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(Research Article)

To study the haematological parameters in culturable carps after exposure to lead acetate and nickel chloride

Shadab Ahmad Khan *

Department of Biology, K.G.N. (P.G. College) Sikandra Rao, Hathras, UP, *India.*

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Abstract

Major environmental problems nowadays refer to heavy metals which are known to deteriorate the natural phenomenon of all the living beings of the ecosystem. The present study was carried out in two freshwater fishes, *Catla catla* and *Labeo rohita* exposed to Lead acetate and Nickel chloride to determine the lethal concentration (Lc50) for 96 hour. The 96h LC50 for both the species was computed as 14.35 mg/L (*L. rohita*) and 16.75 mg/L (*C. catla*). The same fishes were treated with sub-lethal concentrations for determining the hematological parameters. All the variations in parameters were dose dependent and were in parenthesis. The experiment was carried out for four weeks and observed that erythrocytes count, hemoglobin and the hematocrit value increased gradually with exposure time while the leukocytes count decreased. Serum protein and serum glucose were found to be affected significantly. Lead acetate seems to be more effective than the nickel chloride.

Keywords: *C. catla*; *L. rohita*; Heavy metals; Lead acetate; Nickel chloride; Hypoxic condition

1. Introduction

According to the U.S. Geological Survey major rivers' water is polluted and predominantly contained with pesticides (Kole *et al*., 2001). Water contamination is mostly the result of agricultural and urban runoff, where pesticides find their way through leaching in soil or by direct discharge of contaminated wastewater (Ikehata and El-Din 2005). The resultant water pollution poses demonstrated risks to aquatic ecosystems (UNEP 2016). The suitability of water for a particular use depends on the type and amount of some specific impurities which might be in the form of heavy metals discharge from the industries present around that will in some way affect the whole community of the aquatic fauna and flora. Waste management hierarchy has made for recycling the waste material for improving the quality of water. This water quality standard has been proposed by a number of agencies; including Environmental Protection Agency (EPA) of USA and World Health Organization (WHO) which are beneficiated to fish culture (Singh and Kumar 2009). Lead as an immunotoxicant has an adverse effect on human and animal health as neurological dysfunction (Nordberg *et al.,* 2007; Al *et al.,* 2011). Mobarak (2008) pointed out that level of lead produce a neuro-behavioural deficit reduced the fertility and delay in sexual maturity. Recently, notable report have indicated that lead can cause neurological gastrointestinal, reproductive, circulatory, immunological, histopathological and other physiological disability in animals (Berrahal *et al.,* 2007; Abdallah *et al.,* 2010; Mobarak and Sharaf 2011; Al-Kahem *et al.,* 2011). Blood parameters are often measured by the clinical diagnosis of fish physiology is applied to determine the sub- chronic effects of pollutants because of their contamination in the environment.

According to Al-Akel *et al.,* (2010) shown that the toxicants in the aquatic environment, change the water the health and other biological activities of the human beings and the aquatic fauna as well as flora. Fish live in a very intimate contact with aquatic fauna, and are therefore very susceptible to physical and chemical changes which may alter the blood quality and their components as well (Wilson and Taylor 1993; Vosyliene 1999). The present study therefore, assessed

* Corresponding author: Shadab Ahmad Khan

the hematological parameters of the culturable fish *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton) exposed to Lead and Nickel as a comparative study.

2. Material and methods

Fresh and healthy collected fish species of *L. rohita* (weight, 67.5±1.00 g and mean length, 11.0±1.5 cm) and *C. catla* (mean weight, 82.5±1.00 g and mean length, 13.7±1.2 cm) were collected and acclimatized in the Laboratory condition in two different big aquarium with oxygenation under a photoperiod of 12hL: 12hD for two weeks before starting the experiment. The water condition like, Temperature, pH, Dissolved Oxygen and hardness analysed weekly which were 21.5±1.0 °C; 7.8±0.45; 6.8 mg/L and 210.5±6.5 mg/L as Calcium Carbonate ($CaCO_2$) respectively. On completion of two weeks as acclimatization period, the selected 10 fishes in different aquarium with a capacity of water 30L/aquaria were kept. A stock solution of the two chemical were prepared and diluted to the test water to get the required concentrations. These fishes were exposed to 96 hrs. The dead fishes were immediately removed and their number was counted. The value of L_C50 for 96 hrs for both the fishes was calculated by the method of Finney, 1971. Earlier the same method was applied by several other workers (Al-Akel and Shamsi 2000; Ahmad 2012; Shamsi 2014). A control set was also run parallel with the same number of fishes and with the same quality of water but without using the toxicants. The experiment was run in triplicate. The water was aerated and the feeding was stopped.

After finding the L_C50 for both the fish species with Lead and Nickel, the fishes were exposed to four different sub-lethal concentrations like, 2.0, 4.0, 6.0, 8.0mg/L with *L. rohita* and 3.0,6.0,9.0 and 12.0 mg/L with *C. catla*. For analyzing the hematological parameters another two sets of experiment with 200 fishes were distributed into 12 groups. 15 fishes for each group were treated to different concentrations of lead acetate and nickel chloride separately with a control set parallel with for comparison. The fishes were feed once daily to satiety. Two fishes from each aquarium were removed after every week, blood samples were collected in heparinized vials by cutting the caudal peduncle. Blood of these two fishes were pooled to get enough quantity of blood. Samples of clotted blood were discarded.

Hemoglobin was estimated by Cyanomethaemoglobin method using diagnostic hemoglobin kit (Kit no. 527-A-Merck). Hematocrit values were determined by micro-hematocrit centrifuge in glass capillaries, using the micro-hematocrit reader (Hawksley and Sons, England). RBC and WBC were counted by using Neubar-hematocytometer after diluting the blood with Dace's and Turk's solution respectively. The remaining blood sample was centrifuged (5000 rpm/10 minutes at 4˚C and the collected plasma was stored at -20˚C till analysed. Glucose, Total Protein were analysed using their respective kits (Biomerieuk, France).

For statistical analysis, the one-way analysis of varience (ANOVA) was applied to test the significance of difference among the different values. If the P value is less than 0.05, it was considered statistically significant.

3. Results and discussion

In a previous report regarding the toxicity (LC50 at 96h) of the foresaid chemicals were computed as, LC50 at 96h in L. rohita with lead was 11.65 mg/L and with Nickel it was 19.95 mg/L while in *C. catla*, with lead was 13.45 mg/L and with Nickel it was 21.70 mg/L, respectively (Shamsi 2014). The difference in toxic potential of these two metals to different species can be related to the differences in susceptibility and the tolerance related to its accumulation, bio transformation and excretion (Johnson and Toledo 1993; Ahmad 2012) Generally, the toxicity varied with respect to species, size of the fish and the duration of exposure (Oh et al., 1991; Dutta et al., 1995; Ahmad 2012). Changes in hematological parameters and serum chemistry (Serum Protein and Serum Glucose) in the control and the exposed group of both the species are characterized in (Table 1 and 2), respectively which are gradually decreased and increase significantly. Generally, toxicants exposure exerts an adverse effect on the hematopoietic organs which alters the blood parameters and are suitable tools for evaluating the effects of chemicals (Cyriac et al. 1989; Roche and Boge 1996). These findings are concordant with the present investigation.

The two species *L. rohita* and *C. catla*, exposed to different concentrations of lead acetate and Nickel chloride indicated an increase in RBC count, Hemoglobin concentration and Hematocrit values compared to the control one (Table I and II). It is documented that under stress condition, the fish became hyper active perhaps to get out of the stressful medium and would require an increased amount of oxygen to meet their energy demand and on the other hand, it secreted an increased amount of mucus and coated the gills to get relief from the irritating effect of the toxicants. In this condition, the fish seems to secrete an increased amount of mucus to coat the gill aperture, this reduces the gaseous exchange through the gills which may cause a hypoxic condition (Al-Kahem *et al.,* 1998; Ahmad 2012). According to the previous information with different chemicals and in several other fish species in hypoxic condition, there is a stress mediated synthesis of more hemoglobin and release a new erythrocytes from erythropoietic organs to improve the oxygen carrying capacity of blood (Mustafa and Murad 1984; Al- Kahem 1994; Al-Akel *et al.,* 2010). Ahmad (2012) have attributed the increased hemoglobin in Cadmium exposed fish, reduced hyperactivity and impaired gills function. In present investigation the total Leukocytes count was decreased, which might be due to multifunctioning of hematopoietic systems caused by the exposure of such chemicals. Changes in the Leukocytes system manifest in the form of leukocytosis with heterohilia and lymphopenia which are characterstic of leukocytic response in animals exhibiting stress. Ahmad (2012) reported reduction in the WBC count of the fish exposed to Chromium and Diazinon. Jaffer Ali and Rani (2009) also reported decreased leukocytes count in carp exposed to Diazinonbased pesticide and tilapia exposed to Phosalone, respectively. Their findings are more or less coinciding with the present work.

Table 1 Variations in Haemological profile of *L. rohita* after exposure to 96 h with Lead and Nickel

Parameter (in Parenthesis)	Lead (mg/L)					Nickel(mg/L)				
	Control	2.00	4.00	6.00	8.00	Control	2.00	4.00	6.00	8.00
Irythrocytes (Cells x 10^6 /mm^3)	1.680	1.720	1.778	1.815	1.954	1.740	1.790	1.910	1.990	2.025
	±0.003	±0.004	±0.011	±0.177	±0.175	±0.004	±0.005	±0.011	±0.011	±0.001
Leucocytes	39.516	38.660	36.900	34.250	32.500	38.510	36.875	34.500	32.680	30.78750
(Cells x 10^3 / mm^3)	±0.006	±0.005	±0.006	±0.004	±0.059	±0.005	±0.008	±0.009	±0.015	±0.019
Haemoglobin (g/100ml)	7.82	7.95	8.20	8.90	9.0	8.25	8.20	8.95	9.25	9.60
	±0.25	±0.20	±0.05	±0.015	±0.22	±0.26	±0.25	±0.15	±0.45	±0.01
Haematocrit (%)	30.90	31.20	33.50	39.00	36.15	32.50	34.60	38.50	39.00	39.50
	±0.19	±0.09	±0.15	±0.25	±0.20	±0.19	±0.42	±0.25	±0.10	±0.10
Serum Protein	46.50	42.80	41.00	39.45	36.50	42.50	40.10	39.00	38.25	35.40
(mg/100ml)	±2.00	±0.39	±0.40	±0.45	±0.50	±0.25	±0.24	±0.39	±0.40	±0.45
Serum Glucose	65.20	68.10	72.65	78.40	80.35	70.15	74.20	78.40	82.50	85.50
(mg/100ml)	±0.41	±0.35	±0.45	±0.58	±0.60	±0.35	±0.40	±0.60	±0.10	±0.45
Triglyceride	1.71	1.50	1.38	1.28	1.05	1.65	1.45	1.25	0.95	0.65
(mg/100ml)	±0.009	±0.015	±0.011	±0.018	±0.050	±0.01	±0.04	±0.01	±0.25	±0.32

Table 2 Variations in Haemological profile of *C. catla* after exposure to 96 h with Lead and Nickel

Parameter (in Parenthesis)	Lead (mg/L)					Nickel(mg/L)				
	Control	2.00	4.00	6.00	8.00	Control	2.00	4.00	6.00	8.00
Irythrocytes (Cells x 10^6 /mm^3)	1.450	1.675	1.80	1.99	2.02	1.65	1.75	1.85	1.90	1.95
	±0.002	±0.004	±0.007	±0.010	±0.025	±0.005	±0.002	±0.002	±0.012	±0.022
Leucocytes	36.66	35.50	32.40	30.50	29.20	38.50	37.40	36.60	33.33	30.90
(Cells x 10^3 / mm^3)	±0.005	±0.002	±0.004	±0.001	±0.019	±0.008	±0.010	±0.025	±0.010	±0.002
Haemoglobin (g/100ml)	7.75	7.80	8.50	9.10	9.25	8.80	8.90	9.10	9.25	9.90
	±0.18	±0.20	±0.21	±0.19	±0.19	±0.22	±0.25	±0.35	±0.35	±0.40
Haematocrit (%)	32.50	34.0	35.50	36.00	37.80	33.80	32.50	32.50	30.15	28.50
	±0.17	±0.19	±0.20	±0.25	±0.30	±0.91	±0.22	±0.25	±0.20	±0.29
Serum Protein	48.50	47.00	46.50	44.50	42.35	46.50	46.00	41.50	41.50	40.00
(mg/100ml)	±0.24	±0.25	±0.30	±0.40	±0.45	±0.32	±0.33	±0.38	±0.39	±0.40
Serum Glucose	72.50	74.00	76.00	79.00	80.50	79.00	80.00	84.50	86.35	94.25
(mg/100ml)	±0.35	±0.30	±0.49	±0.45	±0.42	±0.32	±0.39	±0.40	±0.46	±0.50
Triglyceride	1.68	1.60	1.58	1.50	1.40	1.70	1.65	1.95	1.95	2.0
(mg/100ml)	±0.010	±0.010	±0.001	±0.015	±0.019	±0.110	±0.112	±0.112	±0.145	±0.245

4. Conclusion

Heavy metals are well known to induce deleterious effects on all kind of life under every ecosystem. Freshwater fishes, *C. catla* and *L. rohita* were exposed to Lead acetate and Nickel chloride and Lc50 was determined as 14.35 mg/L and 16.75 mg/L, respectively. At sub-lethal doses hematological parameters were determined which indicate an increase in RBC count, Hemoglobin concentration and Hematocrit values compared to the control. Under stress conditions fish became hyper active with an increased amount of oxygen to meet their energy demand. Fish seems to secrete an increased amount of mucus to coat the gill aperture which reduce the gaseous exchange and may cause a hypoxic condition for tested fishes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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