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Assessment of chromium toxicity in *Cyprinus carpio* through hematological and biochemical blood markers

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Abstract: Static renewal exposure of various sublethal concentrations of hexavalent chromium [Cr(VI)] produced a deleterious effect on some hematological and biochemical parameters of *Cyprinus carpio* with the increase in concentration of metal. *C. carpio* breeders (W = 500 ± 9.5 g; L = 25.60 ± 2.6 cm) were randomly divided into the control group (n = 33) and treated group (n = 198), with 11 breeders/tank (8.84 m3). The groups were distributed into tanks containing 1 of 7 sublethal concentrations of Cr(VI) (0, 25, 50, 75, 100, 125, and 150 mg/L), with 3 replicates per concentration for 6 months. Monthly Cr(VI) levels in the water were determined with an atomic absorption spectrophotometer. The Cr(VI) induced a significant increase ($P < 0.05$) in white blood cells, mean corpuscular volume, erythrocyte sedimentation rate, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and acid phosphatase, indicating anemia. However, red blood cells, hemoglobin, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, glucose, protein, and cholesterol decreased significantly ($P < 0.05$) with the increase in Cr(VI) concentrations from 25 to 150 mg/L. The actual Cr(VI) concentration varied from 36 to 118 µg/L in the tanks exposed to concentrations from 25 to 150 mg/L. The increasing trend was probably due to the toxic effects of the Cr(VI) and activation of the fish immune system to resist against metallic stress and liver damage. The decreasing trend reflected retarded and deteriorated fish health along with severe hemolysis.

Key words: *Cyprinus carpio,* hematological parameters, anemia, chromium, biochemical parameters

Introduction

Water pollution has become a global problem. Heavy metals have long been recognized as serious pollutants of the aquatic environment. They may enter a water body by industrial and consumer waste or even from acidic rain, thereby breaking down soils and releasing heavy metals into streams, lakes, rivers, and ground water (Obasohan et al., 2008). Although trace metals are essential for normal physiological processes, abnormally high concentrations are toxic to aquatic organisms. Living organisms require varying amounts of some heavy metals for their metabolic function; for instance, chromium plays an important role in growth and metabolism (carbohydrates, glucose, cholesterol, and lipids) (Taylor, 1999), but at higher concentrations (200-300 mg/L for freshwater fish), chromium is a highly toxic, mutagenic (Arreola-Mendoza et al., 2006), carcinogenic (Prabakaran et al., 2007), teratogenic (Yousef et al., 2006), highly mobile and incorporating metal in the food chain (Goldoni et al., 2006). Biological interest in chromium has risen due to its prominent role in industrial pollution

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and toxicity. Chromium is a transition metal located in group VI-B of the periodic table. Cr is the 7th most abundant element on earth and the 21st in the crustal rocks (Deb and Fukushima, 1999; Bagchi et al., 2001). Cr abundance in the Earth's crust ranges from 100 to 300 μg/g. Soils may contain between 5 and 3000 μg Cr/g (Depault et al., 2006). The world production of Cr is in the order of 10^7 t/year; 60%-70% is used in alloys, including stainless steel, and 15%-30% is used in chemical industrial processes, mainly leather tanning, wood processing, textiles, jet aircraft, automobiles, hospital equipment, sugar, paper, pulp, ceramics, pigments, and electroplating. In contrast to most metals, chromium is usually soluble under oxidizing conditions, and only limited removal could be achieved by precipitation processes (Canwet et al., 2001). It exists in 2 valence states in nature, i.e. Cr(VI) and Cr(III). In their studies, Bagchi et al. (2002) and Tyrone et al. (2006) demonstrated that Cr(VI) is 700 times more toxic than Cr(III), due to its readily soluble nature.

Hematological and biochemical blood biomarkers are frequently used for detecting and diagnosing sublethal effects in fish exposed to different toxic substances. Blood is highly susceptible to changes in the environment and is a good indicator of environmental toxicity. Cr(VI) has an adverse effect on the hematological and biochemical parameters of fish (Öner et al., 2009; Zaki et al., 2009). Cr(VI) exposure causes significant elevation in transaminases (serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase), acid phosphatase (ACP), and alkaline phosphatase (ALP) (Vosyliene and Jankaite, 2006; Öner et al., 2008). Blood cell changes associated with Cr(VI) include decreased erythrocyte count and hematological indices (Firat and Kargin, 2009). Other changes associated with Cr(VI) exposure include hypercholesterolemia and hypoglycemia; changes in blood cell indices are variable depending on the concentration and period of exposure (Steinhagen, et al., 2004; Prabakaran et al., 2007; Vinodhini and Narayanan, 2009).

Chronic Cr(VI) exposure has been accepted as significantly toxic by the scientific community. However, in this study, an effort is made to check the effect of various sublethal Cr(VI) concentrations on fish blood for 6 months. This will not only determine the abnormalities in the fish blood due to chromium but will also explain which sublethal concentrations are relatively safer and which are relatively fatal for fish.

Materials and methods

The fish, 231 *Cyprinus carpio* breeders (W = 500 ± 9.5 g; L = 25.60 ± 2.6 cm) of both sexes, were kept at 11 breeders/tank (8.84 m^3) in triplicate for each sublethal concentration. They were fed fish feed of 3.5% of their wet body weight [30% protein and 8% carbohydrates; soybean meal (20%), maize gluten (25%), rice polish (15%), wheat bran (30%), molasses (2%), and vitamin premix (8%) twice a day (Jhingran, 1995)]. Aeration was provided with an automatic compressor. The water was removed every other day to siphon off the extra food.

Exposure to chromium

The breeders were separately exposed to various concentrations (25, 50, 75,100, 125, and 150 mg/L) of potassium chromate (K_2CrO_4) in triplicate for 6 months in a static renewal experiment. A parallel control group without Cr was also run in triplicate for comparison. At the beginning of the experiment and at the beginning of each month, the calculated amount of K_2CrO_4 was added to each tank in a dissolved form. The actual Cr(VI) water concentration was determined monthly through atomic absorption for each concentration.

Collection of blood samples

Blood samples were aspirated from the caudal vein (Dacie and Lewis, 1991). Every 2 weeks, 5 fish were randomly selected from each concentration (including the control group) and immersed in 0.5 mg/L MS 222 (3-aminobenzoic acid ethyl ester; Sigma) for 1-2 min, and then about 4 mL of blood was collected from each fish. After removing the needle from the syringe, 2 mL of the blood was gently decanted into a prepared test tube containing dipotassium salt of ethylenediaminetetraacetic acid (EDTA; Sigma) for a final concentration of 1.0 to 2.0 mg EDTA/mL of blood. The tube was rotated gently to ensure complete mixing of the EDTA with the blood. To obtain plasma for biochemical analysis, the other 2 mL of blood was centrifuged at 3500 rpm for 15 min.

Estimation of hematological and biochemical parameters

Hematological and biochemical parameters were determined using commercially available Randox kits (Randox Laboratories Ltd.). Measured variables included white blood cell (WBC) count, red blood cell (RBC) count, packed cell volume (PCV) and hemoglobin (van Kampen and Zijlstra, 1961), mean corpuscular volume (MCV; Dacie and Lewis, 1991), erythrocyte sedimentation rate (Swarup et al., 1986), glucose (Barham and Trinder, 1972), protein (Henry et al., 1974), cholesterol (Richmond et al., 1974), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Bergmeyer et al., 1978), and ACP and ALP (Bessey et al., 1946). The data was statistically evaluated by ANOVA with the help of SPSS 12.0.

Results and discussion

In the control group's water, no Cr(VI) was detected after 6 months. However, 36, 43, 73, 88, 112, and 118 µg/L Cr was detected from the concentrations of 25, 50, 75, 100, 125, and 150 mg/L Cr(VI) with the atomic absorption spectrophotometer. This chromium is absorbed into the fish from the water and significantly alters the hematological and biochemical characteristics of its blood. At sublethal concentrations, it is not only detrimental for an individual organism but also affects the whole community and the trophic level of the food chain (Firat and Kargin, 2009).

The mean WBC count increased significantly (df = 6, 35; F = 13.57; P < 0.01) with increasing Cr(VI) concentrations, from 6000/mm^3 in the control group to as high as 16,000/mm^3 in the fish exposed to 150 mg/L Cr(VI) (Figure 1). The WBC count increased with the increase in concentration. An increase in WBC following chromium exposure appears to be dose-related and may reflect a stress response leading to increased cell production or cell release from the spleen, which sequesters and stores blood cells under resting conditions and releases them into circulating blood during contraction associated with various state of stress.

The RBC count decreased significantly (df = 6, 35; F = 6.19; P < 0.05) with the increase in Cr(VI) concentration. At 150 mg/L, the RBC count of the fish in the control group decreased from (8×10^6)/mm^3 to (5×10^6)/mm^3 (Figure 2). This decrease in RBCs may be due to a decreased rate of RBC production or an increased loss due to hemolysis (Vosyliene and Jankaite, 2006). In a similar study, RBC deficiency in Cr-treated fish was suggested to be due to reduced iron metabolism and absorption (Tejendra and Jagdish, 2004).

Hemoglobin values decreased significantly (df = 6, 35; F = 11.4; P < 0.001) in the fish exposed to 150 mg/L Cr(VI), from 15 g/dL in the control group to 4 g/dL in the exposed fish (Figure 3). The reduction in hemoglobin appears to be dose-related and may reflect changes in hemoglobin synthesis. However, the reduction in RBC count and hemoglobin content in all of the exposed fish was either due to the

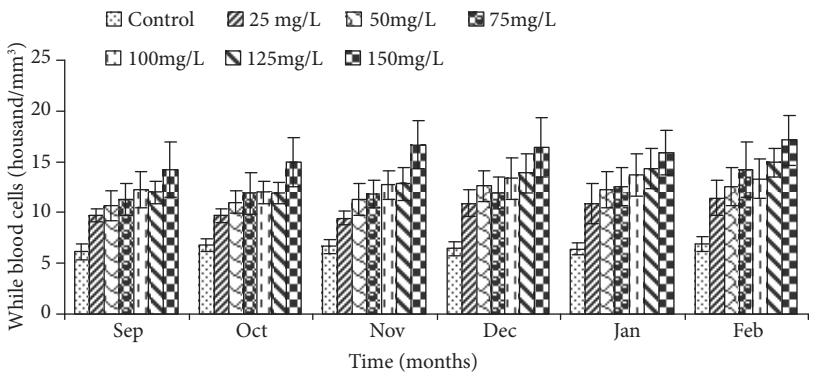

Figure 1. Effect of chronic Cr(VI) exposure on the WBC count of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

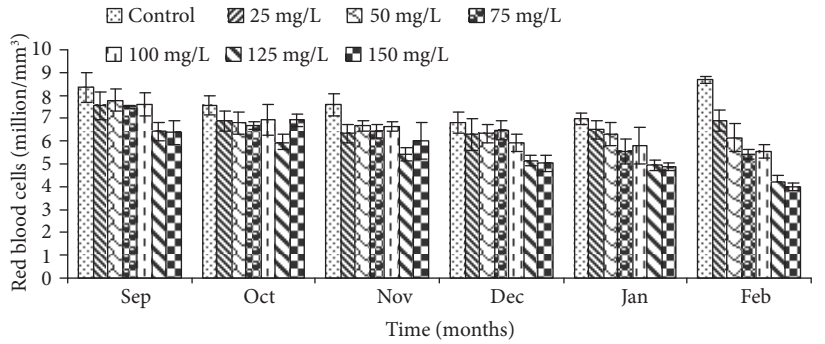

Figure 2. Effect of chronic Cr(VI) exposure on the RBC count of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

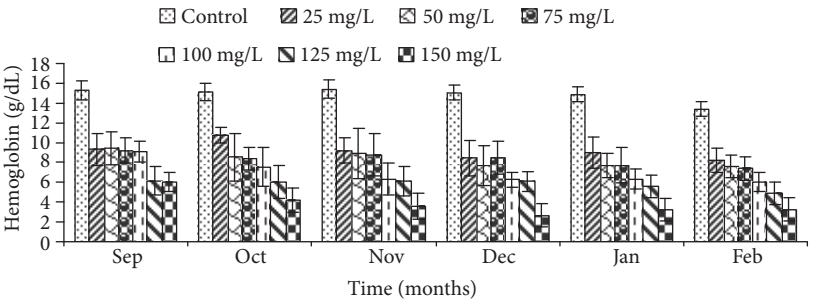

Figure 3. Effect of chronic Cr(VI) exposure on the hemoglobin levels of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

inhibition of hemoglobin synthesizing enzyme or hemolysis that leads to the binding of hemoglobin to plasma haptoglobins. A decrease in the concentration of hemoglobin in the blood is usually caused by the effect of toxic metals on the gills, as well as a decrease in oxygen (Vinodhini and Narayanan, 2009).

In this study, the decrease in hematological indices appeared to be due to the decreased cellular count in the blood after Cr(VI) exposure. The PCV significantly ($df = 6, 35$; $F = 19.6$; $P < 0.01$) decreased from 28% to 13% (Figure 4a), while the MCV increased from 31 to 55 fL (Figure 4b). This increase in MCV may be due to the swelling of the RBCs under Cr(VI) stress. Additionally, an increase in RBC size could be the result of a premature release of immature RBCs in response to anemia. Furthermore, previous work has shown that stress reaction causes an osmotic imbalance. This can diminish the pH of the blood and increase the volume of erythrocytes, subsequently affecting the hematocrit value (Tripathi et al., 2004). Stress reactions can also lead to epinephrine release, which may cause premature release of immature or deformed erythrocytes from the spleen and may affect the hematocrit value (Zaki et al., 2009).

The erythrocyte sedimentation rate increased significantly ($df = 6, 35$; $F = 1.8$; $P < 0.01$) from 1 to 4 mm/h in the exposed fish over time (Figure 5), and this may support the hypothesis that immature RBCs are being released into the circulation following chronic Cr(VI) exposure.

A significant dose-dependent reduction in glucose was observed ($df = 6, 35$; $F = 64.4$; $P < 0.001$), from 95 mg/dL in the control to as low as 52 mg/dL in the fish exposed at 150 mg/L (Figure 6). This gradual reduction of blood glucose in Cr-exposed fish may result from increased energy to cope with oxidative stress (Stahlhut et al., 2006), impairment of

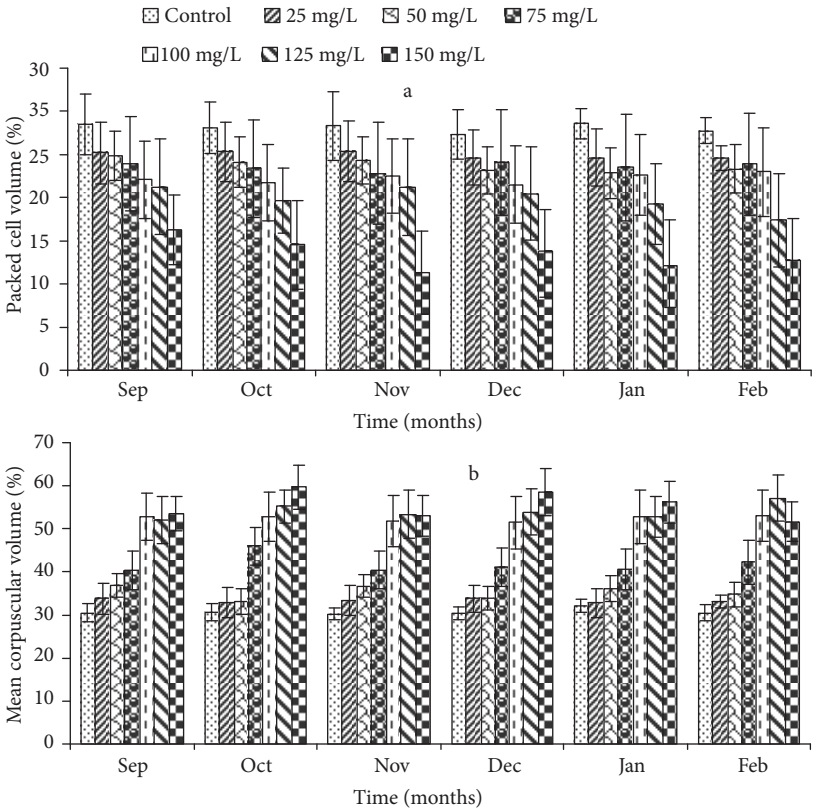

Figure 4. Effect of chronic Cr(VI) exposure on the PCV (a) and MCV (b) of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

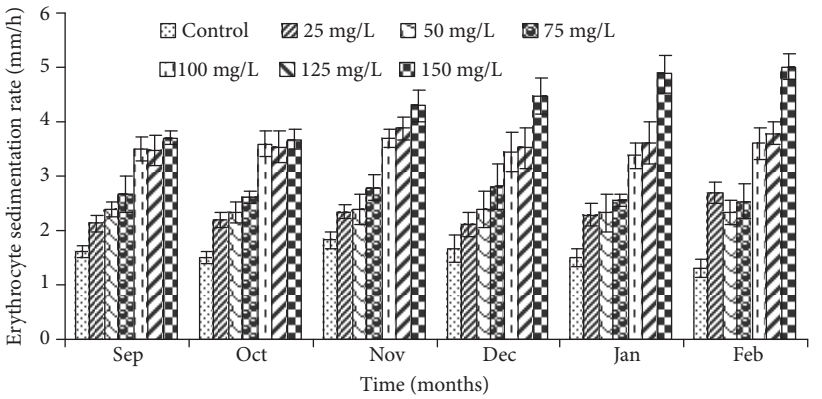

Figure 5. Effect of chronic Cr(VI) exposure on the erythrocyte sedimentation rate of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

gluconeogenesis/glycolysis (Firat and Kargin, 2009), or a reduction of feeding behavior (Kuykendall et al., 2009)

A significant reduction in serum protein content ($df = 6, 35$; $F = 11.2$; $P < 0.001$) was observed in all of the exposed breeders, except for those exposed to 125 mg/L Cr(VI) (Figure 7). Several factors may be involved in this toxic effect, including previously observed hypoglycemia, which decreases protein synthesis (Öner et al., 2009). A similar study noted

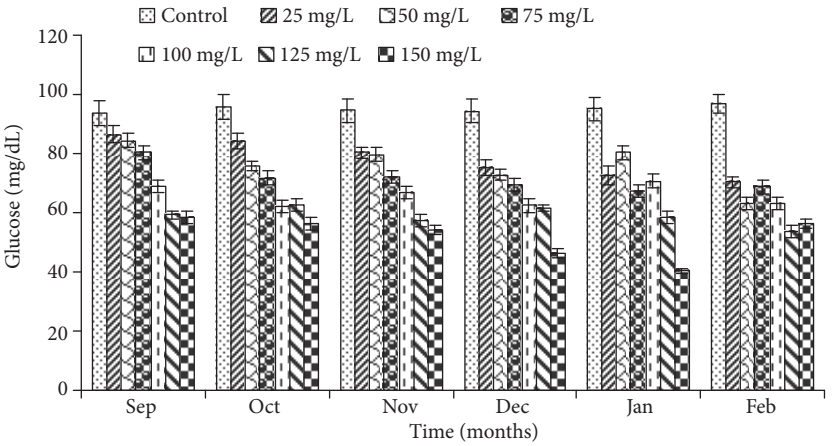

Figure 6. Effect of chronic Cr(VI) exposure on the glucose levels of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

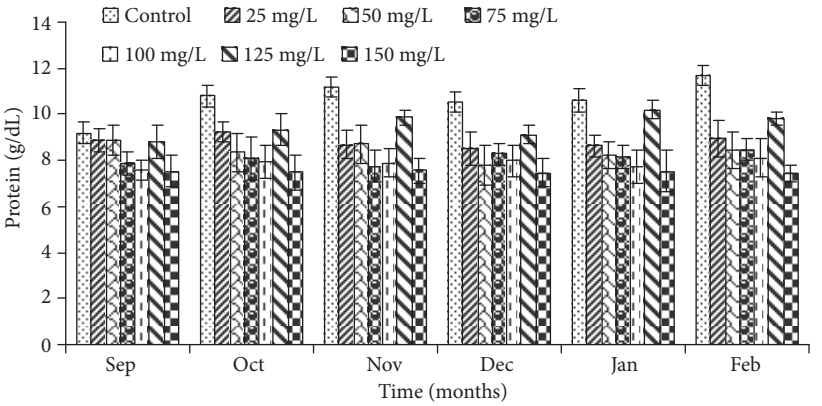

Figure 7. Effect of chronic Cr(VI) exposure on the serum protein levels of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

a reduction in clotting time in *C. carpio*, perhaps reflecting a dose-dependent change in the plasma protein (Fırat and Kargın, 2009; Zaki et al., 2009).

A significant decrease in the total cholesterol (df = 6, 35; F = 89.3; P < 0.001), from 107 to 82 mg/dL, was observed in the blood serum of the exposed fish at concentrations of Cr(VI) from 25 to 150 mg/L (Figure 8). This decrease in cholesterol may reflect liver damage (Vosyliene and Jankaite, 2006; Vinodhini and Narayanan, 2009), caloric restriction, or a reduction in serum proteins such as albumin (Firat and Kargin, 2009).

In an effort to determine the extent of liver toxicity induced by Cr(VI) exposure, the levels of several enzymes were measured, including AST, ALT, ACP, and ALP. AST levels increased significantly (df = 6, 35; F = 113.8; P < 0.05) from 54 to 125 IU/L (Figure 9), while ALT levels increased (df = 6, 35; F = 53.9; P < 0.01) from 51 to 67 (Figure 10a). In addition, ACP levels increased (df = 6, 35; F = 10.7; P < 0.001) from 11 to 29 IU/L (Figure 10b) and ALP levels increased (df = 6, 35; F = 119.2; P < 0.001) from 79 to 122 IU/L (Figure 11). Elevated hepatic enzyme levels may have resulted from leakage of these enzymes from hepatic cells or increased synthesis of liver enzymes. Alteration in the enzymatic activities can result from several types of liver damage, including necrotic hepatocytes or biliary tube obstruction (Öner et al., 2008; Öner et al., 2009; Firat and Kargin, 2009;

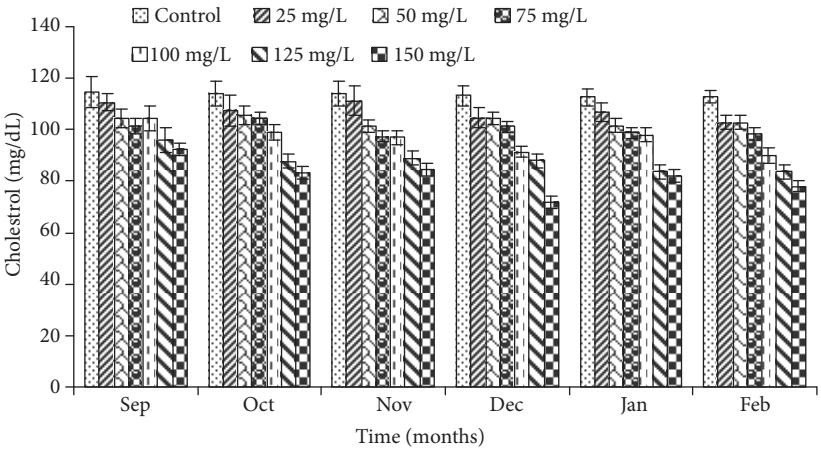

Figure 8. Effect of chronic Cr(VI) exposure on the serum cholesterol levels of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

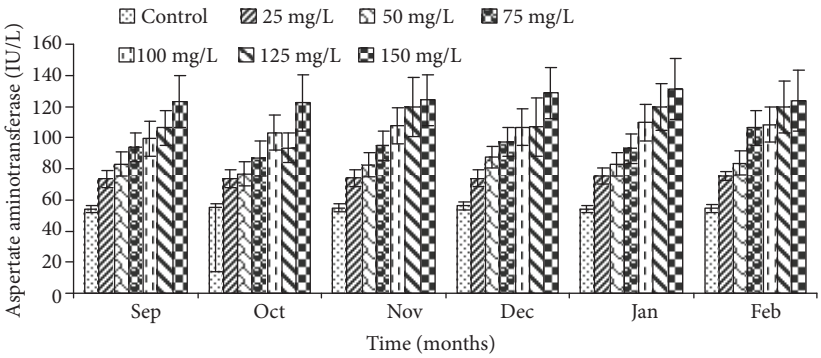

Figure 9. Effect of chronic Cr(VI) exposure on the AST levels of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

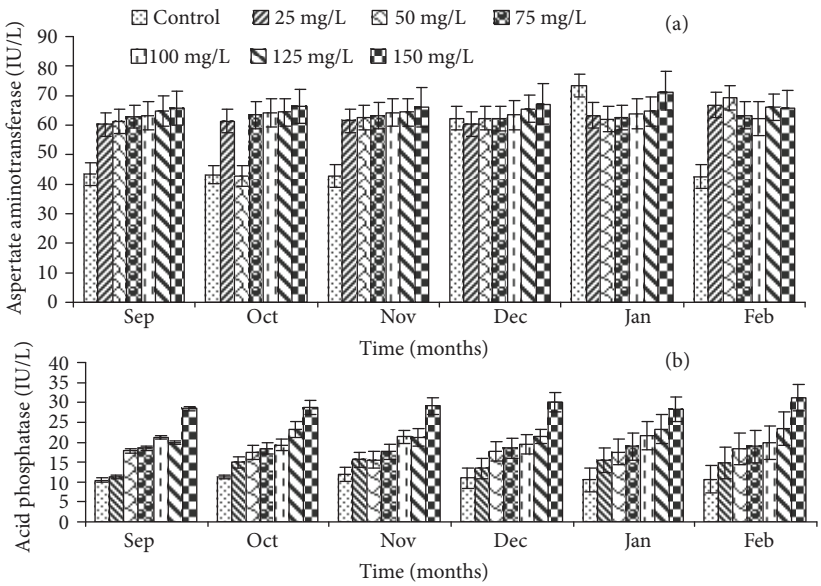

Figure 10. Effect of chronic Cr(VI) exposure on the ALT (a) and ACP (b) levels of *C. carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

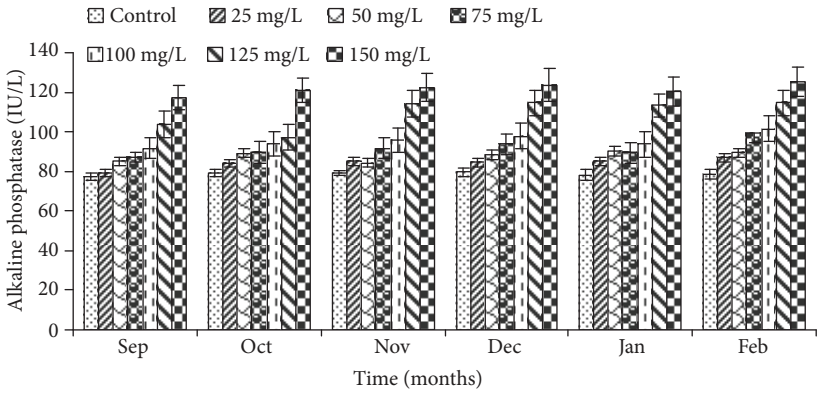

Figure 11. Effect of chronic Cr (VI) exposure on the ALP levels of *Cyprinus carpio*. The values are given as mean ± SD for the 198 treated and 33 control breeders (in triplicate for each concentration).

Shweta et al., 2009). Further work is needed to clarify the toxic effects of Cr(VI) on the hepatic enzymes of chronically exposed fish.

The present results indicate that prolonged exposure to various sublethal concentrations of Cr(VI) induced stress reactions in fish that reduced the immune potential of the fish in a concentration-dependent manner. The changes in the hematological and biochemical parameters indicate that Cr(VI) produced a deleterious effect on fish immunity. This reduced immunological status persisted for a long time and increased the susceptibility of fish to infections.

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