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Review Article

Effect of Chromium Exposure on the Antioxidant Enzymes Activity and Haematological Indices in *Labeo rohita*

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Abstract: Chromium is one of the most common elements in the earth's crust and is used in more than 50 different industries. There are three oxidation states in case of Chromium viz., Cr (II), Cr (III), Cr (VI). Among which Cr (II) is most unstable. Cr (III) and Cr (VI) are the stable oxidation state of Chromium in the environment. Being one of the commonly used metals Chromium and its particulates enter the aquatic medium through effluents discharged from different industries like textiles, tanneries, electroplating workshops, ore mining, dyeing, printing-photographic and medical industries. The discharge from these industries pollutes the waters and affects the biota. 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 LC50/96 hrs) of chromium were investigated in fresh water fish, *Labeo rohita*. *Labeo rohita* common name (Rohu) is a species of fish of the carp family, found in rivers of South Asia. This fish is commonly found in Pakistan, India, Nepal, Bangladesh, Burma, Thailand, China, Kampuchea and Sri Lanka. The rich source of high-quality protein filled with vitamins and omega-3 fatty acids encourage the human being to uptake fish as a major food source. Significant decrease in haematological indices RBC, Hb, PCV, MCH, and MCHC and 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: Chromium, Labeo rohita Fingerlings, Hematological parameters.

INTRODUCTION

Fish are one of the most important food resources and are considered as sources of the primary protein. The concentration of toxic metals in water and negatively affects fish health. These pollutants, which have a negative effect on fish, are released by agriculture, industrial wastewater discharge, raw sewage extraction, chemical waste, and oil spills due to fishing vessels [1]. The chromium concentration in the environment generated by weathering and secondary reactions is a silicate mineral associated with chromate [2]. Chromium concentrations in various environments range from 1 to 3000 mg/kg in soil, 5 to 800 µg / L in seawater, and 0.02 µg/L to 6.0 mg/L in groundwater [3,4]. Hematological parameters are used to effectively monitor the status of fish exposed to various types of toxicity in the aquatic environment [5]. Hematological parameters such as red blood cell count, hematocrit, and hemoglobin concentration are widely used indicators of fish health status under metal toxicity [6]. Hematological indicators, including enzymes, metabolites, nutrients, and inorganic ions, are used to determine cell damage and measure the response to heavy metal exposure [7]. Blood cortisol levels have been widely used as stress biomarkers in fish exposed to heavy metal [8, 9]. Heavy metals can show high toxicity even in low concentration producing cumulative deleterious effects in an aquatic ecosystem [10].

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Principle of Chromium

The principle ore of Chromium is Chromite, from which ferro-chrom alloys and chromium metal are obtained. The chemical formula of the ore is $FeO\cdot Cr_2O_3$ [11, 12]. The metal may be present in divalent (Cr^{+2}), trivalent (Cr^{+3}) and hexavalent form (Cr^{+6}) forms, Cr^{+3} and Cr^{+6} being the most predominant and stable forms [13]. In biological system, Chromium is usually found in the trivalent form [14], and this form (Cr^{+3}) is reported as an essential element in mammals as it takes effective role in glucose, lipid, and protein metabolism [15]. Due to poor membrane permeability, non-corrosiveness and very less tendency to biomagnify in the food chain, the toxicity of trivalent chromium is very low. Hexavalent chromium is considered to be more toxic than trivalent form because of its easy permeability through the cell membrane [12, 14]. Hexavalent Chromium has two main oxy-anion forms CrO_4^{-2} and CrO_7^{-2} which are involved in reversible transformation [16].

$$2\,CrO_4^{-2} + 2\,H_3O^{-} \leftrightarrow CrO_7^{-2} + 3H_2O$$

Labeo rohita common name (Rohu) is a species of fish of the carp family, found in rivers of South Asia. This fish is commonly found in Pakistan, India, Nepal, Bangladesh, Burma, Thailand, China, Kampuchea and Sri Lanka. Its body is deep and dorsal profile is more concave than abdomen. Blunt snout. Generally, one pair of small maxillary barbells is present and sometimes there is a second rostral pair is present. Lateral line scales are 40-42. Color of the body is bluish or brownish along the back and silvery on the sides and beneath. Usually a red mark is present on each scale [17]. In Pakistan, the fresh water reservoirs have been contaminated due to few contaminants counting overwhelming metals. The main reason of water pollution in Pakistan is the release of untreated industrial effluents that result in high level of pollution within the surface water as well as ground water. Despite metal levels reported for many industrial receiving waters, these metal contaminants get little research interest for their toxicity to freshwater fish species [18].

Pollution caused by heavy metals is a serious problem for the environment due to their toxicity, insistency, bioaccumulation, and bio magnifications equity. Heavy metal contamination in the environment results from different natural and anthropogenic sources. The anthropogenic sources include agricultural and industrial activities, combustion of fossil fuel and gasoline, mining [19]. Common indicators of oxidative stress are enzymes of the antioxidant defense system, which have the role of detoxification through the removal of free radicals and protecting the organism under stress these include superoxide dismutase, glutathione peroxidase and catalase. Chromium is one of the most common pervasive pollutants in the aquatic environment, but the pure metallic form is absent naturally [20]. Chromium has three oxidation states Cr (2+), Cr (3+) and Cr (6+). Among these Cr (2+) is most unstable while Cr (3+) and Cr (6+) are the stable oxidation state of Chromium in the environment. Chromium and its particulates enter the aquatic medium from different industries such as textiles, tanneries, ore mining, dyeing, and medical industries, as it is a commonly used metal. Its toxic form is Hexavalent chromium (+6) it can promptly passes cellular membranes and then reduced to trivalent (+3) form [21].

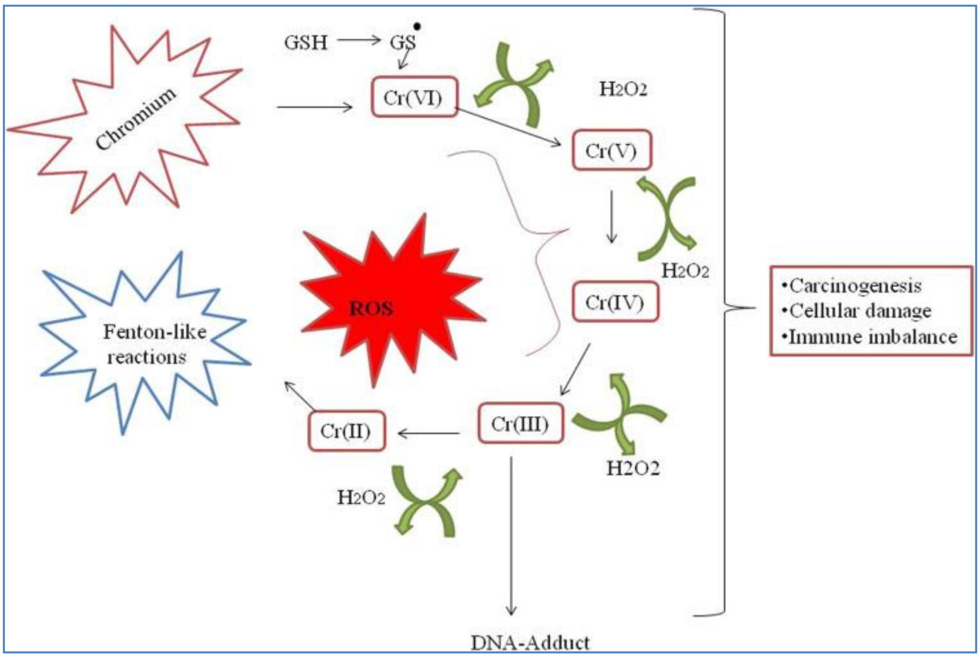

Fig-01: Hexavalent chromium overview [22]

Hematological alterations produced on exposure to sub-lethal concentration (1/10th of LC50/96 hrs) of chromium in fresh water fish, *Labeo rohita* for 7days and 30 days respectively. Results emitted statically significant decrease in RBC, Hb, PCV, MCH, and MCHC while the WBC and MCV values increased in all the experimental fishes when compared to the control with an increase in exposure days [23]. acute toxicity tests (96-hr LC50 and lethal concentration) of chromium (Cr) and cadmium (Cd) on two fish species viz. Channa marulius and Wallago Attu fingerlings for the determination of antioxidant enzymes activity. The results showed activity of superoxide dismutase increased with increasing metallic ion concentrations in the test mediums for both fish species while the activity of catalase and peroxidase decreased by increasing the concentration of metallic ions in the test mediums. The fish kept in control conditions showed maximum activity of catalase and peroxidase [24].

Effect on hematology and immune system

Immuno-haematological study on African mouth breeder (Oreochromis mossambicus) [25] That several phenomena like decrease in lymphocyte and leucocyte count, reduction in spleen weight, suppression in in vivo immune responses etc. are evident in hexavalent chromium exposure. Two freshwater fishes (Cyprinus carpio and Salmo trutta L.) have been exposed for 38 weeks to 1–10 μg/l of potassium dichromate to find out the influence of chromium on humoral immunity by O'Neill [26]. The primary and secondary humoral responses have been found to be diminished for MS2 bacteriophage in that experiment. In Salmo trutta, the primary antibody response has also been found to be diminished by 10%, whereas, in secondary antibody response the value rises by 50%. In carp, the serum proteins level has reported to be reduced by 25%. [27]. In the same study, common carp has appeared to be more sensitive to Chromium than trout. On the other hand, prolonged exposure to Chromium (VI) is shown to induce adaptability in fish. Haematological studies on chronically chromium (0.098 mg/l) exposed Tilapia sparrmanii have confirmed that no significant changes take place in leukocytes or erythrocytes counts but haemoglobin concentrations decrease significantly [28].

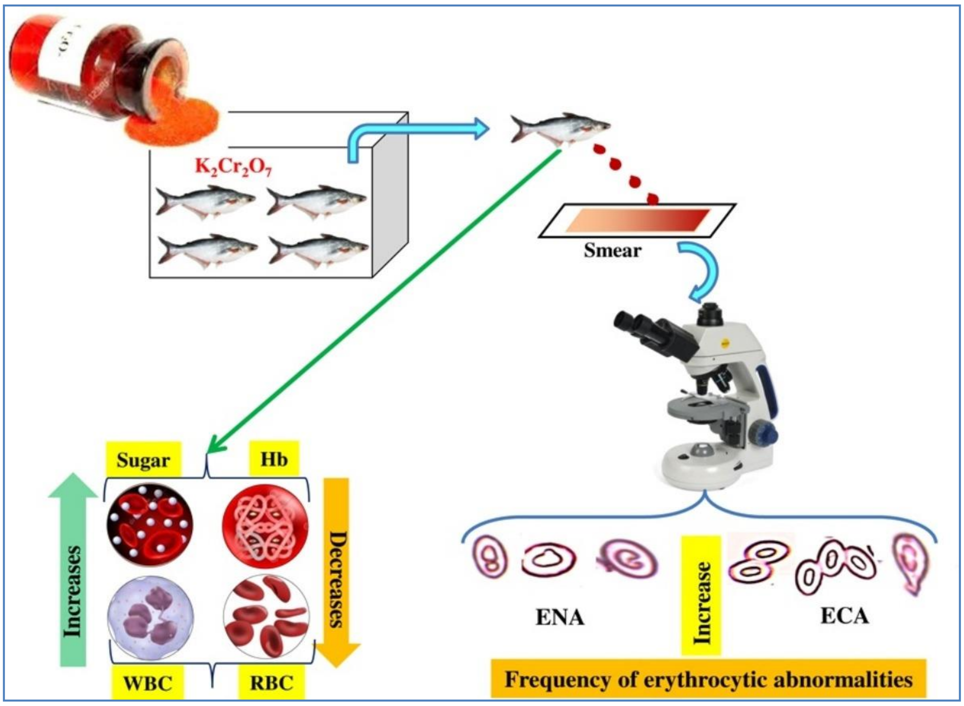

Fig-02: Acute effects of chromium on hemato-biochemical parameters [29]

Effect on enzyme activity

Long-term exposure of chromium exerts some dose-duration depended effects on different enzyme activities. Channa punctatus to 2.6 mg/l of the metal for 60–120 days to determine the activity of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), pyruvate dehydrogenase (PDH) on its different organs like kidney, brain, liver, gill, intestine and muscles. They have reported that the activity of LDH decreases significantly in liver and kidney in case of 60 days exposure. These lipid peroxides and hydroxyl radicals may cause cell membrane damage and thus destroy the cell [30]. Though, chromium is believed to be essential for some metabolic performances of living organisms but the ultimate necessity of chromium still remains as a debatable subject as huge number of laboratory studies have shown that, apart from various toxic effect of Cr (III), the trivalent chromium may also cause allergy, Some of the Cr (III) compounds have been reported to possess toxic even genotoxic effects for humans. It has also been reported that chromium possesses some feototoxic and embryotoxic effects. The metal may have some effects on reduction in implantation rate in case of exposed organisms [31]. It also exerts some effect on ovarian physiology and ovulation [32].

Chronic toxic effects of chromium

Long term exposure to hexavalent chromium exhibit several alterations in behavior, physiology, cytology, histology and morphology. Decrease in antibody production and lymphocyte count, reduction in spleen weight [33], DNA damage, decrease in Growth and survival rate [34], reduction in protein level, diminished humeral responses [28], increase in blood and muscle lactic acid [29], decrease in larval growth and embryo survival rate [25] and erosion in fin and fin-ray morphology [35] have been reported to be the major identified chronic effects of Chromium in different experimental conditions for different experimental fishes. Most of the aforesaid symptoms are found as concentration and duration dependent.

Effect on physiology and growth

The concentration of the metal seems to have an insignificant effect on growth. Increased concentration of chromium (from 24 to 120 µg and 54–266 µg/l) for 105–134 days exposure has been shown to affect both survival and growth rate significantly [36]. Physiological alterations are also identified after exposure to ≥120 µg/l of chromium. Phenomenon of DNA breakage has been testified after exposure to a concentration lower concentration of chromium at different pH values (0.2 mg/l at pH 6.5 and 2.0 mg/l at pH 7.8) induces mortality of embryo and mild problem in hatching [38]. Glycogen content in gill, liver and muscles of fish Labeo rohita has been reported to decline after hexavalent chromium exposure [39]. In Channa striatus, Chromium intoxication lowers the glycogen level in gill, liver and kidney altering some biochemical mechanisms of the fish [40].

Biomarkers of chromium toxicity in fish

Biomarkers are measurable indicators of some biological state or condition. There are several biomarkers of chromium intoxication in fresh water fishes at various investigatory levels. Stress proteins like metallothionine take longer time to express in case of chromium exposure at sublethal concentrations [41, 42]. In L. rohita, a 96h-LC50 exposure to a concentration of hexavalent chromium (39.4 mg/l) significantly declines the tissue glycogen, total protein, and total lipid content in liver, muscle, and gill tissues of the fish [39]. In case of L. rohita, the micronucleuss percentage has been found to range from 0.16 to 0.32 in control group; whereas, a maximum of 2.48% has been found in case of a sub-lethal (1/10th 96 h LC50) concentration after 60 days exposure [43]. On the other hand, H. fossilis has been found to adapt more successfully in chromium induced environment. The average value of micronuclei percentage has been found to be 2.208 ± 0.061 in case of a sub-lethal (1/10th LC50) concentration exposure [44].

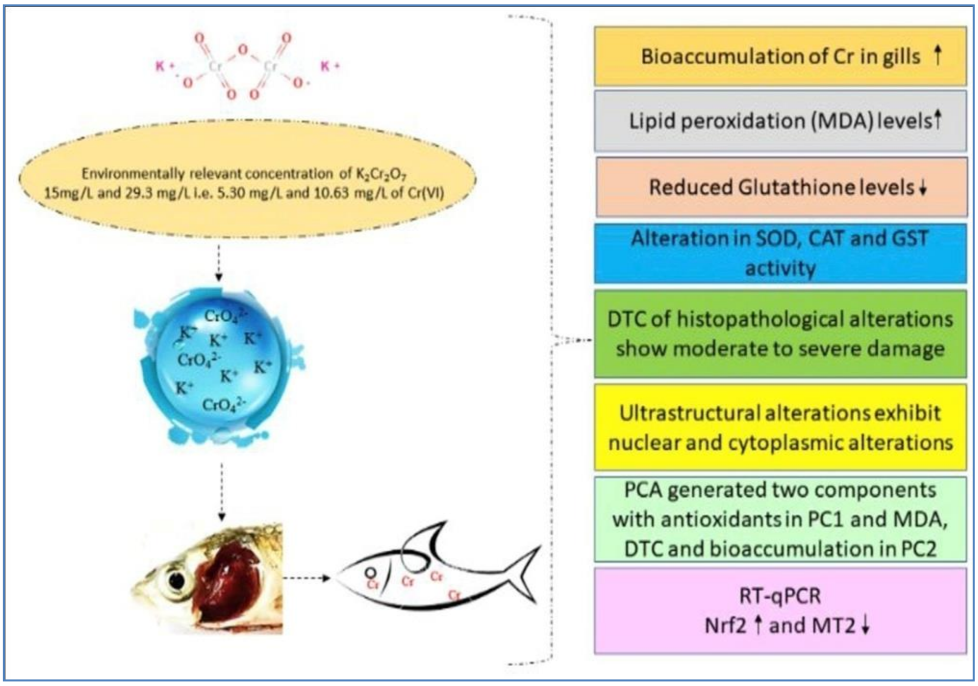

Fig-03: Hexavalent Chromium exposures induced toxic effect on antioxidant level and enzyme activity [46]

Table-01: Effect of chromium exposure, enzyme activity and haematological indices in different species of fishes.

Species	Experimental design	Results	References
Oreochromis niloticus	Twenty one fry tilapia *Oreochromis niloticus* 6-8 cm in length and weight 8-11g were divided into 3 groups each group consists of 7 fishes. Group 1 was exposed to 10 mg/L of cadmium chloride solution (CdCl2) in tap water. Group 2 exposed to 5mg/L of cadmium chloride solution in tap water for 7 days	Histological alterations on liver tissues were in form severe fatty vacuolations, generalised necrosis of hepatocytes, fatty changes, congestion of liver siusoids and central veins. Intestines showed severe congestion of submucosal blood vessels and sloughing of mucosal epithelium. Kidney showed severe glumerular shrinkage and necrosis, lymphocytic infiltration in the distal renal convoluted tubules.	[47]
Catla catla	A group (n=15) of fish were kept in metal exposed medium while control group was kept in metal free water. Chemically pure chloride compound of lead (Pb+2) was used to prepare the solution. The experimental fish *C. catla* was exposed to 96h LC50 concentration (31.25 mgL-1) of lead determined By for 4-day.	Results showed that the POD activity was augmented in all selected organs of Pb+2 exposed fish in relation to control. The POD activity in organs of fish followed the order: Brain>liver>gills>kidney>heart>muscle.	[48, 49]
Mystus vittatus	study the effect of sublethal concentrations (10% and 30%) of heavy metal, arsenic on the haematological profile, condition factor, hepatosomatic and gonadosomatic index of *Mystus vittatus* after exposure to 30 days. The LC**50** for arsenic trioxide for 96 hours was calculated using probit method and found was 3.20 ppm. The LC**50**values of arsenic for 24, 48, 72 and 96 hours were 4.71, 4.16, 3.68 and 3.25 ppm, respectively.	study shows that the haematological parameters *viz*, RBCs count, haemoglobin (Hb) and packed cell volume (PCV) along with haematological indices *viz*, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and granulocytes were significantly decreased simultaneously the WBCs count, agranulocytes and Clotting time (CT) was significantly increased with increasing concentration and exposure period. Arsenic decreases all the three parameters (CF, HSI and GSI) compared with control. Changes in haematological profile, CF, HSI and GSI might reflect anemia, metabolic and physiologic disturbances under the effect of metal.	[50]
Mystus vittatus	The healthy *M. vittatus* ranging from 7.0-8.0 cm in length and weighting 8.0-9.0 gm the effect of sublethal concentrations (10 and 30%) of heavy metal, arsenic on the triglyceride and cholesterol contents in liver and muscles of *Mystus vittatus* after exposure to 30 days.	A significantly decreased lipid and increased cholesterol content of liver and muscles tissues of arsenic induced *M. vittatus* suggested that lipid might have undergone lypolysis, and increased in cholesterol could be due to alteration of steroid biosynthesis during the stressful situation in the intoxicated fishes.	[51]
Labeo rohita	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 (1/10th of LC50 96hrs, 10ppm) of Potassium dichromate for 7days and 30 days	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.	[52]
Cyprinus carpio	the 96 h LC50 for MnSO4 was determined as 5.6 mg/l, and for of CrCl3 as 17.05 mg/l. Fish were exposed to two sub lethal concentrations of MnSO4 i.e. 1.12 mg/l, and CrCl3 i.e. 3.41 (20%, respectively of LC50 value). The carps were randomly distributed in three different glass tanks with a density of 16 fish per tank having 120 L of water. One tank was labeled as control group and the other two were	Bioaccumulation was highest in the gills followed by intestine > muscles > skin > bones. The concentration of hematocrit (HCT), hemoglobin (HGB), Red Blood Cells (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was significantly higher at 96 h ($P < 0.01$) after exposure to manganese and chromium, while the concentration of platelets	[53]

Species	Experimental design	Results	References
	labeled as treated groups. i.e. (Mn treated) and (Cr treated). Treated tanks were then exposed to the concentration of 1.12 mg/l for $MnSO_4$ and 3.41 mg/l for $CrCl_3$.	(PLT) and white blood cells (WBCs) was lower at 96 h of exposure. The highest concentration of serum cholestero, serum reatinine, low density lipid was observed at 24 h. Serum glutamic-oxaloacetic transaminase (SGOT) (19 ± 0.13), and serum albumin were at the highest level at 72 h (3.19 ± 0.07) ($P < 0.01$) post exposure.	
Labeo rohita	A total of 120 fresh water fish (*Labeo rohita*) having three months of age and about 200-225 body weight (gm) After 15 days of acclimatization period placed in eight groups (A-H). Each experimental group contained 15 fish. LC50 and lethal doses of different heavy metals s lead, cadmium, chromium and in combination (Pb +Cr), (Cr+Cd), (Cd+Pb) and (Pb+Cr+Cd) and their binary and tertiary mixtures for Labeo rohita was determined prior to start of experiments	Increased surface breathing, loss of coordination, rapid opercular movement, erratic swimming, air gulping, jerking movement and tremors were noted in fish. Histopathological observations of gills of various fish exhibited severe microscopic alterations like Disruption and disorganization of primary lamellae, disruption of cartilaginous core, congestion, necrosis of lamellar epithelial cells and infiltration of leukocytes in gills of fish exposed to heavy metals were observed.	[54,55]
Labeo rohita	four groups of fingerlings of *Labeo rohita* were exposed to different doses of $PbCl_2$ viz. 96-h, 2/3rd, 1/4th and 1/5th of LC50, separately, for 30 days	Significantly increased peroxidase activity was observed in gills and liver of *Labeo rohita* after exposure to $PbCl_2$ due to all doses as compared to the control. All results were statistically significant at $p<0.05$. Fish liver exhibited significantly ($p<0.05$) higher activity of enzyme than that of gills. The physico-chemical variables viz. pH, dissolved oxygen, carbon dioxide, total hardness, calcium, magnesium and total ammonia of the test media varied significantly at $p<0.05$, that exerted significant effects on peroxidase activities in gills and liver of fish.	[56]
Labeo rohita	the healthy fingerlings of *Labeo rohita* were divided in four groups, each group containing 10 fishes Group A : Control group, not exposed to any chemicals. Group B : exposed to 0.5 ml/L *Aloe vera* juice.Group C : Exposed to 0.5 ml/L *Aloe vera* Juice and 100 µg/ L arsenic. Group D: exposed to 100 µg/ L arsenic.	A significant decrease in enzymatic activity of GPT, GOT, ACP and ALP was noted in liver and muscle tissues. The level of protein, lipids and glycogen also decreased, whereas the amount of protease and free amino acids profoundly increased.	[57]
Labeo rohita	the fingerlings of Labeo rohita were divided in four groups containing 10 fishes in each group A) Control group: fed with fish pallets and was not exposed To any chemicals. B) Fish + Aloe vera juice: exposed to 0.5 ml/L Aloe vera juice. C) Fish + Aloe vera juice+Chromium: exposed to 0.5 ml/L Aloe vera Juice + 1.5 mg / L Chromium D) Fish + Chromium: 1.5 mg / L Chromium	A significant decrease in enzymatic activity as GPT, GOT, ACP & ALP was noted in liver and muscle tissues. The level of protein, lipids and glycogen were also found decreased, whereas protease and free amino acids were profoundly increased.	[58]

CONCLUSION

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***.** Trivalent chromium is essential component of different enzymes whereas, hexavalent chromium with the bio-membrane permeable capacity is found to have toxic impact on fresh water fishes. In case of acute exposure at 50% lethal concentration, fishes have been found to lose their body balance with restlessness, lowered breathing rate and higher rate of mucus secretion. Haematological alteration such as decreased haemoglobin percentage, decreased RBC count can be considered as biomarker.

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