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Research Paper

Haematological investigation on freshwater teleost *Labeo rohita* (Ham.) following aquatic toxicities of Cr (III) and Cr (VI)

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Abstract

The effect of Cr(III) and Cr(VI) toxicity were studied on the haematology of freshwater teleost *Labeo rohita* exposed for24h, 48h and 96h LC50. Haematological parameters viz,. RBC, WBC, Haemoglobin (Hb), Haematocrit, Mean Cell Volume (MCV) Mean cell Haemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were studied. Changes in haematological parameters such as total leucocytes and erythrocyte counts, haemoglobin and haematocrit were apparent in the fish exposed to both trivalent and hexavalent chromium. The response of fish to chromium in general was dose dependent in both the Cr^{3+} and Cr^{6+} exposed fish. MCH and MCHC values were significantly affected. The study with a specified concentration of Cr^{3+} and Cr^{6+} has made it clear that there is differential toxicity based on the valence states of chromium; the hexavalent form is found to be more toxic. The results and discussion in details.

Keywords: Chromium, Toxicity, *Labeo rohita, Haematology*

Introduction

Heavy metals entering aquatic ecosystem through industrial wastes, mining, agricultural development, and fossil fuel combustion can have a wide range of adverse effects on aquatic communities [18]. Several studies have shown that exposure of fish to heavy metals leads to a number of disturbed physiological processes [12,13,21,25,32,39,42,45,46,51]. Chromium (Cr) is a metallic element belonging to the first transitional series of the periodic table. Elemental chromium has a CAS Registry Number of 7440-47-3. The three most stable forms in which chromium occurs in the environment are the 0 (metal and alloys), +3 (trivalent chromium), and +6 (hexavalent chromium) valence states. In the +3 valence state, the chemistry of chromium is dominated by the formation of stable complexes with both organic and inorganic ligands [19]. In the +6 valence state, chromium exists as oxo species such as CrO_3 and CrO_4^{2-} that are strongly oxidizing[8]. The toxicity of chromium to aquatic life was intensively investigated during previous decades, and a considerable amount of experimental data was compiled and reviewed [11, 20, 22, 44] the effects of chromium and other heavy metals to freshwater species of plants, invertebrates, fish and animals. Chromium compounds are known to have toxic, genotoxic, mutagenic and carcinogenic effects on man and animals [33, 41, 49].

The toxicity of chromium on the haematological parameters of a variety of freshwater fishes has been well documented [15, 16, 27, 35, 39]. Haematological parameters have successfully been implemented to determine the effect of industrial effluent [30, 37] and heavy metals [17, 28, 30]. In view of this, short-term acute toxicity tests were performed on *Labeo rohita* over a period of 96h to determine the LC_{50} value so as to elucidate the acute effects of trivalent and hexavalent chromium on the haematological parameters of freshwater teleost *Labeo rohita*.

Materials and Methods

The present investigations, the both sexes of freshwater teleost *Labeo rohita* (8.2 – 9.6cm in length and 9.7 – 12.8 gm in weight) were used and they were procured from Siraco Fish Farm, Nerinjipet, Erode District, Tamil Nadu. The test animals were maintained in large glass aquaria at 25±1º C under diurnal lighting conditions under 12L: 12D photoperiod (lights on from 6.00 am to 6.00 pm) and acclimatized to the laboratory conditions for 28 days (normal day during the testing period ranged from 11.30h light and I2.30h dark). An overhead Philips Fluorescent lamp (120 cm, 40W) provided a light intensity of *ca.* 0.65 X10 quanta s cm at the water surface [34]. During the period of acclimatization the fish were fed with commercial fish food *ad libitum.* Food and water were changed daily with an intermittent aeration with an aerator to ensure sufficient oxygen supply and the timing of the renewal was never constant to avoid the possibility of entrainment of any circadian rhythm by a fixed handling schedule. Feeding was discontinued and all fish were fasted for 24h prior to experimentation to keep the experimental animals in a more or less similar metabolic state. The 96 hour LC50 value was determined following Finney's Probit Analysis[14] with 5 concentrations *i.e.,* 20, 30, 40, 50 and 60ppm for both Cr^{3+} and Cr^{6+} individually.

The acute toxicity testing with fish followed the current recommended procedure of the Committee on Methods for Toxicity Testing with Aquatic organism [4]. One tenth and one fifth of the 96 hour LC50 concentrations were chosen as sublethal concentrations for studies at 24h,48h and 96 h exposure to the trivalent (as chromium (III) oxide) and hexavalent chromium (as Potassium dichromate) and the values are given as Mean ± SE of 5 individual observations. Test solutions were renewed daily with fresh water. Water chemistry of renewed and exchanged solutions was measured daily according to the method of APHA, 1995[3].

Hematological indices which form very important parameters for the evaluation of fish physiological status under metallic stress were studied in the fourth series of experiments following exposure to one fifth, one tenth of the 96hLC50 concentration of Cr^{3+} and Cr^{6+} and specified concentration of Cr^{3+} and Cr^{6+}. RBC, WBC values were obtained by employing Microcell Counter. The hemoglobin concentration was determined using the cynamet-hemoglobin method [6] and the hematocrit was determined according to the method described by Korzhuev [26]. Mean cell volume (MCV), Mean cell Haemoglobin (MCH), and Mean cell haemoglobin concentration were determined adopting standard universal formulae. Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the formulae described by Dacie and Lewis [10].

Results and Discussion

From the experiments, no mortality was observed over the 96 hour LC50 period of study in the control. The mortality percentage was of the order 8, 16, 32, 44 and 52 in the case of fish exposed to 20mg, 30mg, 40mg, 50mg and 60mg of chromium (III) as chromium trioxide. In respect of fish exposed to chromium (VI) of the same concentration the percent mortality was higher in each of the concentration studied over their counterparts exposed to chromium (III). The fiducial limit with 95 percent confidence from the variance in respect of LC50 studies in chromium (III) exposed fish was found to be, LC50 = 57.074, the upper limit 63.480 and the lower limit 52.619 whereas the fiducial limit for their counterparts in respect of chromium (VI) was of the order LC50 = 36.222, upper limit is 41.408, lower limit is 31.322 which is suggestive of the pronounced toxicity of chromium (VI) as against chromium (III) (Table 1 and 2 Figure 1- 4).

Table 1: 96h LC^{50} value for *Labeo rohita* following exposure to different concentrations of Cr^{3+} as Chromium trioxide

	Dose mg/L	log(D)	n	r	M	M′	Probit	Expected probit	95% fiducial limits of probit	
									lower	upper
Control	0		25	0	0	0				
Experimental	20	1.30102	25	2	8	8	3.5947	3.5351	3.3217	3.7484
”	30	1.47712	25	4	16	16	4.0056	4.1015	3.9769	4.2261
”	40	1.60205	25	8	32	32	4.5327	4.5034	4.411	4.5958
”	50	1.69897	25	11	44	44	4.8493	4.8151	4.709	4.9213
”	60	1.77815	25	13	52	52	5.05	5.0698	4.9328	5.2069

The regression equation is: y = -0.6500 + 3.217x
SE of slope = 0.8300
LC50 = 57.074
Lower 95% fiducial limit of LC50 = 52.619
Upper 95% fiducial limit of LC50 = 63.480

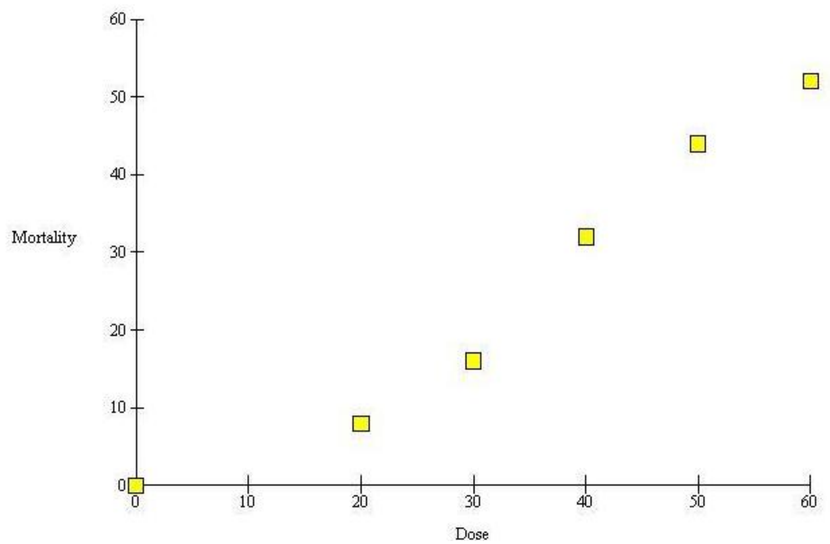

Figure 1: Mortaliy curve for *Labeo rohita* following exposure to different Concentrations of Cr^{3+}

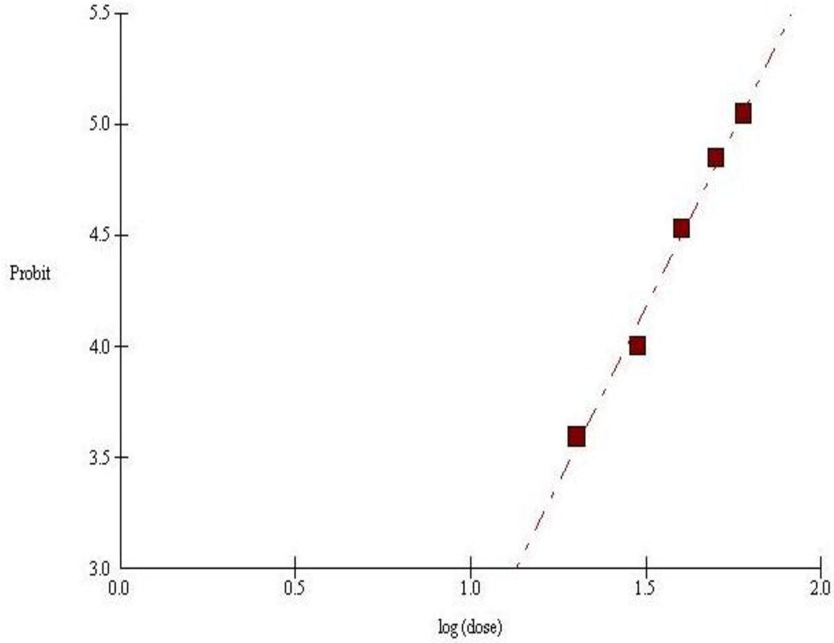

Figure 2: Probit regression line for Cr3+ for *Labeo rohita* following exposure to different concentrations of Cr^{3+}

Table 2: 96h LC^{50} value for *Labeo rohita* following exposure to different concentrations of Cr^{6+} as Potassium dichromate

	Dose mg/L	log(D)	n	r	M	M′	Probit	Expected probit	95% fiducial limits of probit	
									lower	upper
Control	0		25	0	0	0				
Experimental	20	1.30102	25	5	20	20	4.1585	4.0147	3.5834	4.446
”	30	1.47712	25	8	32	32	4.5327	4.6873	4.4337	4.941
”	40	1.60205	25	14	56	56	5.1507	5.1646	4.9468	5.3823
”	50	1.69897	25	17	68	68	5.4673	5.5347	5.2628	5.8067
”	60	1.77815	25	21	84	84	5.9944	5.8372	5.4888	6.1855

The regression equation is : y = -0.9549 + 3.820x
SE of slope = 0.762
LC50 = 36.222
Lower 95% fiducil limit of LC50 = 31.322
Upper 95% fiducial limit of LC50 = 41.408

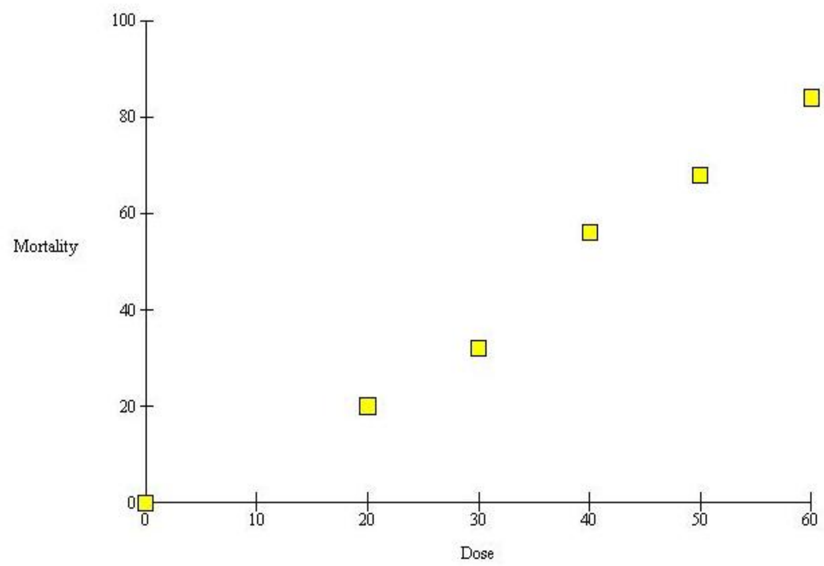

Figure 3 : Mortality curve for *Labeo rohita* following exposure to different Concentrations of Cr^{6+}

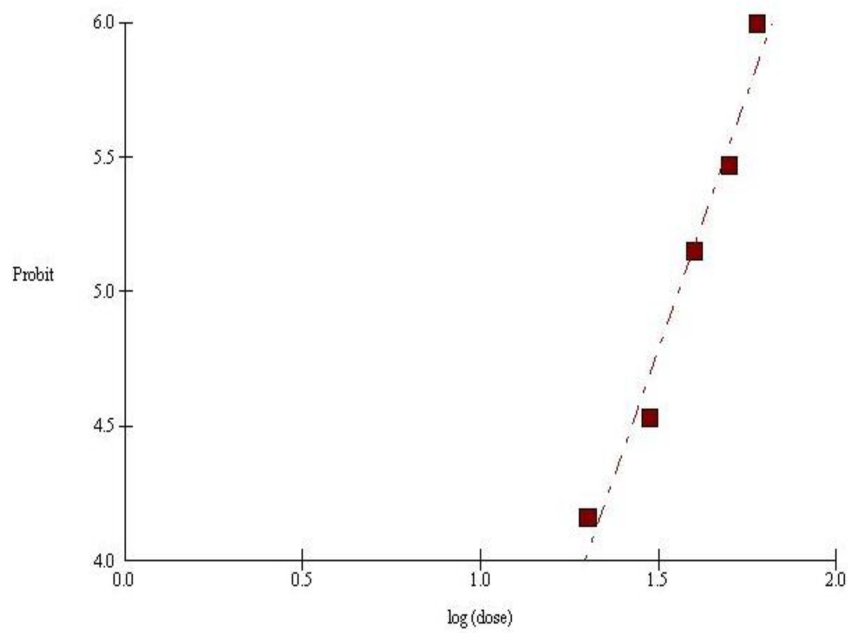

Figure 4: Probit regression line for Cr6+ for *Labeo rohita* following exposure to different concentrations of Cr^{6+}

The mortality ranged from 8% to 52% in fish exposed to Cr^{3+} and it was found to be 20 to 84 in fish exposed to Cr^{6+}. The mortality percentage increased with a corresponding increase in the toxicant concentration and also duration of the exposure demonstrating both time and concentration dependent responses. Further the mortality rate was found to be higher in Cr^{6+} exposed fish than Cr^{3+} exposed fish eliciting the fact that Cr^{6+} is more toxic than Cr^{3+}.

Haematological indices form crucial parameters for the evaluation of fish physiological status under metallic stress. The alterations in blood indices depend to a large extent on the concentrations of heavy metals and duration of exposure of fish to them. It is well known that haematological indices are of different sensitivity to various environmental factors and chemicals. The count of erythrocytes is quite a stable index and the fish body tries to maintain this count within the limits of physiological standards, using various physiological mechanisms of compensation, especially under stress.

Table 3: Variations in the haematological parameters following exposure to one fifth of the 96hLC50 concentration of Cr^{3+} as Chromium trioxide and Cr^{6+} as potassium dichromate in freshwater teleost fish *Labeo rohita*

Parameter	Treatment	Exposure period (hours) 24		48		96	
		Cr^{3+}	Cr^{6+}	Cr^{3+}	Cr^{6+}	Cr^{3+}	Cr^{6+}
Red blood cell (10^6 mm^{-3})	C	1.211±0.135	1.396±0.147	1.302±0.131	1.409±0.127	1.374±0.138	1.417±0.141
	T	1.246±0.142* (+2.89)	1.191±0.125** (-14.68)	1.357±0.142* (+4.22)	1.153±0.136** (-18.16)	1.489±0.121* (+8.36)	1.046±0.152** (-26.18)
White blood cell ($10^3 mm^{-3}$)	C	21.38±0.117	21.38±0.146	21.56±0.121	21.56±0.147	21.74±0.142	21.74±0.161
	T	19.43±0.152* (-9.12)	18.46±0.138** (-13.65)	19.07±0.127** (-11.54)	17.93±0.129** (-16.83)	17.69±0.152** (-18.62)	16.41±0.137** (-24.51)
Haemoglobin (g 100 mL^{-1})	C	8.62±0.143	8.76±0.161	8.84±0.172	8.43±0.148	8.97±0.124	8.94±0.143
	T	9.36±0.162* (+8.58)	7.12±0.171** (-18.72)	9.78±0.137** (+10.63)	6.62±0.152** (-21.47)	10.29±0.143** (+14.71)	6.28±0.152** (-29.75)
Hematocrit (%)	C	31.42±0.142	36.45±0.153	31.65±0.124	36.64±0.174	31.93±0.164	36.58±0.122
	T	32.74±0.142 (+4.20)	28.19±0.153** (-22.66)	33.78±0.126* (+6.72)	26.58±0.165** (-27.45)	35.27±0.731** (+10.46)	22.71±0.169** (-37.91)
MCV (μ^3m)	C	259.45±0.147	261.10±0.162	243.08±0.171	260.04±0.158	232.38±0.127	258.15±0.153
	T	262.76±0.137* (+1.27)	236.69±0.163* (-9.34)	248.93±0.159* (+2.40)	230.52±0.172** (-11.35)	236.87±0.148* (+1.93)	217.11±0.129** (-15.89)
MCH (pg/cell)	C	71.18±0.141	62.75±0.157	67.89±0.176	59.82±0.147	65.28±0.162	63.09±0.174
	T	75.12±0.182* (+5.53)	59.78±0.159* (-4.73)	72.07±0.132* (+6.15)	57.41.±0.174* (-4.02)	69.10±0.162* (+5.85)	60.03±0.183* (-4.85)
MCHC (g 100 mL^{-1})	C	27.43±0.128	24.03±0.181	27.93±0.147	23.00±0.181	28.09±0.154	24.43±0.128
	T	28.58±0.183* (+4.19)	25.25±0.121* (+5.07)	28.95±0.164* (+3.65)	26.14±0.128** (+13.65)	29.17±0.117* (+3.84)	27.65±0.164** (+13.18)

Values are Mean + SE of 5individual observations
Values in parentheses denote percent increase (+) or decrease (-) over control
* *Highly significant p<0.001: * Significant p<0.05

Table 4: Variations in the haematological parameters following exposure to one tenth of the 96hLC50 concentration of Cr^{3+} as Chromium trioxide and Cr^{6+} as potassium dichromate in freshwater teleost fish *Labeo rohita*

Parameter	Treatment	Exposure period(hours)					
		24		48		96	
		Cr^{3+}	Cr^{6+}	Cr^{3+}	Cr^{6+}	Cr^{3+}	Cr^{6+}
Red blood cell (10^6 mm^{-3})	C	1.191±0.118	1.379±0.132	1.223±0.172	1.387±0.163	1.241±0.182	1.408±0.184
	T	1.216±0.146* (+2.00)	1.246±0.185* (-9.64)	1.261±0.153* (+3.10)	1.219±0.174** (-12.11)	1.309±0.139* (+5.47)	1.145±0.149** (-18.69)
White blood cell (10^3mm^{-3})	C	20.91±0.182	20.91±0.116	21.16±0.163	21.16±0.183	21.27±0.129	21.27±0.187
	T	19.56±0.171* (-6.45)	19.17±0.185* (-8.32)	19.14±0.191* (-9.54)	18.89±0.147** (-10.72)	18.43±0.121** (-13.35)	17.48±0.137** (-17.81)
Haemoglobin (g 100 mL^{-1})	C	7.46±0.127	8.42±0.162	7.69±0.138	8.51±0.185	7.86±0.145	8.65±0.172
	T	7.81±0.182* (+4.69)	7.34±0.136* (-12.82)	8.18±0.192* (+6.37)	7.23±0.166** (-15.04)	8.74±0.147** (+11.19)	6.94±0.139** (-19.76)
Hematocrit (%)	C	33.51±0.118	35.12±0.162	33.59±0.183	35.84±0.127	33.68±0.182	36.39±0.172
	T	34.45±0.192* (+2.80)	29.44±o.183** (-16.17)	35.12±0.153* (+4.55)	29.17±0.171** (-18.61)	36.47±0.164* (+8.28)	28.48±0.149** (-21.73)
MCV (μ^3m)	C	281.36±0.172	254.67±0.137	274.65±0.176	258.39±0.191	271.39±0.152	258.4±0.1585
	T	283.30±0.168* (+0.68)	236.27±0.147* (-7.22)	278.50±0.184* (+1.40)	239.29±0.159* (-7.39)	278.60±0.152* (+2.65)	248.73±0.182* (-3.76)
MCH (pg/cell)	C	62.63±0.154	61.05±0.167	62.87±0.125	61.35±0.182	63.33±0.161	61.43±0.147
	T	64.22±0.116* (+2.53)	58.9±0.138* (-3.52)	64.86±0.162* (+3.16)	59.31±0.171* (-3.32)	66.76±0.118* (+5.41)	58.86±0.183* (-4.18)
MCHC (g 100 mL^{-1})	C	22.2±0.1776	23.97±0.158	22.89±0.149	23.74±0.125	23.33±0.171	23.77±0.148
	T	22.67±0.116* (+1.84)	24.93±0.127* (+4.00)	23.29±0.163* (+1.74)	29.17±0.192** (+22.87)	23.96±0.157* (+2.70)	24.36±0.147* (+2.48)

Values are Mean + SE of 5individual observations
Values in parentheses denote percent increase (+) or decrease (-) over control
* *Highly significant p<0.001: * Significant p<0.05

In the present study haematological profiles present a very interesting picture. (Tables 3-5) Fish exposed to one fifth Cr^{3+} registered an increase in respect of TEC, haemoglobin percent and hematocrit. The percent increase in the count was found to be 2.89 at 24h, 4.22 at 48h, and 8.36 at 96h of exposure with the values at 1.246±0.142, 1.357±0.142, and 1.489±0.121 (10^6mm^{-3}), respectively. A similar trend of increase was noticed in fish exposed to one tenth of 96h LC50 concentration of Cr^{3+} with values recorded at 1.216±0.146, 1.261±0.153, and 1.309±0.139 (10^6mm^{-3}) at 24h, 48h and 96h, respectively and with a percent increase of 2.00, 3.10 and 5.47. Fish exposed to a specified concentration (15mg/L) has registered a percent increase of 4.20, 6.14 and 11.15, respectively at 24h, 48h and 96h, with values of 1.461±0.137, 1.502±0.142 and 1.589±0.125 (10^6mm^{-3}).

Table 5: Variations in the haematological parameters following exposure to specified 96hLC50 concentration of Cr^{3+} as Chromium trioxide and Cr^{6+} as potassium dichromate in freshwater teleost fish *Labeo rohita*

Parameter	Treatment	Exposure period (hours) 24		48		96	
		Cr^{3+}	Cr^{6+}	Cr^{3+}	Cr^{6+}	Cr^{3+}	Cr^{6+}
Red blood cell (10^6 mm^{-3})	C	1.402±0.127	1.402±0.132	1.415±0.163	1.415±0.161	1.426±0.147	1.426±0.128
	T	1.461±0.137* (+4.20)	1.173±0.116** (-16.33)	1.502±0.142* (+6.14)	1.143±0.158** (-19.22)	1.589±0.125** (+11.15)	1.013±0.173** (-28.96)
White blood cell (10^3mm^{-3})	C	21.35±0.181	21.35±0.162	21.58±0.148	21.58±0.137	21.76±0.146	21.76±0.151
	T	18.96±0.126** (-11.19)	18.09±0.172** (-15.26)	18.65±0.181** (-13.57)	17.73±0.174** (-17.84)	18.34±0.142** (-15.71)	16.23±0.129** (-25.41)
Haemoglobin (g 100 mL^{-1})	C	8.87±0.118	8.87±0.183	8.95±0.131	8.95±0.153	9.08±0.122	9.08±0.127
	T	9.96±0.146** (+12.28)	7.03±0.172** (-20.74)	10.32±0.184** (+15.30)	6.78±0.138** (-24.80)	11.91±0.173** (+31.16)	6.31±0.159** (-30.50)
Hematocrit (%)	C	36.18±0.132	36.18±0.147	36.24±0.117	36.24±0.162	36.47±0.142	36.47±0.172
	T	38.23±0.171* (+5.66)	27.31±0.164** (-24.51)	38.96±0.118* (+7.50)	25.71±0.127** (-29.05)	41.24±0.174** (+13.07)	22.07±0.159** (-39.48)
MCV (µ^{3}m)	C	258.05±0.125	258.05±0.172	256.11±0.181	256.11±0.143	255.75±0.138	255.75±0.176
	T	261.67±0.171* (+1.40)	232.82±0.163* (-9.77)	259.38±0.128* (+1.27)	224.93±0.137** (-12.17)	260.18±0.129* (+1.73)	217.86±0.147** (-14.81)
MCH (pg/cell)	C	63.26±0.147	63.26±0.129	63.25±0.162	63.25±0.142	63.67±0.174	63.67±0.168
	T	68.17±o.183* (+7.76)	59.93±0.176* (-5.26)	68.70±0.154* (+8.61)	58.88±0.128* (-6.90)	74.95±0.149** (+17.71)	62.29±0.135* (-2.16)
MCHC (g 100 mL^{-1})	C	24.51±0.162	24.51±0.137	24.69±0.128	24.69±0.174	24.89±0.122	24.89±0.171
	T	26.05±0.128* (+6.28)	25.74±0.153* (+5.01)	26.48±0.173* (+7.24)	26.17±0.155* (+5.99)	26.45±0.172** (+6.26)	28.59±0.128** (+14.86)

Values are Mean + SE of 5individual observations
Values in parentheses denote percent increase (+) or decrease(-) over control
* *Highly significant p<0.001; * Significant p<0.05

Fish exposed to one fifth of LC50 concentration of Cr^{3+} registered decline in the WBC count. The values were found to be 19.43±0.152, 19.07±0.127and 17.69 ± 0.152 (10^3mm^3), respectively for 24h, 48h and 96h, respectively with a percent decrease of 9.12, 11.54 and 18.62 over the period of study. A similar trend of decline throughout the period of study was noticed in fish exposed to one tenth of 96h LC50 concentration of Cr^{3+}. The percent decline was of the order 6.45%,9.54-% and 13.35% following 24h, 48h and 96 h of exposure with values recorded at 19.56±0.171, 19.14±0.191 and 18.43±0.121($10^3 mm^3$). Fish exposed to a specified concentration of Cr^{3+} (15mg) as well exhibited a declining trend in respect of WBC count. The values were found to be 18.96±0.126, 18.65±0.181and 18.34±0.142($10^3 mm^3$), following 24h, 48h and 96h of exposure with a decline of 11.19%, 13.57% and 15.71%.

Hb concentration in respect of fish exposed to one fifth of the 96hLC50 concentration of Cr^{3+} registered an increase registering values at 9.36±0.162, 9.78±0.137 and 10.29±0.143 ($g100mL^{-1}$) at 24h, 48h and 96h respectively with a percent increase over the control of 8.58, 10.63 and 14.71. A trend on the same line was observed in respect of fish exposed to one tenth of 96h LC50 concentration of Cr^{3+}. The percent increase was found to be 4.69, 6.37 and 11.19, respectively at 24h, 48h, and 96h of exposure over their control counterparts. Fish exposed to a specified concentration of Cr^{3+} as well recorded a % increase of 12.28, 15.30 and 31.16with values recorded at 9.96±0.146, 10.32±0.184 and 11.91±0.173 ($g100mL^{-1}$) at 24h, 48h, and 96 h of exposure.

Hematocrit determination as well registered an increase in values following exposure to one fifth, one tenth and even at the specified concentration. The values were found to be 32.74±0.142, 33.78±0.126 and 35.27±0.731 (%) respectively for fish exposed to one fifth of the 96h LC50 concentration with a % increase of 4.20, 6.72 and 10.46 over their respective control counterparts.

MCV, MCH and MCHC values were all found to be on the increasing side following exposure to one fifth, one tenth and even at specified concentration of 96h LC50 concentration of Cr^{3+}. The values were found to be 262.76±0.137, 248.93±0.159 and 236.87±0.148 ($\mu^3 m$) with a percent increase of 1.27, 2.40 and 1.93 for MCV in respect of fish exposed to one fifth of the 96h LC50 concentration of Cr^{3+} over their control counterparts over the entire period of study. The values were found to be 64.22±0.116, 64.86±0.162 and 66.76±0.118 (pg/cell) in respect of MCH with a percent increase of 2.53, 3.16and 5.41 following exposure to one tenth of 96h LC50 concentration of Cr^{3+}. The specified concentration of Cr^{3+} as well registered increased values for MCHC registering values at 26.05±0.128, 26.48±0.173 and 26.45±0.129 ($g100mL^{-1}$) with a percent increase of 6.28, 7.24 and 6.26, respectively at 24h, 48h and 96h over their control counterparts.

Fish exposed to Cr^{6+} exhibited a different pattern. All the haematological indices except MCHC were found to decline following exposure to one fifth, one tenth of 96hLC50 concentration of Cr^{6+}. A similar trend was noticed as well for fish exposed to the specified concentration of Cr^{6+} (15mg/L). The RBC count registered a percent decline of 14.68, 18.16 and 26.18 over their control counterparts registering values at 1.191±0.125, 1.153±0.136and 1.046±0.152 ($10^6 mm^{-3}$) at 24h,48h and 96h of exposure to one fifth of the 96hLC50 concentration. The percent decrease was observed to be 9.64, 12.11 and 18.69 in respect of fish exposed to one tenth of the LC50 concentration of Cr^{6+} and 16.33, 19.22, and 28.96 in the case of fish exposed to a specified concentration of Cr^{6+} over their respective control counterparts.

The pattern for Haemoglobin and hematocrit as well showed a profound decline of these parameters in fish following exposure to one fifth of 96hLC50 of Cr6+concentration with a percent decline of 18.72, 21.47 and 29.75 over their respective control counterparts for haemoglobin, and the percent decline was found to be 22.66, 27.45 and 37.91 for hematocrit The same was found to be the case in respect of fish exposed to one tenth of the 96h LC50 concentration of Cr^{6+}. The values were found to be 29.44±0.183, 29.17±0.171 and 28.48±0.149% at 24h, 48h and 96h of exposure eliciting a percent decrease of 16.17, 18.61 and 21.73 over their control counterparts. Fish following a specified concentration of Cr^{6+} recorded a percent decline of 24.51, 29.05 and 39.48 registered values at 27.31±0.164, 25.71±0.127 and 22.07±0.159% for the period of study over their respective control counterparts. MCV and MCH values were all found to have decreased phenomenally in the case of fish following exposure to one fifth and one tenth of the 96h LC50concentration and the specified concentration as well. The declining trend in respect of Cr^{6+} exposed fish was found to be quite interesting and to be in conformity with the studies on several of the heavy metals studied.

Earlier works also reported a fall in RBC count, haemoglobin percent and packed cell volume (PCV) and decrease in MCH, MCHC and MCV in freshwater fishes exposed to cadmium, zinc and nickel indicating anaemia, erythropenia and leucopoiesis [38,48]. However fish exposed to Cr^{3+} offered a differing picture of increase in all haematological parameters. The decrease in the case of haematological parameters except MCHC in the case of fish exposed to Cr^{6+} and the increase in these all parameters in the case of fish exposed to Cr^{3+} were all found to be dose dependant and temporal related; the fish exposed to Cr^{6+} eliciting all the more an enhanced toxicity effect suggesting differential toxicity effect of the valence states. The toxicity of metals has been studied in a variety of fish and numerous chemical and biological parameters have been examined. One such parameter is the haematology which gives valuable information on the physiological reactions of a fish to changes in the external environment [7]. Haematological studies conducted to determine the effect of hexavalent chromium on fish physiology showed increases in hematocrit and haemoglobin levels [37,40,47]. The TEC, haemoglobin per cent and hematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH)and Mean cell haemoglobin concentration have all declined in *Labeo rohita* exposed to Cr^{6+} in both the one fifth and one tenth of the 96hLC50. However the decline was more pronounced in fish exposed to one fifth concentration. In Cr^{3+} exposed fish in both one fifth and one tenth concentrations all the haematological parameters increased except WBC, and the increase was found to be more pronounced in fish following exposure one fifth of the 96hLC50 concentration than those exposed to one tenth concentrations. In both the Cr^{3+} and Cr^{6+} exposed fish the WBC count decreased appreciably, the decline more pronounced following exposure to Cr^{6+}.

This may be due to an anaemic state of the fish which could be possibly due to iron deficiency and its consequent decreased utilization for haemoglobin synthesis. This may be compared with a similar study on *Labeo rohita*, which also reported hypochromic microlytic anaemia under lead chloride stress [23]. Decrease in TEC, haemoglobin per cent, hematocrit and MCH were also reported in *Channa punctatus* exposed to both copper and chromium and this decrease is more pronounced in fishes exposed to chromium suggesting that the metal induces acute anaemia under toxic conditions [31].

MCV gives an indication of the status (or the size) of red blood cells and reflects a normal or abnormal cell division during the formation of red blood cells [28]. Statistically significant decreases in MCV indicate shrinkage of the red blood cells. This could be a result of hypoxia, stress or impaired water balance, microcytic anaemia or a large concentration of immature red blood cells that have been released from the erythropoietic tissue. A significant increase in MCV in fish exposed to Cr^{3+} indicates that the red blood cells have swollen due to hypoxia, osmotic stress or macrocytic anaemia [28].

A decrease in the total white blood cell count in fish exposed to both Cr^{3+} and Cr^{6+} may be the result of increased secretion of corticosteroid hormones [12]. The secretion of these hormones is considered to be a non-specific response to any environmental stressor and is a fundamental mechanism in the increased susceptibility of fish to disease when they are suffering from stress due to pollution [50]. Such a mechanism may be operative in chromium – induced decline in WBC count. Haemoglobin is the respiratory pigment of red blood cells that combines with O_2 and CO_2 and acts as a buffer. Haemoglobin is therefore closely associated with red blood cell counts and haematocrit. An increase in haemoglobin concentration observed in fish exposed to Cr^{3+}could be a mechanism whereby the body attempts to absorb more oxygen from the surrounding medium to meet the increased oxygen demand [9,46] ascribes this increased oxygen demand as indicative of impaired gill function or a rise in metabolic rate.
In the present study, the anaemia could be thought of as probably due to structural alterations of heme resulting in altered haemoglobin synthesis. Further the inhibitory effect of chromium on the enzyme system in the synthesis of haemoglobin cannot be ruled out as suggested in earlier studies [24]. Fish exposed to one fifth and one tenth of 96h LC50 concentration has clearly established that hexavalent chromium is more toxic than trivalent chromium and induced cumulative deleterious effects at various vital functional sites like, haematological indices and biochemical profiles.

Haematological studies conducted to determine the effect of hexavalent chromium on fish physiology showed increases in hematocrit and haemoglobin levels following exposure to both one tenth and one fifth 96hLC50 concentrations of Cr^{3+}. This finding can be corroborated with the observations of [37,40,47].
In the present study, marked increase in RBC count, Haemoglobin, Hematocrit, MCV , MCH, and MCHC resulted in fish exposed to Cr^{3+} while the these parameters except MCHC recorded decrease following exposure to Cr^{6+} denoting enhanced impact. WBC in both the cases registered lowered values. The

series of experiment where same concentration (15mg/L) of Cr^{6+} and Cr^{3+} was used clearly established that the valence state of chromium rather than concentration has an important role to play in several of the metabolic activities. Influence of Cr^{6+} has been well established as exemplified by the remarkable changes in the cellular constituents of blood in fish more than Cr^{3+}.

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