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Effect of Hexavalent Chromium on the WBCs of the Fresh Water Fish, *Labeo rohita*

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Abstract

Industrial effluents containing heavy metals may reach aquatic systems either through direct discharge or surface runoff and cause damage to aquatic organisms affecting their immune system and health. Hence the present study has been undertaken to observe the effects of hexavalent chromium on the WBCs of the fresh water fish, *Labeo rohita*. WBCs play a major role in the immune response of the fish. For acute toxicity determination, healthy fish were subjected to static bioassays. The 24, 48, 72, and 96hr LC50 values were 50.88, 42.03, 28.09 and 10.87 ppm respectively. The fish were exposed to 0.5, 1, 1.5 and 2 ppm for 20 days. Differential count of WBCs and total WBC count were determined after every five days for twenty days. Lymphocytes exhibited a decline while the other cells and total WBC count exhibited an increase due to hexavalent chromium exposure. The results were subjected to two way analysis of variance.

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Introduction

Fishes are the most important biomonitoring agents for the evaluation of toxic components accumulated in polluted aquatic ecosystems. They help to understand the nature as well as the changes of aquatic ecosystems in an effective manner. They are highly sensitive to environmental changes in particular to aquatic pollution when compared to other aquatic organisms. The impact of heavy metals is reflected heavily on the physiology and immunology of fish. The accumulation of heavy metals inside the fish body leads to chronic or acute diseases in human beings while consuming them [1,2,3,4,5]. Hence, comprehensive knowledge towards the physiology and immunology of fish are very much handy with reference to the impacts of heavy metals and aquatic pollution.

Haematological parameters are the vital components that can be used as effective tool to analyse the physiological and pathological conditions of fish. Variations in haematological parameters of fish are mainly because of aquatic conditions and species, age, sexual maturity and health conditions of fish [6]. Several studies have been carried out with relevance to fish physiology and pathology by various researchers. In particular, studies relevant to haematology are very resourceful to know the association of blood parameters with environmental changes. The haematological parameters such as RBC, WBC, Hb and PCV values are under the control of several factors including environmental conditions. Heavy metals enter in to the fish from the uptake of spoiled food through their alimentary canal or gills and external body surface. Later on, the heavy metals are transported via blood stream to the vital organs and tissues of the fish body where they are stored. Appearance of dead fish over the surface of water is the visible indication of highly polluted water, but unhealthy fish is the only indicator of sublethal pollution. Very minute level of pollution may not show any illness signs; however, it reduces the fish populations, which leads to long term reduction and extinction of the population from that ecosystem [7,8,9,10].

Heavy metal pollution is one of the major problems all over the world due to industrial and agricultural practices. They are well known for inducing oxidative stress and/or carcinogenesis by provoking free radicals/ reactive oxygen species. The form in which the heavy metals found in water is the most significant factor in the level of toxicity to fish. The level of toxicity is more when the heavy metal concentration is high which paves the way for various diseases in subsequent times. Heavy metal also affects the physiology and biochemical activities of fish, which are not only an essential ecosystem component, but also serve as food source. Earlier studies showed that fish and shellfish are the important contributors to consumer intake of some contaminants due to their presence in the aquatic environment and their accumulation in the flesh of fish and shellfish [11,12,13]. Vinodhini and Narayanan [14] studied the effect of heavy metal pollutants such as cadmium, chromium, nickel and lead on common carp (*Cyprinus carpio*) and observed the significant level of influence of toxic heavy metals on biochemical and haematological parameters of fish body. Effect of zinc on the haematological parameters of fresh water fish was also studied [15]. Several studies regarding the impact of heavy metals on fish revealed that haematological parameters can be used as indicators to find out the effect of heavy metal pollution in aquatic systems [16,17,18]. In this context, the present study has been designed to find out the effect of hexavalent chromium on the WBCs of the fresh water fish, *Labeo rohita*.

Materials and Methods

The Indian major carp, *Labeo rohita* is a fish of the carp family Cyprinidae, found commonly in rivers and freshwater lakes in and around the South Asia and South-East Asia. It is a herbivore and is treated as a delicacy in Orissa, Bihar and Uttar Pradesh. In fact, the Kayastha community of Uttar Pradesh treats it as one of their most sacred foods: to be eaten on all auspicious occasions.

Stock of fish was procured from a local fish farm in Madurai, Tamil Nadu, India. They were acclimatized to laboratory conditions for about two weeks in well water. During the period of acclimation they were fed on algal and artificial fish feed. Only fish of equal size and weight (20 to 25g) were selected for the experiments.

Chromium

Hexavalent chromium can be obtained from

different salts. For this study, crystals of Potassium dichromate ($K_2Cr_2O_7$) were used as the source of chromium. 2.8g of $K_2Cr_2O_7$ was dissolved in 1000 ml of distilled water to get 1 ppt solution and it was kept as a stock solution for the future study.

Test Medium

The test medium was the bore well water into which the stock solution of chromium can be mixed in varying volumes in order to get different experimental concentrations and these were used for the estimation of LC_{50} values for 24, 48, 72 and 96 hr. The temperature of the water used in this study ranged between 25 and 30 ^{0}C and pH of the water ranged between 7.5 and 8.5. For the experimental purpose stock solution was used to prepare different working ppm concentrations by dissolving desired volume of ppt solution in 1000 ml of bore well water as regularly till the end of experiment.

Experimental Design

After the completion of acclimation, all the healthy fishes were selected for experimental purpose. The acute toxicity of hexavalent chromium was assessed by determining the LC_{50} value with triplicate sets. In each set, different concentrations of chromium were used to estimate the LC_{50} value by observing the mortality of fish for different exposure periods.

Estimation of LC_{50}

The acute toxicity of chromium was estimated with static renewal bioassay procedure [19]. Different concentrations of chromium were selected and in each concentration, ten fishes were introduced to find out the percent mortality. The mortality in all the concentrations was recorded after 24, 48, 72 and 96 hours of exposure. The percent mortality was recorded in wide and then narrow ranges of different concentrations of chromium for 24 48, 72 and 96 hours of exposure. The LC_{50}value for different exposure periods was obtained by applying probit analysis.

Selection of Sublethal Concentrations

From the obtained 96 hour LC_{50} value of chromium, four sublethal concentrations namely 1/40th, 1/20th, 1/15th and 1/10th of 96 hr LC_{50} value were selected tor long term exposure to study the haematological changes in the fish, *L. rohita*. By this method 0.5, 1.0, 1.5 and 2 ppm concentrations of hexavalent chromium were selected as sublethal concentrations. The test media were daily changed. Along with these sublethal concentrations control group of fishes were also maintained side by side throughout the period of experiment In all these control and sublethal concentrations (0.5, 1, 1.5 and ppm), ten fishes were introduced for the experiment.

Haematological Studies

From the control and four sublethal concentrations, each fish was sacrificed and blood samples were taken for the estimation of haematological parameters by using standard procedures [20,21,22] and MS-Excel (Version: 12.0.6219.1000).

Results

The percent mortality of *L. rohita* exposed to different concentrations of chromium was noted. No mortality was noted in 5ppm concentration till 96 hr, whereas 100% mortality occurred in 80 ppm within 24 hr of exposure. The LC_{50} values for 24, 48, 72 and 96 hr are listed in Table 1. The LC_{50} values observed decreased with increase in the duration of exposure. Total WBC count of *L. rohita* exposed to different concentrations of hexavalent chromium is exhibited in Figure 1. In the control group of fishes, 6900 cells/mm^3 were counted in all the exposure periods. Whereas in toxicant exposed fishes changes were observed. The effect of hexavalent chromium was more in higher concentration of prolonged exposure compared to lower concentration of chromium. The changes were noted in all the concentrations for all exposure periods with increased WBC count.

Table 2 exhibits neutrophil count of *L. rohita* exposed to different concentrations of hexavalent chromium. The normal neutrophil count is 65%. Hexavalent chromium affected the neutrophil count. When the concentration increased the neutrophil count also increased and increased neutrophil count was also noted with increasing exposure period. Lymphocyte count of *L. rohita* exposed to different concentrations of hexavalent chromium is presented in Table 2. The increasing concentrations of hexavalent chromium caused decrease in the lymphocyte count. At the same time increase in the exposure period also resulted in a decrease in the lymphocyte count. The depletion of lymphocytes was more in higher concentrations and

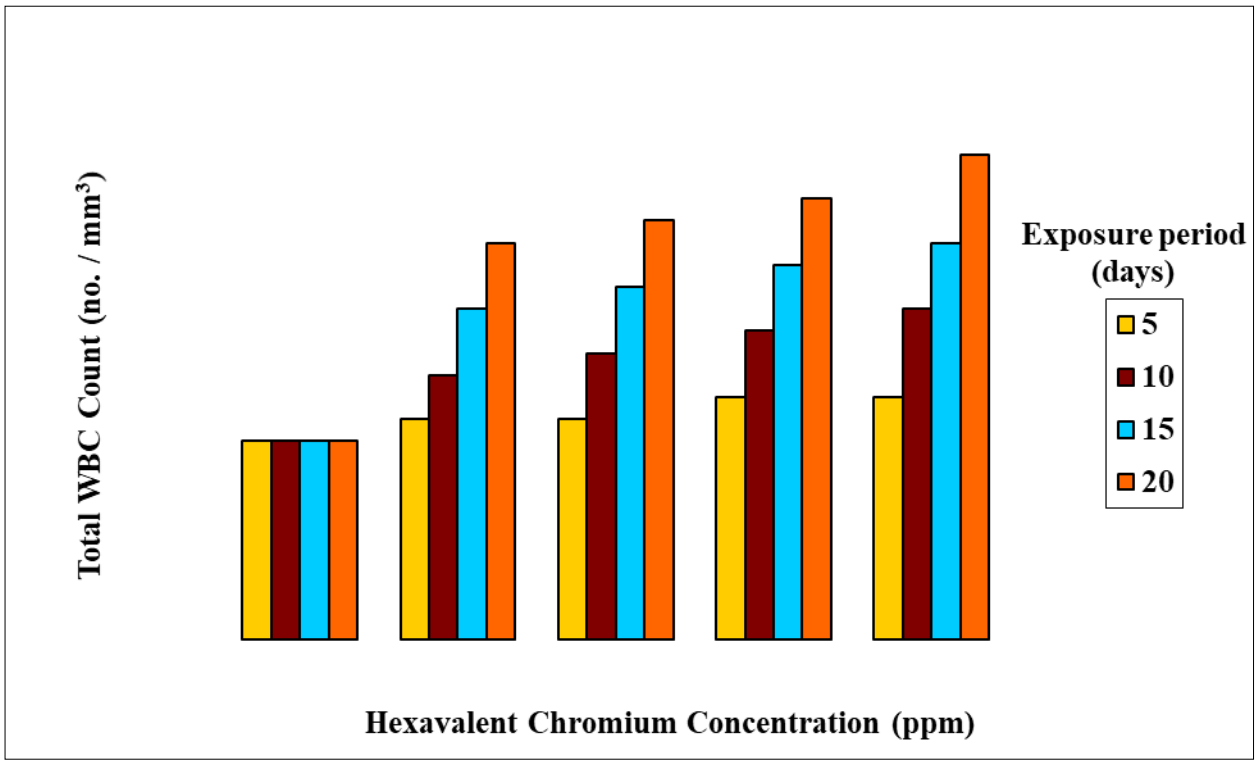

Figure 1. Effect of hexavalent chromium on the WBC count (number / mm^3) of *L. rohita*

Table 1. Acute toxicity test results of hexavalent chromium to *L. rohita*

Exposure Periods (hr)	LC_{50} (ppm)	95% fiducial limits		Regression equation (Y=a+bX)	Slope Function (S)	Chi square test result (0.05 level)
		Lower (ppm)	Upper (ppm)			
24	50.88	48.35	53.55	-4.28+5.44 X	1.52	Not Significant
48	42.03	39.82	44.36	-3.03+4.95 X	1.59	Not Significant
72	28.09	25.67	30.72	5.05+3.1 X	2.09	Not Significant
96	19.87	17.79	22.20	1.34+2.82 X	2.25	Not Significant

Table 2. Effect of hexavalent chromium on the different types of WBCs (%) of *L. rohita*

WBC Count (%)	Hexavalent Chromium Concentration (ppm)	Exposure Period (days)				
		0	5	10	15	20
Basophil	0.5	0	0	0	1	1
	1.0	0	0	0	1	1
	1.5	0	0	0	1	1
	2.0	0	0	0	1	2
Eosinophil	0.5	4	4	4	4	4
	1.0	4	4	5	4	4
	1.5	4	4	5	5	5
	2.0	4	5	5	5	5
Neutrophil	0.5	65	66	66	68	73
	1.0	65	67	68	71	76
	1.5	65	69	70	74	78
	2.0	65	70	72	77	81
Monocytes	0.5	0	0	0	1	1
	1.0	0	0	0	1	1
	1.5	0	0	0	1	1
	2.0	0	0	0	1	1
Lymphocytes	0.5	31	30	30	26	21
	1.0	31	29	27	23	18
	1.5	31	27	25	19	15
	2.0	31	25	23	16	11

longer exposure periods.

Eosinophil count of *L. rohita* exposed to hexavalent chromium is presented in Table 2. The normal count of eosinphil is 4%. The increase in hexavalent chromium concentration and exposure period caused an increase in the eosinophil count. During prolonged exposure at higher concentration, the eosinophil count was more in number. Table 2 presents monocyte count of *L. rohita* exposed to hexvalent chromium. Change in the monocyte count was found in higher concentration for prolonged exposure periods. During the initial period there were no changes observed in all the concentrations of hexavalent chromium. The changes were observed only after 20 days period of exposure. Concentration of hexavalent chromium had no effect on monocyte count. Regarding exposure period, changes were noted only in 15 and 20 days exposure but not in earlier exposure periods.

Basophil count of *L. rohita* exposed to hexavalent chromium is shown in Table 2. The basophil count changed only at higher concentrations and prolonged exposure periods. There were no changes observed in initial exposure periods. During prolonged exposure at higher concentration, much increase in basophil count was noticed.

Table 3 exhibits the two way ANOVA for the factors with the variables, exposure period and hexavalent chromium concentration. Variables such as exposure period and hexavalent chromium concentration caused significant variations with neutrophil and lymphocytes which were statistically significant at 5% level while variations for basophil, eosinophil and monocytes were not statistically significant.

Discussion

The present study reveals that chromium is acutely toxic to the fish *L. rohita* and the mortality rate increases with increase in the concentration of chromium. The mortality rate also increased with increasing duration of exposure. The LC_{50}value of chromium for 96 hr is 20 mg/l. The LC_{50} value of chromium for 96 hr is about 6 times higher than that of *Lepidocephalichthys thermalis* [23]. Acute toxicity of mercury has been studied in *Sarotherodon mossambicus* [24]. The result indicated mercuric chloride impairing oxidative and transphosphorylative activities during acute mercury toxicosis in fish. Toxicity of mercuric chloride to *Channa punctatus* has been shown to depend upon the concentration and duration of exposure [25]. When concentration and duration of exposure increased, the fish exhibited anamolous behaviour and a dose and time dependent mortality rate. The 96 hr LC_{50}Value of zinc to *Oreochromis mossambicus* was 2 times higher than that of the present study. But the range of toxicity varies for different species and for different toxicants. Furthermore, several factors like pH, hardness, alkalinity, equilibration and kinetics involved in the chemical reaction determine the toxic efficacy.

One of the recent areas of research in toxicology is concerned with the fate of the chemicals inside the organism. A chemical, which enters into an organism in a natural way, has to pass through certain barriers which separate the external medium from the internal medium. The barriers are skin, respiratory surfaces and intestine. Apart from these, blood also carries these toxicants, accumulates at different tissues and alters the haematological parameters. The study relevant to fish blood has been increasingly reported in the field of toxicology and environmental monitoring because it acts as an indicator of physiological and pathological variations. The blood in the gill has direct contact with water medium and any unfavourable change in water could be reflected in the circulatory system. These studies could be used to indicate the health status of fish as well as the water quality [26,27,28].

A toxicologist studying the kinetics or dynamics of a chemical in an organism is interested in knowing the routes of entry, translocation mechanism and the fate of the chemical as to its metabolism, accumulation and elimination. The concentrations of copper, iron, zinc and lead were studied in two important penaeid prawns from Chilka lagoon namely *Penaeus monodon* and *P. indicus*. The maximum concentrations of Fe, Zn and Pb in the skeleton of *P. monodon* were 3.20, 0.125, 0.802 and 0.123 ppm respectively and in *P. indicus*, they were 2.99, 0.680 and 0.120 ppm respectively [29]. Heavy metals are being introduced into aquatic environment through industrial processes; sewage disposal, soil leaching and rain fall. These metals are relatively toxic

Table 3. Two way analysis of variance (ANOVA) for the factors with the variables, exposure period and hexavalent chromium concentration

Factor	Source of Variation	SS	df	MS	Calculated F - value	Table value at 5% level	Level of significance
Basophil	Exposure Period	0.15	3	0.05	1	3.490	Not Significant
	Hexavalent Chromium Concentration	6.2	4	1.55	32	3.259	Significant
Eosinophil	Exposure Period	2	3	0.67	5.33	3.490	Significant
	Hexavalent Chromium Concentration	1.3	4	0.33	2.6	3.259	Not Significant
Neutrophil	Exposure Period	81	3	27	11.57	3.490	Significant
	Hexavalent Chromium Concentration	339.2	4	84.8	36.34	3.259	Significant
Monocytes	Exposure Period	8.88 E-16	3	2.96 E-16	- 4.0	3.490	Not Significant
	Hexavalent Chromium Concentration	4.8	4	1.2	-1.6E+16	3.259	Not Significant
Lymphocytes	Exposure Period	114.6	3	38.2	11.54	3.490	Significant
	Hexavalent Chromium Concentration	544.7	4	136.2	41.16	3.259	Significant
Total WBC	Exposure Period	1348 E3	4	337 E3	10.01	3.259	Significant
	Hexavalent Chromium Concentration	15135 E2	3	5045 E2	14.99	3.490	Significant

even at fairly low concentration and affect the survival of fishes and other aquatic organisms [30].

WBCs were found to increase with increasing concentration and duration of exposure to chromium. This may be because of the role of the leucocytes in engulfing the foreign materials resulting in phagocytosis. So this was supported with increasing concentrations and durations, the leucocyte count being increased in *Heteropneustes fossilis* exposed to carbaryl and methyl parathion pesticides [31]. WBCs increased with increasing concentrations and duration of exposure to copper. WBCs also increased with increasing concentration and duration of exposure to Lambda-cyhalothrin [32].

Neutrophils, monocytes and basophils were found to be increased. The level of increase was gradual with increase in the concentration and duration of chromium exposure. DDT induced the haematotoxicity in *Clarias batrachus* with gradual decline in total RBC, WBC count, haemoglobin content and oxygen carrying capacity of RBC [33]. Elevation of neutrophil count was observed in fish exposed to pesticide [30, 31]. Similar trend was reported in *O. mossambicus* species. The WBC count increased with increasing duration and concentration of pesticide in the medium [34].

In the present study the gradual decline of lymphocytes was found with increasing concentration and duration of exposure with reference to the control fish. Same results were observed in *L. rohita* exposed to copper and zinc at neutral and acidic pH [35]. Levels of leucocytes were inereased with increase in the toxicant exposure duration and concentration in *Cyprinus carpio* exposed to mercury [36]. Increase in TLC in the test fish could be due to stimulated lymphophoiesis and /or enhanced release of lympohocytes from lymphomyeloid tissues. The increase in lymphocyte number in treated fish is also probably for the removal of cellular debris of necrosed tissue at a quicker rate as reported by McLeay and Brown [37]. However it is obvious even at low level, any toxicant will induce the elevation of leucocyte upto a certain level with permissible concentrations. Metals disrupt chemical communication system. Chemical alarm response of juvenile fathead minnows after embryonic copper exposure was reported. Exposure to elevated copper concentrations during embryonic development is sufficient to impair chemosensory function during developing life stages. Inability to detect nearby predators by olfaction could lead to ecological perturbations in populations inhabiting metal contaminated ecosystems [38].

Haematological parameters of the fish are the indicators to find out the quality of the water and health of the fish. Hexavalent chromium caused remarkable changes in different haematological parameters and affected the health of the fish, which led to the death of fish. Thereby the fish becomes unsafe for edible purposes and the water becomes unsuitable for potable and recreation purposes.

Conclusion

The 24, 48, 72, and 96hr LC50 values of hexavalent chromium to *L. rohita* were 50.88, 42.03, 28.09 and 10.87 ppm respectively. When exposed to sub-lethal concentrations of hexavelent chromium for 20 days, the fish exhibited decline in the levels of lymphocytes while an increase in the levels of other types of WBCs and total WBC count.

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