significant variations (p>0.05) in all periods. Similar significant decrease was reported in Hb level by Christensen et al. (1977) in Brook trout *Savelinus fontinalis* exposed to lead for 60 days. In contrary, Larsson et al. (1985) reported no reduction in Hb level in the white fish *Coreganus* sp. exposed to Pb. According to Mustafa (2012) the reduction in Hb content in fish exposed to toxicant could also be due to the inhibitory effect of toxic substance on the enzyme system responsible for synthesis of Hb.

In general, metals can affect haematological parameters of fish via osmotic changes which impose hemodilution (an increase in the volume of plasma, resulting in a reduced concentration of red blood cells in blood) or hemoconcentrations (an increase in the concentration of blood cells resulting from the loss of plasma or water from the bloodstream) (Sanchez-dardon et al., 1999). In general, spleen is responsible for this change. Because, it, serving as a potent blood storage organ in some teleost, sequestering blood cell under resting conditions and releasing them to circulating blood associated with various stress (Murugan, 2008). The reduction in erythrocyte count, PCV and haemoglobin could be attributed to haemodilution of blood due to the damage of some organs (Salman, 2014). Changes in haematological parameters such as PCV, RBCs and Hb, can be interpreted as a compensatory response that improves the oxygen carrying capacity to maintain gas transfer, also indicates a change in the water blood barrier for gas exchange in gill lamellae (Jee et al., 2005).

Fish Behaviour

Fish showed abnormal behaviour approximately 1h after exposure to various concentrations of lead acetate, Signs of abnormality exhibited in the increase of swimming activity, hypersensitivity, loss of equilibrium, hyperactivity,

hyper-excitability, increased operculum movement, erratic swimming, frequent jumping, swimming at the water surface and spiralling, convulsion. Attempts to escape were seen, accompanied with trials of hitting the walls of the aquarium before finally sinking to the bottom. Behavioural abnormalities were observed in all treatments, but severity of signs increased with high concentrations of lead acetate. Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may occur due to accumulation of acetylcholine in synaptic and neuromuscular junctions (Rao et al., 2005). Loss of equilibrium follows erratic and darting swimming movements, might berelated to the inhibition of brain cytochrome C oxidase activity, causing cytotoxic hypoxia, thus causing brain damage to the region associated with the maintenance of equilibrium (Salman, 2014).

Growth Rate

The results of growth rate of Bunni fish are presented in Table 2. Body weight data after 60 days showed a significant decrease (P≤0.05) in all treatments except T4, compared with control group. The highest reduction for body weight was recorded in T1, T2 and T3 respectively, Fish in the control group and T4, however, showed a significant increase (P≤0.05) in final body weight compared to other treatments .T1 showed significant decrease (p≤0.05) in body weight after 60 days compared with T2, T3 and T4. There was no significant difference between T2 and T3. Fish growth is an indicator of populations' life conditions that could be used to detect stress due to contamination (Al-Rudainy and Kadhim, 2012).

Table 2: Average body weight values ± S.D. of *M. sharpeyi* exposed to different concentrations of lead acetate for 60 days

	Body weight(g)	
Treatments	Average initial weight (g)	Average final weight (g)
Control	12.06±0.55 **A**	15.75±0.03 **a**
T1	13.30±0.60 **A**	8.16±0.44 **d**
T2	12.00±0.70 **A**	10.32±0.57 **c**
T3	13.22±0.20 **A**	10.10±0.04 **c**
T4	13.00±0.56 **A**	12.01±0.54 **b**

*Different vertically alphabetic letters represent significant variations at (p≤0.05) between treatments.

Decrease in body weight might be due to decrease their food uptake under toxic environmental conditions to lower the energetic costs of digestion. Data showed fish loss appetite of food increased with a high concentration of lead acetate. Depression in appetite is a common response of fish to stress and intermittence of feeding for longer periods; it can have a clear impact on growth and reproduction (Astm, 2008). A substantial growth reduction caused by toxicant stress has important implications for survival in the natural situations (Dembele et al., 2000).

CONCLUSION

In conclusion, the results of this study showed that sub lethal levels of lead acetate affect blood parameters, growth and behaviour of *B. sharpyei*. Overall, changes in the haematological profiles indicate an attempt by the fish to adapt in an environment with increased requirement for oxygen leading to further knock-on effects on other body systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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