Toxic effect of chromium on leucocytes of *Labeo rohita* after administration of human B erythrocytes as antigen

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Abstract

The toxic effect of chromium on leucocytes of *Labeo rohita* after primary and primary boosted antigen administration was measured. The antigen was used in the form of human B erythrocytes. In the present studies the fish were exposed to sub lethal concentration of 12 mg/l. of chromium. The sampling was done after 2, 4 and 6 weeks of primary and primary boosted antigen exposure. As a result there was found significant increase in TLC. In case of DLC there was increase in monocytes, neutrophils, basophils and eosinophils while a significant decrease in lymphocytes was observed as compared to untreated groups.

Keywords: Chromium, Leucocytes, *Labeo rohita.*, B erythrocytes, Toxicity

Introduction

The contamination of aquatic environment by heavy metals is one of many problems of environmental toxicology. The danger of these chemical elements comes from the fact that they are not biodegradable and therefore as they separate in environment they accumulate along the food chain until it reaches man. Some heavy metals present the phenomenon of biomagnification and their concentration increases progressively during their passage through the different rings of biological food chain. Certain aquatic organisms can bioconcentrate heavy metals up to levels 100,000 times greater than those present in water in which they live. (Abate *et.al.*, 2003). Toxic effects of metals on aquatic ecosystems range from complete loss of biota to subtle effects on rates of growth, population, reproduction and mortality. (Hodson, 1987).Moreover hematological and enzymatic values are also effected by these toxic substances. (Post, 1987). Chromium is one such heavy metal released to environment by mining, smelting and industrial uses. Its hexavalent form is water insoluble and is more readily absorbed across cell membranes. (Hodgson and Levi, 2000). In toxic doses it causes adverse effects on body systems and tissues of organisms leading to abnormalities and even mortality. When released in aquatic environment it disturbs the biodiversity including fish. Fish is the important part of aquatic food chain and any abnormality in it can damage the whole food chain. Chromium along with other effects disturbs the hematology of fish. In the present work the effect of chromium is determined on the total and differential leucocyte count of *Labeo rohita* which are important defense mechanisms of body against foreign agents.

Material and Methods

The farmed fish *Labeo rohita* of 11 ± 0.634 cm length and 13 ± 2 g weight were used for this study. No of fish used in experimental and control aquaria. Twenty fish were placed in each control and experimental aquariam. In the experimental aquaria, the temperature was maintained at 27 ± 2^0C and pH at 7.8 ± 0.1. The toxicant chromium was used in form of potassium dichromate in which hexavalent chromium is present. The hexavalant form is considered more toxic. The blood sample was taken from

fish by cardiac puncture for the study of total and differential leucocyte count.

Total leucocytes count

For leucocytes count blood was obtained through 1ml disposable syringe, which was coated with some crystals of EDTA at its piston. The blood in the syringe was gently shaken and then transferred to eppendorf (1.5 ml).The total leucocytes count was obtained by Shaw method using Neubauer counting chamber by using solution A and solution B as fish blood diluting fluid. The blood was drawn up to 0.5 marks in thoma pipette. The blood was diluted 20 times by using solution A and B. The solution A was drawn in until the bulb was half filled and solution B was then drawn in up to mark 11. After gentle shaking, the counting chamber was loaded with this diluted blood and counting was done under the microscope in large squares present at four angular points of Neubauer counting chamber (Schaperclaus, 1991). The number of W.B.C. was calculated by using the following formula:

No. of W.B.C. / ml = N/V × 20

Where

N = Number of cell in 4 big squares

V = Volume of ruling chamber for W.B.C.

20 = Dilution factor

(Schaperclaus, 1991)

Differential Leucocytes Count:

After taking the blood from fish (without EDTA) a thin film was prepared on glass slide according to the method described by Schaperclaus (1991). This smear was stained with Sudan Black B and Giemsa. First these smears were air dried and fixed for five minutes with formalin vapors. The samples were then immersed in Sudan Black B for 10-20 minutes. They were then washed with absolute alcohol several times. The slides were then stained in Giemsa for 10-15 minutes and then washed with distilled water. (Murad and Houstan (1988). Roberts (1989) and Mughal and Malkani (2004). Different types of leucocytes were counted on slides by selecting a clear area of film. A minimum of 100 cells were counted in a complete strip of film. The percentage of respective cells was then calculated (Schaperclaus, 1991 and Lucky, 1977).

The data was analyzed by using ANOVA and L.S.D. (least significant difference) (Sokal and Rohlf, 1981). $P > 0.05$ were considered as significant.

Experimental Plan

The experiments were conducted in following manner. The LC_{50} was calculated by Read and Munch method (1987) after 96 hours and it appeared to be 26.4 mg/l.

Effects of sub-lethal concentrations of chromium on immune system during acute phase were determined by conducting two experiments involving primary and primary boosted responses. **Group A** served as controls as it received no toxicant and antigen. This group was sampled with every treated group and was examined for TLC, DLC.

In primary phase two groups were made. **Group B** received no toxicant but a single dose of antigen and after two, four and six weeks it was examined for both parameters. **Group C** was exposed to two daily exposures of 12mg/l. of chromium and an antigen administration on day3. This group was also examined after two, four and six weeks of antigen administration for total and differential leucocyte count.

In primary boosted phase there were again two groups. **Group D** received no toxicant but two doses of antigen with a gap of 12 days. This group was analyzed

for both parameters after two, four and six weeks of second antigen administration. **Group E** had two daily exposures of 12mg/l. of chromium and then antigen was administered on day 3 and 15. After two, four and six weeks of second antigen administration this group was analyzed for TLC and DLC.

Results

i. Total Leucocyte Count

The total leucocytes count was significantly different in exposed groups from control.

Group A had the TLC of $4.81\times10^3 \pm 0.016$ cells/ mm^3. In group B, TLC after two weeks of antigen was $8.1\times10^3 \pm 0.012$ cells/ mm^3 throughout study period and after four and six weeks it became $8.7\times10^3 \pm 0.036$ cells/ mm^3 and $8.5\times10^3 \pm 0.040$ cells/ mm^3 respectively. Group C had TLC value of $8.4\times10^3 \pm 0.018$ cells/ mm^3, $9.1\times10^3 \pm 0.028$ cells/ mm^3 and $8.9\times10^3 \pm 0.012$ cells/ mm^3 after 2, 4 and 6 weeks respectively.

Group D had total leucocytes count of $8.38\times10^3 \pm 0.015$ cells/ mm^3, $8.44\times10^3 \pm 0.0098$ cells/ mm^3 and $8.08\times10^3 \pm 0.008$ cells/ mm^3 after 2, 4 and 6 weeks after second antigen administration respectively. In group E, TLC after 2, 4 and 6 weeks of second antigen administration was $8.78\times10^3 \pm 0.015$ cells/ mm^3, $9.2\times10^3 \pm 0.020$ cells/ mm^3 and $9.0\times10^3 \pm 0.015$ cells/ mm^3 respectively. (Table 1).

ii - Differential Leucocytes Count

The differential leucocytes values in treated groups were significantly lower than control groups. In group A the lymphocyte number was 92.3 ± 0.4. Group B had 94.8 ± 0.0057, 97.2 ± 0.192 and 98 ± 0.127 lymphocytes after 2, 4 and 6 weeks respectively. Group C had lymphocyte number 87.28 ± 0.059 after 2 weeks of antigen administration which further reduced to 76.2 ± 0.017 and 72 ± 0.179 after 4 and 6 weeks respectively. Group D had lymphocyte number 96.6 ± 0.098, 97.2 ± 0.080 and 93 ± 0.126 after 2, 4 and 6 weeks respectively of second antigen administration. Group E had lymphocyte number of 76 ± 0.18, 74.2 ± 0.196 and 74 ± 0.179 after 2, 4 and 6 weeks of second antigen administration (Table 2).In case of monocytes there was significant increase in treated groups as compared to control groups. Group A, the control group, showed 4.8 ± 0.44 monocytes in 2, 4 and 6 weeks respectively. Group B had 4.98 ± 0.283 monocytes after two weeks of antigen administration respectively. which was later increased to 22 ± 0.0067 and 5.9 ± 0.457 after 4 and 6 weeks. In group C the monocytes were 6.18 ± 0.0067, 7.6 ± 0.40 and 8 ± 0.16 after 2, 4 and 6 weeks of antigen administration respectively. Group D after 2, 4 and 6 weeks of second antigen administration showed similar trend in monocytes number of 5.1 ± 0.283, 5.8 ± 0.336 and 5.7 ± 0.60 respectively. In group E, after two weeks, monocytes number was 7.5 ± 0.439 which increased to 8.1 ± 0.415 and 8.3 ± 0.42 after 4 and 6 weeks respectively (Table 3). Neutrophils showed a significant increase in all treated groups when compared to control group A which had 0.4 ± 0.219 neutrophils. In group B this number increased to 0.48 ± 0.335, 0.52 ± 0.304 and 0.52 ± 0.335 after 2, 4 and 6 weeks respectively of antigen administration. Group C showed 0.7 ± 0.0057 neutrophils after 2 weeks, 2.3 ± 0.56 after 4 weeks and 2.78 ± 0.21 after 6 weeks. The group D had 0.56 ± 0.179, 0.54 ± 0.41 and 0.52 ± 0.179 values of neutrophils after 2, 4 and 6 weeks respectively of second antigen administration. In group E the value further changed to 2.85 ± 0.010 after 2 weeks, 2.78 ± 0.067 after 4 weeks and 2.48 ± 0.04 after 6 weeks (Table 4). In control group A the basophils number

Table 6: Effect of chromium exposure followed by antigen administration on EOSINOPHILS of *Labeo rohita.*

GROUP	TREATMENTS			EOSINOPHILS MEAN ±S D		
	Toxicant	1st Antigen	2nd Antigen	2 weeks	4 weeks	6 weeks
A	–	–	–	0.8 ± 0.179	0.8 ± 0.179	0.8 ± 0.179
B	–	+	–	*0.89 ± 0.057	*0.91 ± 0.034	*0.90 ± 0.022
C	+	+	–	*1.18 ± 0.014	*1.9 ± 0.101	*2.0 ± 0.059
D	–	+	+	*0.93 ± 0.011	*0.93 ± 0.042	*0.90 ± 0.051
E	+	+	+	*2.0 ± 0.057	*2.3 ± 0.093	*2.8 ± 0.071

P < 0.05

Discussion

Studies on leucocytes revealed that the leucocytes count first increased on exposure to chromium followed by antigen stimulation, which later on decreased. The possible reason for this increase may be the stress caused by chromium to lymphoid organs. Chromium is a good stressor and can enhance this effect on the animals (Almeida and Barajas, 2002). In addition chromium can make fish more susceptible to infection and high concentration of this metal damages the tissue as well (EIFAC, 1983). The other possibility for this increased number of leucocytes could be due to the stress caused by heavy metal in fish. Chromium may be recognized as the foreign toxicant against which this leucocytosis may provide defense or this increase might be in response to possible infection or tissue damage caused by chromium. The later decrease in leucocytes number after 6 weeks might be due to the dilution of chromium in tissues or may be due to activation of some other defense mechanisms in the body. Gill and Pant (1987) also obtained similar results in hematological studies due to chromium in fresh water fish *Barbus conchonius*. Hashmi (1999) also demonstrated increase in total leucocytes count in *Labeo rohita* after mercury intoxication. Alkahen (1995) showed that increase in W B C occurred in response to tissue damage induced by cadmium chloride in catfish. There are different types of leucocytes, which show differential behavior in body defense. The lymphocyte number in exposed groups was lower than that of non-exposed. This decrease might be due to the stress induced by chromium. According to Ganong (1999) the glucocorticoids are released during stress, which in turn decrease the number of circulating lymphocytes and also inhibit lymphopoiesis in lymphoid organs. It may be possible that the toxic effect on lymphoid organs in fish might have caused this decrease in lymphocytes number. Rajaram, *et. Al,.* (1995) and

Table 2: Effect of chromium exposure followed by antigen administration on LYMPHOCYTES of *Labeo rohita*

GROUP	TREATMENTS			LYMPHOCYTES MEAN ± S D		
	Toxicant	1st Antigen	2nd Antigen	2 weeks	4 weeks	6 weeks
A	–	–	–	92.3 ± 0.4	92.3 ± 0.4	92.3 ± 0.4
B	–	+	–	*94.8 ± 0.0057	*97.2 ± 0.192	*98.0 ± 0.127
C	+	+	–	*87.28 ± 0.059	*76.2 ± 0.017	*72.0 ± 0.179
D	–	+	+	*96.6 ± 0.098	*97.2 ± 0.080	*93.0 ± 0.126
E	+	+	+	*76.0 ± 0.18	*74.2 ± 0.196	*74.0 ± 0.179

P < 0.05

Table 3: Effect of chromium exposure followed by antigen administration on MONOCYTES of *Labeo rohita.*

GROUP	TREATMENTS			MONOCYTES MEAN ±S D		
	Toxicant	1st Antigen	2nd Antigen	2 weeks	4 weeks	6 weeks
A	–	–	–	4.8 ± 0.44	4.8 ± 0.44	4.8 ± 0.44
B	–	+	–	*4.98 ± 0.283	*5.22 ± 0.0067	*5.9 ± 0.457
C	+	+	–	*6.18 ± 0.0067	*7.6 ± 0.40	*8.0 ± 0.0.106
D	–	+	+	*5.1 ± 0.283	*5.8 ± 0.336	*5.7 ± 0.60
E	+	+	+	*7.5 ± 0.439	*8.1 ± 0.415	*8.3 ± 0.42

P < 0.05

Table 4: Effect of chromium exposure followed by antigen administration on NEUTROPHILS of *Labeo rohita*.

GROUP	TREATMENTS			NEUTROPHILS MEAN ± S D		
	Toxicant	1st Antigen	2nd Antigen	2 weeks	4 weeks	6 weeks
A	–	–	–	0.4 ± 0.219	0.4 ± 0.219	0.4 ± 0.219
B	–	+	–	*0.4 8 ± 0.335	*0.52 ± 0.304	*0.52 ± 0.335
C	+	+	–	*0.7 ± 0.0057	*2.3 ± 0.56	*2.78 ± 0.21
D	–	+	+	*0.56 ± 0.179	*0.54 ± 0.41	*0.52 ± 0.179
E	+	+	+	*2.85 ± 0.010	*2.78 ± 0.067	*2.48 ± 0.04

P < 0.05

Table 5: Effect of chromium exposure followed by antigen administration on BASOPHILS of *Labeo rohita*.

GROUP	TREATMENTS			BASOPHILS MEAN ± S D		
	Toxicant	1st Antigen	2nd Antigen	2 weeks	4 weeks	6 weeks
A	–	–	–	1.2 ± 0.179	1.2 ± 0.179	1.2 ± 0.179
B	–	+	–	*1.6 ± 0.098	*1.94 ± 0.0098	*1.9 ± 0.0099
C	+	+	–	*2.8 ± 0.034	*3.1 ± 0.54	*3.0 ± 0.071
D	–	+	+	*2.1 ± 0.033	*2.0 ± 0.030	*1.9 ± 0.219
E	+	+	+	*3.8 ± 0.121	*3.4 ± 0.053	*3.4 ± 0.107

P < 0.05

appeared to be 1.2 ± 0.179. In group B basophils were 1.6 ± 0.098 after 2 weeks which further increased after 4 and 6 weeks to 1.94 ± 0.0098 and 1.9 ± 0.0099 respectively. Group C had basophils number of 2.8 ± 0.034, 3.1 ± 0.054 and 3.0 ± 0.071 after 2, 4 and 6 weeks respectively of antigen administration. After 2, 4 and 6 weeks of second antigen administration. In group D the basophils had value of 2.1 ± 0.033, 2 ± 0.030 and 1.9 ± 0.219 respectively. The group E showed 3.8 ± 0.121 basophils after 2 weeks, 3.4 ± 0.053 after 4 weeks and 3.4 ± 0.107 after 6 weeks of second antigen (Table. 5). Eosinophils in control group A had value of 0.8 ± 0.179, in group B. It increased to 0.89± 0.057 after 2 weeks, 0.91 ± 0.034 after 4 weeks and 0.90 ± 0.022 after 6 weeks of antigen administration. Group C showed eosinophils number of 1.18 ± 0.014, 1.9 ± 0.101 and 2.0 ± 0.059 after 2, 4 and 6 weeks respectively of antigen administration. In group D the values decreased to 0.93 ± 0.011 after 2 weeks and to 0.93 ± 0.042 and 0.90 ± 0.051 after 4 and 6 weeks respectively of second antigen administration. The group E had 2.0 ± 0.057, 2.3 ± 0.093 and 2.8 ± 0.071 eosinophils after 2, 4 and 6 weeks respectively after toxicant and antigen administration. (Table. 6).

Table 1: Effects of chromium exposure followed by antigen administration on TLC of *Labeo rohita*.

GROUP	TREATMENTS			T L C (10^3/MM^3) MEAN ± S D		
	Toxicant	1st Antigen	2nd Antigen	2 weeks	4 weeks	6 weeks
A	–	–	–	4.81 ± 0.016	4.81 ± 0.016	4.81 ± 0.016
B	–	+	–	*8.1 ± 0.012	*8.7 ± 0.036	*8.5 ± 0.040
C	+	+	–	*8.4 ± 0.018	*9.1 ± 0.028	*8.9 ± 0.012
D	–	+	+	8.38 ± 0.015	*8.44 ± 0.0098	*8.08 ± 0.008
E	+	+	+	*8.78 ± 0.015	*9.2 ± 0.020	*9.0 ± 0.015

P < 0.05

Balamurugan, *et. al.*, (2000) showed that chromium induced decreased lymphocytes so this could be the reason of decreased lymphocytes in blood. Inflammation was found in liver and kidney due to chromium exposure, which may have induced non-specific response against stress. This may be the cause of increased neutrophils. The neutrophils are involved in phagocytosis and increase in their number may enhance phagocytosis to get rid of chromium molecules. Eosinophilia, basophilia and monocytosis are the conditions produced during allergic reactions and infections. Cr intoxication may have resulted in such responses. The results of differential leucocytes revealed that chromium might have caused stress leading to infection and inflammation, which may result in abnormal number of leucocytes. Similar results were obtained by Khangarot, *et.al.*, (1998) when they exposed fresh water catfish *Saccobranchus fossilis* to sub toxic levels of chromium. Arunkumar *et.al.*, (2000) reported similar changes in differential leucocytes counts after chromium exposure to African mouth brooder fish, *Oreochromis mossambicus*. Abnormal number of differential leucocytes in different fishes exposed to heavy metals were reported by various authors (Mughal and Malkani (2004a), Pandima Devi, *et.al.*, (2003), Mancuso and Hueper (1951), Dinakaran (1997), Dethloff and Bailey (1998) and Dethloff, *et.al.*, (2001).

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