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Effect of Heavy Metal Chromium on Haematological Parameters of *Clarias batrachus*

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Abstract

The toxic effects of chromium in edible fish are a source of worldwide concern. The purpose of this study was to keep track of chromium.The level of chromium toxicity in the exposed *Clarias batrachus* caused toxicity was determined by Haematological examination of blood. For 60 days, a sublethal dosage of chromium trioxide (1/10 concentration of 96 hr10 LC50) was administered. Fish were fed a specific diet at a rate of 2% body weight per day.

In whole blood, haemoglobin, haematocrit, erythrocyte, and leucocyte counts were performed in comparison to the control group, there was a 22.77 % decrease after 60 days of exposure. The number of white blood cells (WBCs), red blood cells (RBCs), and haemoglobin (Hb), as well as the time it takes for blood to clot, all decrease. Antibody production and ion-dependent ATPase activity have both been reduced. Glycogen, total lipid, and total protein levels are all dropping in the liver, muscle, and gills. The amount of glycogen in the liver decreases. Hyperglycemia response. The vitality of cells is dwindling. The activity of ALA-D (aminolevulinic dehydratase) rises. WBC, RBC, Hb, MCV, PVC, reactive oxygen species ROS, and bacterial susceptibility all increase when the spleen-to-body ratio rises. The blood glucose levels of the treated fish steadily increased after 24, 48, and 72 hours at various concentrations in the current study. There was a significant increase was observed only after 15 days ($p<0.01$) and ($p<0.001$) of exposure periods. However, after 15 days ($p<0.01$) and ($p<0.001$) of exposure durations, a substantial increase was detected.

Keywords: *Channa punctata*, chromium, Haematology.

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Introduction

Chromium is commonly found in its trivalent form (Langard *et al*.1979) and this form (Cr^{+3}) is thought to be an important element in mammals because it helps in glucose, lipid, and protein metabolism. Trivalent chromium has low toxicity due to its weak membrane permeability, non-corrosiveness, and low potential to biomagnify in the food chain. The physiological reaction of fish to changes in their external environment may be determined by a haematological analysis of their blood. Haematological variables are well-known for their clinical usefulness in prognosis and diagnosis, as well as the relative ease with which they may be sampled (Deshmukh, 2016).According to Dhara (2014), chromium (VI) binds to plasma proteins and participates in transportation after passing through the cell membrane via the sulphate ion channel. The metal then accumulates physiologically in many organs. The overall pattern of Cr(VI) distribution in fish: Avenant-Oldage (2000)Gills > Liver > Skin > Muscles. The bioconcentration of chromium in fish muscle, gills, and liver has been shown to rise with increasing medium concentration and exposure duration(Mallesh *et al*.2015).In an experimental setting, the liver is thought to be the major storage and detoxifying site for chromium, (Heath 1987). The bile of the experimental organism (*Clarias batrachus*) exposed to a metal-contaminated diet and environment had a greater content of metals. According to Gauglhofer and Bianchi (1991), this storage is mostly stabilised by protein or short peptide linkage, such as glutathione linkage. The principal route of chromium or its compound elimination in fish is through faeces Dhara (2014). It has been discovered that the pH range has a significant impact on the bioavailability of metals in fish. Abbasi *et al.* (1995) on teleost, *Nuria Henricus*, support this claim. Van Der Putte *et al.* (1981) showed that the pH of the surrounding water has a significant impact on Chromium buildup in the tissues of Rainbow trout (Salmo gairdneri). Hogendoorn-roozemond (1977) have also reported that gill contains more amount of chromium at pH-6.5 than other internal organs whereas, the reverse is evident at pH-7.8.Comparative studies have revealed that Chromium concentration remains higher in gill than in other organs at the same pH.

As a result, fish haematology is utilised to illustrate faulty physiological systems in fish (Adakole, 2012). In numerous fish species, haematology is used to determine their health state. Because blood is a sensitive sign of stress, piscine haematology is rising in popularity. Chromium (Cr) is an insoluble trivalent element that may be found in rocks, animals, plants, and soil. Intense industrialisation and other anthropogenic activities have resulted in the global presence of soluble Cr(VI), which is rapidly leached from soil to groundwater or surface water, in quantities exceeding acceptable values.

Cr(VI)ecotoxicity is connected to its capacity to have a range of negative impacts on biological systems, including fish. Cr(VI) poisoning is a danger to aquatic life in aquatic environments. This study discusses Cr environmental 's fate and transport, as well as its acute and chronic impacts on fish. Environmental fate and transport, as well as its acute and chronic impacts on fish, generate a variety of negative effects in biological systems. In aquatic habitats, Cr(VI) poisoning is a serious threat to aquatic life. This study looks at the fate and transit of environmental Cr, as well as its acute and chronic effects on fish.

Cellular, metabolic, and genetic toxicity of Cr(VI). This study attempts to make sense of the voluminous data on Cr(VI) toxicity in diverse fish species. Such information is immensely helpful to scientists and government authorities working in environmental contaminant risk assessment and management as a guide to the best course of action for restoring ecosystems and, as a result, preserving human health. Cr aquatic toxicity is influenced by both biotic and abiotic causes. The kind of species, age, and developmental stage are biotic variables. The abiotic variables are temperature, Cr concentration, Cr oxidation state, pH, alkalinity, salinity, and hardness of the water.

Furthermore, the sensitivity of a particular organism is determined by the metal's lethal and sub-lethal concentrations, as well as its speciation.

Materials and Methods

For a few days, *Clarias batrachus* were kept in aquaria to acclimate to laboratory settings. The fish were kept as control specimens in one tank and were fed and housed under identical conditions as the experimental fish, with the exception that they were not given the heavy metal compound dosage. The experimental toxicants were selected from an inorganic salt of the heavy metal chromium, potassium chromate.

Exposure intervals of 7, 15, 30, and 60 days were used in the trial. There were eight different experiments set up. The fifth set was administered a sublethal dosage (1/10 concentration of 96 hr 10 LC50) of chromium trioxide for 45 days to assess the chronic effects. Fish were fed a specific diet at a rate of 2% body weight each day. Heparinized needles were used to take blood from the caudal vessels of control and experimental fish. In whole blood, haemoglobin, haematocrit, erythrocyte, and leukocyte counts were determined. Erythrocytes are oval, small structures that range in diameter from 7 microns to 36 microns in fish blood, as shown in African Lung Fish i.e. Protopterus.

Results and Discussion

Ichthyologists are becoming interested in fish haematology. Clinical fish haematology has only been practised for about 2-3 decades, and exclusively in Western nations. Despite the fact that it is an analytical technique, fish haematology is still not widely employed in India, and it is only used when there is a stable epidemiological issue among fishes or with their environment, resulting in mass death (Chadha and Sharma, 2015).In freshwater fish, Cr(VI) induces behavioural, histological, and immunological alterations. In fish RBC, Cr(VI) causes micronuclei, binucleated cells, and DNA breaks. It also causes endocrine disturbance in fish, affecting T3, T4, TSH, and cortisol levels, among other things. Fish with chronic exposure to Cr(VI) develop hyperglycemia and hyperlactatemia. Chronic exposure to Cr alters enzyme activity (VI)(Bakshi and Panigrahi 2018).

Table 1: Alternation of haematological parameters of *Clarias batrachus* exposed to Cr^{+3}

Haematological Parameters	Exposure periods				
	Control	7 days	15 days	30 days	60 days
Hb%	10.10	11.11	11.90	12.84	7.80
RBC ($10^6 mm^{-3}$)	1.77	1.66	1.59	1.42	1.23
WBC($mm^3 x10^3$)	6.40	11.90	12.98	14.04	14.80
CT(sec)	42.00	36.66	34.30	20.40	46.41
PCV%	22.51	27.60	33.61	45.18	17.51
ESR	9.60	8.70	5.60	4.83	15.00
TEC	3.61	4.38	8.76	8.25	2.44
TLC	61.08	55.10	42.41	32.55	29.00
MCH	35.10	29.00	15.90	17.70	38.45
MCV	82.33	75.29	46.80	59.03	103.96
MCHC	41.04	36.72	30.98	25.62	35.40

Haematological parameters, such as MCV, MCH, and MCHC, have received minimal attention. Only a small number of species from diverse regions of the world have normal values for these criteria. The respiratory pigment heamoglobin, which is found in erythrocytes, transports oxygen in fish blood. Haemoglobin has the same physiological purpose in fish as it does in mammals, and it has a consistent around 65,000 molecular weight. These numbers are a useful

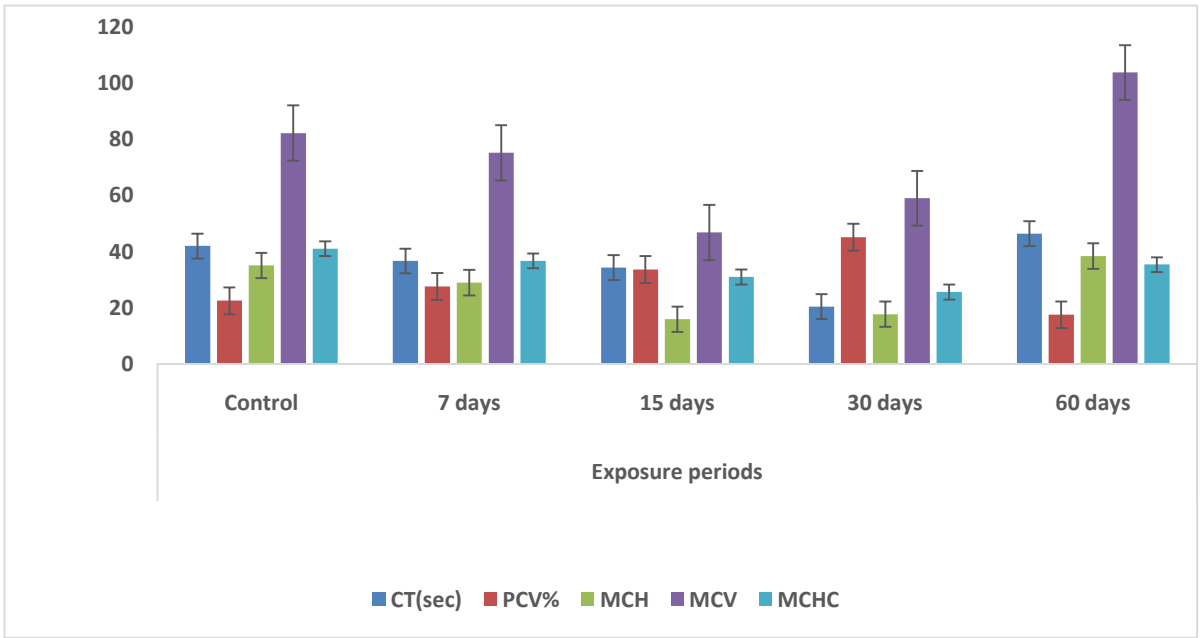
indicator of the blood's physiological state, including ecological, biological, and physiopathological factors.

Hexavalent chromium is believed to be a hazardous metal having mutagenic, carcinogenic, and other negative effects on biota, despite the fact that it is a ubiquitous element in the environment and trivalent chromium is also required for life. Hexavalent chromium is reduced metabolically within the cell when it enters. The cytoplasmic preponderance of trivalent chromium is the end effect of this process (Blasia *et al.* 2000). Various reactive intermediates are generated during these metabolic events, which have been shown to be harmful to the integrity of the DNA helix resulting in lethal effects in the afflicted individual (Wang *et al.*1997).

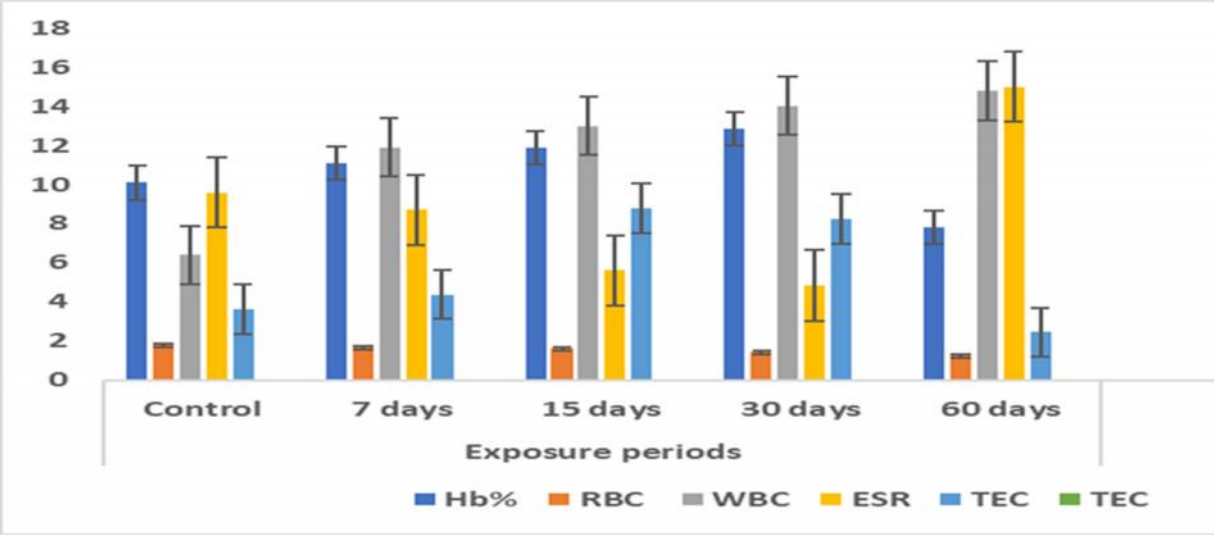
During this process, several intermediate chromium metabolites migrate to the nucleus and interact with DNA, resulting in the ultimate detrimental impact, according to the same scientists. The physiological, behavioural, histological, biochemical, genetic, and immunological conditions of the experimental organism are all affected by chromium, according to the researchers. Hexavalent chromium, which has a bio-membrane permeability capability, is hazardous to freshwater fish. Fishes were observed to lose their body balance with restlessness, slowed breathing rate, and increased mucus production after being exposed to a 50 percent fatal dosage. Reduced haemoglobin percentage and RBC count are two haematological changes to consider.

The blood of a freshwater teleost, *Clarias batrachus*, was used in this study to see how exposure to 1/10th of the lethal concentration of heavy metal Chromium (VI) affected various blood parameters like haemoglobin percent (Hb percent), Clotting Time (CT), Packed Cell Volume (PCV), Erythrocytes Sedimentation Rate (ESR), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Mean Corpuscular Haemoglobin. The percent value of haemoglobin is determined by the total erythrocyte count.

Graph 1: Alternation of haematological parameters of *Clarias batrachus* exposed to Cr+3



Graph 2: Alternation of haematological parameters of *Clarias batrachus* exposed to Cr+3



The numerical values of haemoglobin percent in this investigation were greater than the control group. After 7, 15, and 30 days of exposure, there was an increase of 10%, 17.82%, and 12% in contrast to the control group, and a decline of 22.77 %, with a fall in PCV 22.21 %, TEC 32.40 %, TLC 55.52 %, and MCHC 10%. After 60 days of exposure, however, there are increases in CT 9.50%, MCH 9.5 %, MCV 35.10 %, and ESR 56.25 % (Table No. 1, Graph No. 1 & 2).

In comparison to the control group, there was a 23.3 % drop after 60 days of exposure. However, only after 15 days ($p<0.01$) and ($p<0.001$) of exposure did a meaningful rise emerge. Joshi *et al.*, (2002), found similar results. Different concentration of chromium has an immediate effect on fertilisation. The number of white blood cells (WBCs), red blood cells (RBCs), and haemoglobin (Hb), as well as the time it takes for blood to clot, all decrease. Antibody production and ion-dependent ATPase activity have both been reduced. Glycogen, total lipid, and total protein levels are all dropping in the liver, muscle, and gills. The amount of glycogen in the liver decreases. Hyperglycemia response. The vitality of cells is dwindling. The activity of ALA-D (aminolevulinic dehydratase) rises. WBC, RBC, Hb, MCV, PVC, reactive oxygen species ROS, and bacterial susceptibility all increase when the spleen-to-body ratio rises. The blood glucose levels of the treated fish steadily increased after 24, 48, and 72 hours at various concentrations in the current study. This rise is due to glycolysis, which occurred as a result of fish responding to stress and converting glucose to meet their energy needs. Hussein and Nadim (2003) found that increased production of hormones such as catecholamines and glucocorticoids leads to an increase in glycolysis, resulting in a high glucose level in the blood.

Fish, like other vertebrates, respond to stress by evoking a widespread physiological response that includes an increase in stress hormones and subsequent alterations that assist the animal to maintain its normal or homeostatic condition (Barton 2002). Increases in plasma cortisol, catecholamines, and glucose levels, as well as increases in branchial blood flow and muscle activity, are all part of this reaction (Barton and Iwama 1991). This study also finds a change in plasma glucose. It was discovered to be negligible at low chromium concentrations but behaves according to time. Salahuddin and Khola (2013) have similar findings. However, in high concentrations, it causes structural harm to the fish, affecting their growth, development, and survival. As a result, blood glucose has been used as a stress indicator. Silver money (1974). Finally, variations in blood glucose suggest that they might be employed as markers of chromium-related stress in fish exposed to high chromium levels in the water. The results were all statistically significant.

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