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Impact of Tannery Effluent, Chromium on Hematological Parameters in a Fresh Water Fish, *Labeo Rohita* (Hamilton)

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Abstract

Chromium is one of the heavy metals present in the Tannery effluent. Chromium is known to cause various health effects. The health hazards associated with exposure to chromium are dependent on its oxidation state. The hexavalent form is toxic than trivalent form. The hematological alterations produced on exposure to sub-lethal concentration (1/10th of LC_{50}/96 hrs) of chromium were investigated in fresh water fish, Labeo rohita for 7days and 30 days respectively. Results revealed statically significant decrease in RBC, Hb, PCV, MCH, and MCHC in all the experimental animals when compared to the control with an increase in exposure days. In contrast to this, the WBC and MCV values were significantly increased. The decrease in hematological parameters clearly indicates that the exposed fishes have become anemic due to heavy metal exposure.

Keywords: Tannery effluent, chromium, labeo rohita, hematological parameters.

Introduction

Pollution has become a serious threat and has brought drastic changes and ill-effects to the growing population as well as the mother Earth. Pollution of aquatic eco-systems is a major global problem since the past two decades. Rapid urbanization and industrialization has led to increased disposal of pollutants such as heavy metals, radio nuclides and various types of organics and inorganics into the aquatic environment. Extensive industrialization has measurably influences the quality of water of lakes, ponds and rivers all over the world. Industrial wastes constitute the major source of metal contamination of the aquatic environment[1].

Heavy metals constitute the major contaminants. Metals are important pollutants, because they are not eliminated from the aquatic ecosystems by natural processes like organic pollutants and are enriched in mineral organic substances. Metal contaminants are introduced into aquatic system through smelting process, effluents, sewage and leaching of garbage which cause serious damage to the aquatic fauna[2,3]. The Tannery industry has shown tremendous expansion during the last 25 years and plays an important role in India's foreign exchange earnings. However it adds pollutants to the aquatic environment. The tannery waste waters continue to cause negative effects on the aquatic organisms as they also have endocrine disruption effects. Tanners use large number of chemicals during the process, discharging toxic wastes into rivers and thereby degrading agricultural lands. The uncontrolled release of tannery effluents increases health risks to target and non-target organisms.

Tannery effluents are considered to be more dangerous than all the other industrial wastes as pointed out by Arora H.C[4]. The major constituent of tannery effluent is Chromium, a heavy metal which is highly toxic to aquatic fauna[5]. Chromium is an important pollutant from tannery effluent and causes deleterious effects on non-target organisms resulting imbalance of an ecosystem. Chromium compounds are used in ferrochrome production, pigment production, electroplating and tanning. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms[6,7]. Fish is an important component of human nutrition, and those from contaminated sites present a potential risk to human health. Metals can accumulate in aquatic organisms including fish and persist in water and sediments. Fishes are the simple and reliable biomarkers of pollution of aquatic bodies. When fish are exposed to elevated levels of metal in a polluted aquatic ecosystem, they tend to take these metals up from their direct environment[8]. The blood parameters have been used as a sensitive indicator of stress in fish exposed to different water pollutants, toxicants and effluents etc. Hematological and biochemical profile in fish is a sensitive index for evaluation of fish metabolism under metallic stress. Blood is a good bio- indicator to study the problem in organ function. The measurement of hematological changes of fish under exposure to any toxicant may be used to predict its effect upon chronic exposure. The blood parameters get affected on account of metal toxicity. The oxygen carrying capacity of blood, RBC in fishes often varies with life history, habits and environmental conditions. In recent years, hematological variables were used more when clinical diagnosis of fish physiology was applied to determine the external stressors and toxic substances as a result of close association between the circulatory system and external environment[9]. Hence the objective of the present investigation is to determine the changes in different hematological parameters of the fresh water

fish, *Labeo rohita* exposed to sublethal concentration of chromium.

Material and Methods

Test Animal: Healthy live specimens of *Labeo rohita* (average length 10±2cms and average weight 15±2gms) were obtained from local ponds and transported to the laboratory treated with 0.05% $KMnO_4$ solution for 2 minutes to avoid dermal infection, kept in large cement tanks and supplied with clean de-chlorinated tap water. In laboratory the fish were acclimatized for about 2 weeks prior to the experiment with a photoperiod of 12:12 light and dark cycle with constant aeration and filtration. During the period of acclimatization, the fish were fed with commercial fish feed to satiety twice daily. The laboratory tap water was analyzed for physico-chemical parameters by adopting standard methods[10].

Test Chemical: Analytical grade Chromium as Potassium di chromate supplied by BDH (India) was used as a metal toxicant throughout the experiment.

Experimental design: Fishes were divided into 3 groups containing 10 fishes each with the I group serving as control without any treatment, the group II, III fish were exposed to sublethal concentration ($1/10^{th}$ of LC_{50} 96hrs, 10ppm) of Potassium dichromate for 7days and 30 days after determining LC50 value[11]. After stipulated period, the control and experimental fishes were washed with tap water and dried using blotting paper before collecting the blood sample. Blood was drawn by cardiac puncture using $2cm^3$ disposable plastic syringe and 21 gauge disposable needle. After collection the blood was immediately transferred to glass vials containing 1% EDTA solution. Blood samples were used for the measurement of RBC (Erythrocyte), WBC, PCV (Haematocrit), Hemoglobin (Hb) concentration. The methods employed for determination of different hematological parameters were RBC and WBC count by Neubauer's improved Hemocytometer using diluting fluids[12], PCV by Wintrobe's method and Hb using Sahli's hemoglobinometer. The haematological Indices, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using the formula of Baker F.J. and Silverton R.E. given below[13] given below:

$$\text{Mean Corpuscular Volume (MCV) } \mu m = \frac{\text{Haematocrit (\%) x 10}}{\text{Erythrocyte count (mm3)}}$$

$$\text{Mean Corpuscular Haemoglobin (MCH) pg} = \frac{\text{Haemoglobin (g\%) x 10}}{\text{Erythrocyte count (mm3)}}$$

$$\text{Mean Corpuscular Haemoglobin Concentration (MCHC) \%} = \frac{\text{Haemoglobin (g\%) x 100}}{\text{Haematocrit (\%)}}$$

Statistical Analysis: All measurements were performed in average of three replicates. Data obtained was analyzed using the SPSS/PC+ Statistical package (ver.11.5). Significant difference between control and experimental groups were determined using Duncan's test for multiple range comparisons[14]. Results were considered as statistically significant at 95% confidence level ($p<0.05$).

Results and Discussion

In the present study the Physico-chemical parameters of water such as pH, Dissolved Oxygen, and Alkalinity etc., were presented in table-1. The exposure of fish, *Labeo rohita* to sublethal concentration of Potassium dichromate for 7days and 30 days caused significant alterations in hematological parameters are represented in table-2. The results reveal that the Red blood corpuscels (RBC), Haemoglobin (Hb), Haematocrit (PCV), MCH, and MCHC values were significantly decreased after 7days and 30days exposure periods when compared to control, being statistically significant ($p<0.05$). On contrast to this, the White blood cell (WBC), and MCV values were found statistically significant increase ($p<0.05$) in experimental fish when compared with control.

Table-1
Physico-chemical parameters of Tap water used in the Laboratory for sub lethal tests

Parameter	Values
pH	7.6±0.1
Temperature	27±2ºC
Dissolved Oxygen	6.52±0.2 mg/lit
Alkalinity	32.5±1.5 mg/lit
Turbidity	0.22±0.02 mg/lit
Salinity	0.25±0.05 mg/lit
Free Carbondioxide	2.35±0.04 mg/lit

The fishes exposed to sublethal concentrations of heavy metal chromium in the present investigation showed remarkable hematological alterations. Hematology is used as an index of fish health status in number of fish species to detect different stress conditions like diseases, hypoxia, and exposure to metals and pollutants etc.[15,16]. The statistically significant decrease ($p<0.05$) is not uncommon in fish exposed to sublethal concentrations of metals, toxicants and therapeutic agents. The general reduction in blood parameters is an indication of anaemia.

The heavy metal induced significant decrease in RBC, Hb and PCV. The RBC count coupled with low haemoglobin content may be due to destructive action of pollutants on erythrocytes. Our results are in good agreement with the earlier works reported[17,18]. The decrease in haemoglobin concentration indicates the fish inability to provide sufficient oxygen to the tissues[19]. In support to our present work, it is found that there was a decline in the values of RBC, Hb and PCV in fishes exposed to combined metal solution[20]. Significant decrease in

the RBC, Hb and PCV of fishes exposed to heavy metals was also noticed[20,21]. A specific toxic effect on fish blood and tissues occurs due to various heavy metals and toxins which enters the aquatic environment. Prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants[22]. Our results are in good concurrence with the earlier works of Buckley *et al*., and Palanisamy *et al*.[23,24]. The alterations in these hematological indices may be due to a defence reaction against toxicity through the stimulation of erythropoises.

The anemic conditions in fish may be detected using haematocrit [25]. The PCV values always decrease when a fish loses appetite or become diseased or stressed. At present, the distinct decrease in the level of Haemoglobin and PCV after exposure to heavy metal chromium clearly suggests a haemodilution mechanism possibly due to gill damage or impaired osmoregulation. The haemodilution has been interpreted as a mechanism that reduces the concentration of the irritating factor in the circulatory system[26]. Our result are in line with Smit *et al.*[27] that heavy metal exposure results in the decrease in RBC count, Hb and PCV values are due to the impaired intestinal absorption of iron. The increase in WBC in the present study has been attributed to several factors like increase in thrombocytes, lymphocytes or squeezing of WBC's in peripheral blood. Increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of fishes exposed to toxicant. High WBC counts indicate damage due to infection of body tissues, severe physical stress as well as Leukemia. Similar increase was reported by Banerjee and Banerjee[28] in Channa punctatus due to Copper sulphate and Potassium dichromate induced toxicity[29,30] in Channa punctatus exposed to Copper.

The erythrocyte constants MCV, MCH, and MCHC offer relationship on size, form and Hb constants of erythrocytes. They allow the determination of morphological anaemia that whether Normocyte, Macrocyte or Microcytic anaemia. The alterations in the haematological indices i.e. increase in MCV and decrease of MCH and MCHC in the present study may be due to a defense against the toxic effect of chromium and in turn due to decrease in RBC's, Hb and PCV and the disturbances occurred both in metabolic and haemopoitic activities in fish. Increase in MCV and WBC count suggests that the anemia is of macrocytic type[31]. The MCV gives an indication of the status of size of Red blood cell and reflects an abnormal or normal cell division during erythropoises. The increase in MCV may be attributed to the swelling of erythrocytes as a result of Hypoxic condition or Osmotic stress or Macrocytic anaemia in fishes exposed to metal pollution[32.] Our results are in line with the findings of Larsson *et al.*[33]. The decrease in MCH and MCHC in the present study clearly indicates that the concentration of hemoglobin in RBC is reduced. The MCH is a good indicatior of Red Blood Cell swelling [34]. The significant decrease in the MCHC values in the present study may be due to swelling of RBC or decrease in hemoglobin synthesis. The present results are in line with the previous findings of Ovie Kori-Siakpere [35].

Table-2
Haematological parameters of control and experimental fresh water fish, *Labeo rohita* exposed to Tannery effluent, Chromium

Haematological parameters	Control	Experimental	
		7 days	30 days
RBC (cumm)	1.27±0.012	1.22±0.009* (-3.937)	1.08±0.008* (-14.961)
WBC (cumm)	6.18±0.011	7.63±0.007* (+23.463)	9.47±0.008* (+53.236)
Hb (%)	4.2±0.063	3.9±0.105* (-7.143)	3.0±0.052* (-28.571)
PCV (%)	10.7±0.075	10.3±0.089* (-3.738)	9.2±0.105* (-14.019)
MCV (cuμ)	84.38±0.010	85.08±0.073* (+0.829)	86.11±0.008* (+2.050)
MCH (pg)	32.81±0.008	32.23±0.009* (-1.768)	27.78±0.012* (-15.331)
MCHC (%)	38.88±0.011	37.87±0.008* (-2.598)	32.25±0.009* (-17.052)

Values are expressed as Mean ± SD (n=6), * = Significant at $p<0.05$ level, Values in Parenthesis indicates percent change over control.

Conclusion

Thus it is concluded that the present study clearly indicates that chromium, a toxic heavy metal discharge via effluents into aquatic environments caused severe anemia and alterations in hematological indices in the fresh water fish, *Labeo rohita*.

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