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Toxicological impact of hexavalent chromium on hematology and FSH, LH hormones of *Ctenopharyngodon idella*

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Abstract

Heavy metal chromium influences the nutritive value of fish. The aim of this study is to scrutinize the effect of sub lethal dose of Cr (VI) on hematological parameters and endocrine hormones FSH and LH in the widely consumed fresh water fish *Ctenopharyngodonidella*. For this purpose, the experiment was designed to expose the fish to sublethal dose of hexavalent chromium (120 mg/l) for 7 days. After the stipulated time period blood was collected for hematological and hormonal studies and analysed through routine clinical methods. The results indicated a consistent reduction compared to control in the values of white blood cells (14.75%), hemoglobin (1.08%), red blood cells (11.11%), platelets (48.92%), platelet distribution width (6.76%), mean platelet volume (2.24%) and plateletcrit (46.35%). While increasing trend was noticed in hematocrit (59.17%), Mean corpuscular volume (3708.96%), Mean corpuscular hemoglobin (343.42%), Mean corpuscular hemoglobin concentration (96.48%). Among hormones follicle stimulating hormone and luteinizing hormone represented a non-significant elevation of 63.74% and 40.73%. Reduction of blood parameters represents severe hemolysis, anemic condition and retarded fish health. While increasing trend indicates activation of immune system against the Cr (VI) stress. Cr induced reproductive disruption by increasing level of FSH and LH.

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Introduction

The enormous utilization of heavy metals over the previous few decades has inexorably led to enhance the flow of metallic substances in the water. These metals are the matter of concern because of their diversified impact and concentration range that stimulates the toxicological impact on aquatic flora and fauna (Yang and Rose, 2003). Heavy metal pollutants can cause a metabolic derangement in the living system in contact. Heavy metals are considered serious contaminants of the aquatic environment due to their prolonged biological half-life, inherent toxicity even at lower concentration as well as higher bioaccumulation rate (Chaudhary *et al*., 2018).Heavy metal chromium has acquired broad consideration among the scientific community in recent years due to their prospective dangers to human wellbeing (Shuhaimi-othman *et al*., 2010). Chromium exists in water, soil, air, and also in biosphere and is a ubiquitous trace metal.

It is released into the atmosphere from different natural and artificial sources. As soon as it is liberated into the surrounding, chromium easily forms complexes with several other ligands, which creates it much more mobile as compared to other heavy metals (Nanda and Behera, 1996).Chromium exists largely in the oxidation states Cr (III) and Cr (VI), the subsequent hexavalent species are considered more toxic in the environment due to their high solubility and motility (Reynolds, 2004; Vutukuru, 2005). Chromium is a compound of biological interest, which probably plays a role in the metabolism of glucose and lipid as the main nutrient ((Langard and Norseth, 1979). It is recognized to be toxic to living organisms for its bioaccumulation as well as non-biodegradable properties. In accordance to Indian standards, the optimum tolerance of the total Cr for public water supply is approximately 0.05 mg/L. Hexavalent chromium salts have various applications at industrial level but their indiscriminate discharge into the aquatic ecosystem represents severe risk to the development and survival of aquatic fauna, which may also include fish population (Mishra and Mohanty, 2008).

Fish are considered as a superb model for understanding the mechanistic elements related to metal toxicity (Abedi *et al*., 2012). Among fish population, exposure to chemical contaminants may cause boosting or decline hematological ranges. Hematological parameters are incorporated as they are easily calculable in the blood and circulating hormone amounts could be changed by exposure to xenobiotic chemical compounds. Environmental pollution is a critical and increasing issue (Thangam *et al*., 2016). Hematological indices are ready-made tools utilized by Ichthyologists and researchers in numerous parts of the planet earth. This is due to the fact that the fish is closely related to the aquatic environment and the blood will reveal several conditions inside the entire body of fish just before the signs of disease are noticeable (Fernades and Mazon, 2003). Consequently, the aim of our research study was to observe the impact of sublethal chromium (VI) concentration on hematological parameters and on LH and FSH reproductive hormones in grass carp *Ctenopharyngodon idella* following seven days exposure period.

Materials and methods

Experimental fish

Grass carp is abundant in fresh water, collected from the hatchery and transported in large fish containers to the laboratory. They were then released into an aquarium. One aquarium was used for control and the other one was treated with 120mg/L potassium dichromate for seven days. Potassium dichromate was used as a source of chromium. The fish fed on commercial fish pellets containing about 40% protein content. 40% water was changed both for control and test group daily. Test group was added with the required quantity of metal. Blood samples were taken to determine the hematological parameters and hormonal studies.

Hematological and hormonal studies

Collection of blood

The fish were caught very carefully with the help of a tiny dip net, single one at a time with slightest disturbance. Anaesthetizer MS222 was used for anesthesia. Blood samples had been taken from both control and 7 days treated fish by cut on caudal peduncle. Soon after the initial drop of blood is discarded, the freely oozing blood was collected. Blood collection was interrupted quickly as the blood oozing from the cut caudal peduncle started to display the indications of coagulation. After collection of blood, it was immediately shifted to glass vials containing 1% EDTA solution to prevent blood clotting.

For the hematological profile of the control and treated fish, Haemoglobin, Haematocrit, RBC, WBC, platelet counts measured in the whole blood of *Ctenopharyngodon idella*. Erythrocyte indices of fish viz., MCV, MCH and MCHC were also calculated using the method of Dacie and Lewis (1977).Follicle stimulating hormone and Luteinizing hormones have been evaluated by Immunoenzymometric assay.

Statistical analysis

All measurements were performed in average of five replicates. The data obtained were analyzed using SPSS Statistical package (version 16). The results have been stated as mean ± S.E and were evaluated using Student's t-test.

Results

In this study, the exposure of fish, *Ctenopharyngodon idella* to a sublethal concentration of potassium dichromate for 7days caused changes in hematological parameters and hormones. The mean±SD values for control and experimental group under sub-lethal concentrations are depicted in Table 1 and 2.

Table 1. Alterations in hematological indices after 7 days exposure of Grass carp to hexavalent chromium with a dose of 120mg/L.

Hematological indices	Control group (Mean±SE)	Treated group (Mean±SE)
WBC	65.3± 29.271	55.666± 35.623
HGB	6.2± 1.955	6.133± 1.936
RBCs	0.963± 0.813	0.856± 0.821
HCT	10.9± 10.051	17.35± 9.901
MCV	77.476± 21.304	2951.033± 2862.123
MCH	219.503± 106.932	973.333± 567.724
MCHC	382.043± 207.329	750.65± 724.685
RDW- CV	20.866± 0.417	19.733± 0.328
RDW-SD	85.566± 0.371	97.533± 0.796***
PLT	1052± 411.500	537.333± 229.913
MPV	7.4± 0.461	7.566± 0.317
PDW	17.233± 0.409	16.066± 0.666
PCT	0.740± 0.262	0.397± 0.171

Values expressed as a mean of 6 fish. Student "t" test; $p>0.05$ vs. control (non-significant), $p<0.001$ highly significant***.

Abbreviations used: WBC, White blood cells; RBC, Red blood cells; HGB, Hemoglobin; HCT, Hematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW-CV, Red cell distribution width-coefficient of variation; RDW-SD, Red cell distribution width-standard deviation; PLT, Platelets; MPV, Mean Platelet Volume; PDW, Platelet Distribution Width; PCT, Plateletcrit.

The results reveal that white blood cells, red blood corpuscles, hemoglobin, platelets, mean platelet volume, platelet distribution width and plateletcrit decreased after 7days of exposure compared to control. In contrast, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and mean corpuscular volume showed a non-significant increase (p>0.05) in experimental fish compared to control.

The percent change showed a decline in the hematological parameters like white blood cells (14.75%), hemoglobin (1.08%), red blood cells (11.11%), platelets (48.92%), platelet distribution width (6.76%), mean platelet volume (2.24%) and plateletcrit (46.35%). While hematocrit showed an increase of 59.17%, Mean corpuscular volume 3708.96%, Mean corpuscular hemoglobin 343.42%, Mean corpuscular hemoglobin concentration 96.48% are presented in Fig. 1.

Table 2. Alteration in FSH and LH hormones after 7 days exposure of Grass carp to hexavalent chromium with a dose of 120 mg/L.

Hormones	Control group (Mean± SE)	Treated group (Mean± SE)
FSH	1.026± 0.146	1.68± 0.760
LH	1.473± 0.554	2.073± 1.153

Values are expressed as mean of 6 fish Mean± SEM, Student "t" test; p>0.05 vs control.
Abbreviations used: FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone.

Follicle stimulating hormone mean value and standard error for control group was 1.026±0.146 and in treated group was 1.68±0.760 presenting a non-significant increase of 63.74%. Luteinizing hormone mean±SE for control group was 1.473±0.554 and in test group was 2.073±1.153 revealing a non-significant elevation of 40.73% and are represented in Table 2 and Fig. 2.

Discussion

Hematological studies are physiologically and ecologically important for establishing the relationship between characteristics of blood and environment (Ovuru and Ekweozor, 2004) and is regarded as an important mean for the selection of genetically resistant animals to specific diseases and environmental hazards (Mmereole, 2008).Tavares-Dias and De Moraes (2007) stated that one of the easiest approach to evaluate the immune system is to examine modifications in white blood cells count. Another group of researchers investigated decline in WBC in response to heavy metals in fish (Witeska, 2005; Oliveira *et al.*, 2006; Olanike, 2007; *Safahieh et al.*, 2010). Reduced white blood cells count may possibly be due to the production of epinephrine (adrenaline) throughout stress as a response against heavy metal toxicity and deterioration of the immune system (Olanike, 2007). Pal and Trivedi, (2016) also studied the reduction of the total leukocyte count in *Channapunctatus* after treatment with chromium trioxide. The concentration of hemoglobin reflects the body's oxygen supply and the organism itself tries to keep it as much stable as possible (Mallesh *et al.*, 2015). According to the results of this study, Hb appears to be the best indicator of environmental stress. Pal and Trivedi, (2016) studied in *Channa punctatus* that after 7, 15 and 30 days there was reduction in the percentage of hemoglobin i.e 5.02%, 10.06% and 17.61% but after 60 days the hemoglobin decreased by 23.3%. Singh *et al.* (2008) also investigated decline in hemoglobin level after 15, 30 and 45 days exposure period to copper sulphate in *Channa punctatus*. Bhatkar (2011) reported reduction in Hb% in *Labeo rohita* after exposure to chromium chloride for 10, 20 and 30 days.Nussey *et al.*, (1995) described that a decline in haemoglobin concentration indicates the fish's inability to supply sufficient oxygen to the tissues. The prolonged reduction of the haemoglobin content is harmful for the transport of oxygen and any blood dyscrasia and erythrocytes degeneration can be due to pathological conditions in fish exposed to toxic substances

(Shalaby, 2001).Red blood cells play an important role in transporting hemoglobin which carries oxygen to all body tissues (Hibiya,1982). Due to the toxicity of metals, the hemopoietic organs are affected and cannot release normal red blood cells in the general circulation and therefore may be accountable for the severe decline. Reduced lifespan of red blood cells and slower erythropoiesis are accountable for the reduction in the number of RBC due to metal toxicity (Nimmy and Joseph. 2018).Anemic conditions in fish can be detected with hematocrit. Poleo and Hytterod (2003) reported an increase in HCT values for Atlantic salmon, *Salmo salar*, exposed to water rich in aluminum for 3 weeks at pH 9.5.

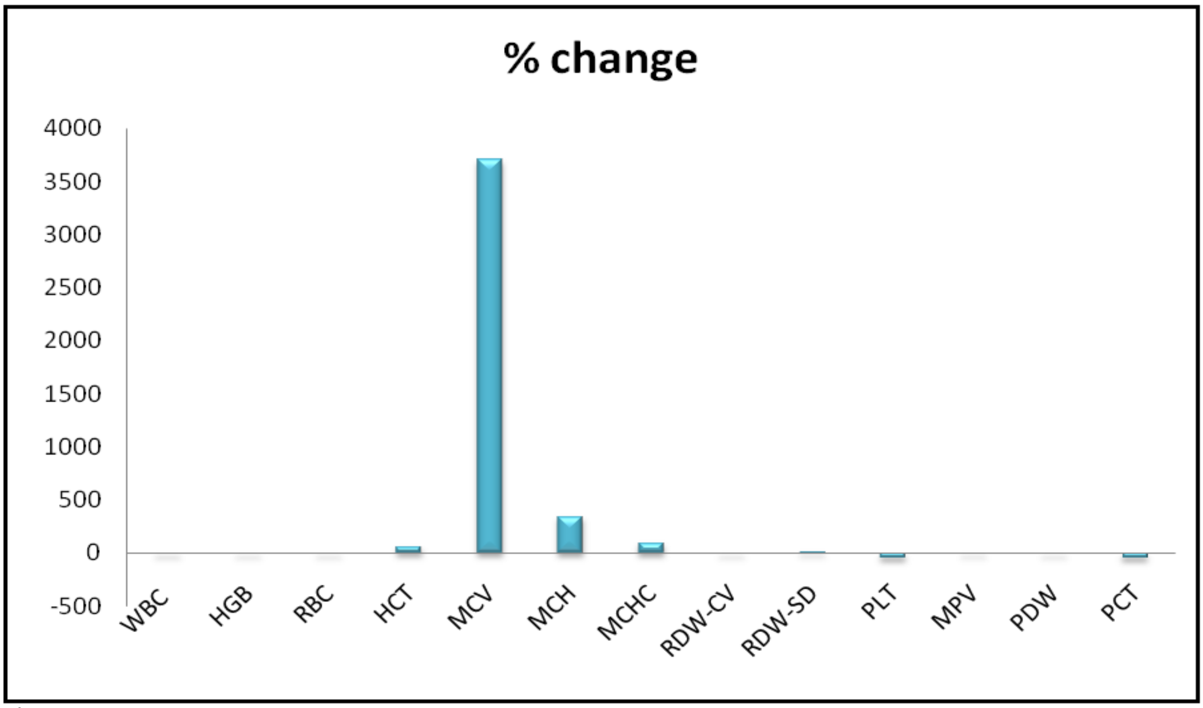

Fig. 1. Hematological indices showing percent increase and decline after 7 days exposure to grass carp with a dose of 120mg/L.

Increase in hemoglobin level and hematocrit concentrations were also noticed in fish after 96-hour exposure to 3 mg/l of copper (Mishra and Srivastava, 1980). In the present study, a marked increase in MCV may be due to a decrease in the total erythrocytes count because MCV and total erythrocyte count display inverse relationship with each other. This is in line with the findings of Shah (2006) in *Tinca tinca*, Olanike (2007) in *Clarias gariepinus*, Sandeep *et al.* (2013) in *Labeo rohita*, and Sharrma and Langer (2014) in *Garragotyala*.

An increase in MCV may possibly be due to the enlargement of red blood cells due to hypoxic condition or osmotic disorders and absorption of electrolytes and water in cells accompanied by acidification of the cytoplasm of red blood cells or macrocytic anemia in fish exposed to metal contamination (Sinha *et al.*, 2000). In another research study, a mixture of copper and zinc fed to *Cyprinus carpio* increased MCH and MCHC (Dhanapakiam and Ramasamy, 2001). Khalesi *et al.*, (2017) demonstrated a substantial change in MCH due to chromium toxicity to *C. carpio*. Similar results have been recorded by Koprucu *et al.* (2006) and Adeyemo (2007) under the aspect of heavy metals and pesticide stress in various species of fish. Significant changes in the MCH values might be due to diminished cellular blood iron, resulting in lowered oxygen carrying capability of blood and ultimately stimulates erythropoiesis (Hodson, 1978). Sudhasaravanan and Binukumari (2015) found a decrease in MCH and MCHC, indicating that the concentration of hemoglobin in red blood cells is reduced. Low platelet concentration could be due to thrombocytopenia or decreased synthesis or increased platelets destruction rate. Minimal platelet concentration also advocates that the clot formation procedure will be delayed, leading to extreme blood loss in case of injury (Purves *et al.*, 2004). Bhatkar (2011) studied the increasing trend of mean corpuscular volume in fish kept at sublethal doses of $CrCl_3$ in comparison to control. These haemotological disorders are a haemopoietic or erythrocyte mobilization response to hypoxemia provoked by heavy metal stress. MCV and MCH have been found to boost incase of macrocyte anaemia (Bomford *et al.*, 1975).

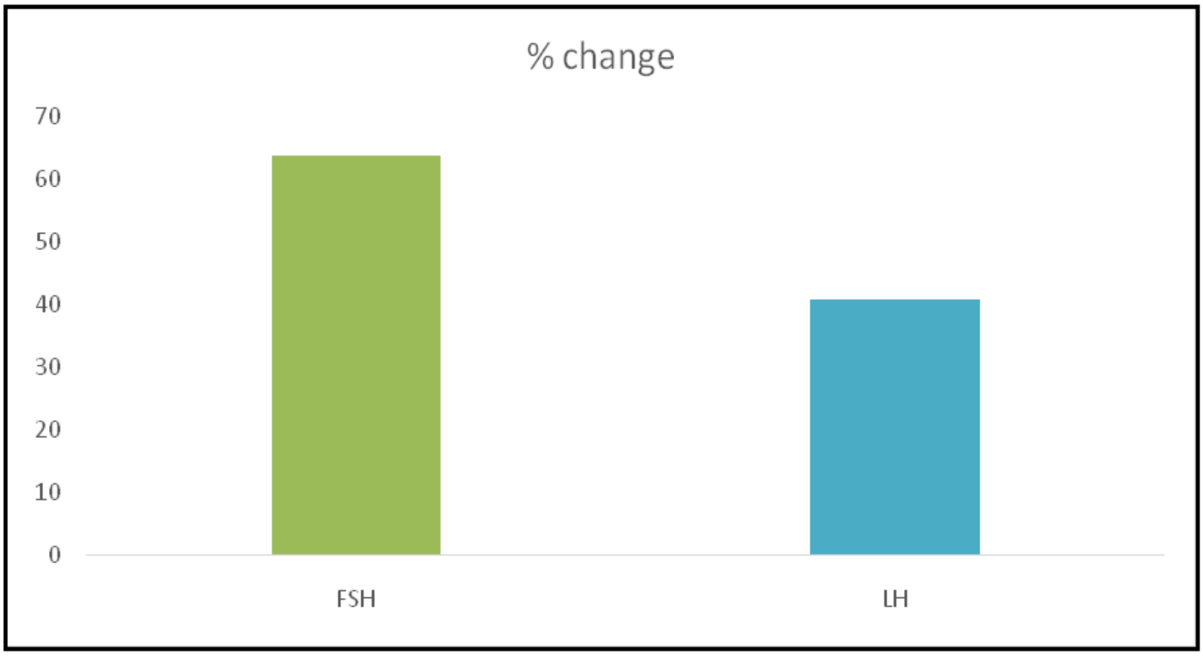

Fig. 2. FSH and LH hormones showing percent increase in grass carp after 7 days exposure to hexavalent chromium.

The production of female and male sex hormones is powered and normalized through the hypothalamic-pituitary gonadal axis. Both LH and FSH are glycoprotein gonadotropins exuded by the adenohypophysis of the pituitary gland in reaction to the gonadotropin-releasing hormone (GnRH) produced by the hypothalamus (Sifakis *et al.*, 2011). A better serum FSH concentration is due to repression of feedback inhibition of anterior lobe of pituitary gland. This suppression could secondarily enhance FSH secretion (Fattahi *et al.*, 2009). High amount of follicle stimulating hormones may result from the direct impact of the pesticides or toxicants on the central nervous system (Hu *et al.*, 2002; Shariati *et al.*, 2008). Ball and Baker (1969) reported that the gonadotropin hormone in different teleosts is inhibited by stress. A slight increase in LH was investigated in fish exposed to 10 or 100µg/l of BPA, whilst exposure to higher concentrations of BPA produced substantial elevation in LH concentrations (Faheem *et al.*, 2017).

Conclusion

According to the results of this research study it is clear that sub lethal toxicity of Cr can lead to a reduction of red blood cells, white blood cells and hemoglobin which leads to an anemic or leukemic condition. LH and FSH are reproductive hormones. Increase in hormonal level might be due to tumors or cancerous tendency in the ovaries due to the carcinogenic nature of chromium. Reduction or increase in their quantity can impact on the breeding capability of fish.

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