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Original Article

Sub-lethal effects of potassium dichromate on hematological and histological parameters in climbing perch, *Anabas testudineus* (Anabantidae)

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Abstract: Chromium, which enters the river through anthropogenic sources, is one of the potent heavy metals. The present study is an attempt to determine the LC_{50} of Potassium dichromate for the climbing perch, *Anabas testudineus* and to study the impact of two sub-lethal concentrations (6 and 12 mg/l) of Potassium dichromate the toxic hexavalent Cr(VI) form of Chromium on this fish through investigating hematological and histopathological parameters. Experimental set up included quadruplicate treatments for each dosage, and the results were compared with control treatments. The results showed that the LC_{50} value at 96 hr was 59.92 mg/l. The fishes exposed to sub-lethal concentrations showed severe abnormalities such as; degeneration of hepatocytes, necrosis of hepatic tissue and extensive haemorrhage in gills and renal tissue. The present study brings out the harmful impact of Cr(VI) in the aquatic environment and necessitates regulations of its inflow to natural water bodies as a management plan to curb its contamination.

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Introduction

Water pollution has been regarded as one of the most important threats globally impacting human health, environment and sustainable development (World Water Assessment Programme, 2018). Although there has been a much understanding on the impact of the addition of anthropogenic contaminants to the environment, the natural aquatic bodies have extensively been polluted with heavy metals released from domestic, industrial and other man-made activities (Conacher et al., 1993; Velez and Montoro, 1998). According to the United States Environmental Protection Agency (USEPA), and the International Agency for Research on Cancer (IARC), these metals are also classified as human carcinogens based on the epidemiological and experimental studies (Tchounwou et al., 2012). Among the various metals, Chromium (Cr) has turned out to be a vital pollutant, which has the potential to be toxic to living organisms due to their bioaccumulation and non-biodegradable properties (Velma, 2009). With increasing industrialization and fewer means for safe disposal, contaminations of Cr in the aquatic environment is often being regarded as a menace in India. The situation gets even worse since its availability in nature either as dichromate in acidic environments or as chromate in alkaline environments has rampantly infiltrated into the drinking water system (Risikesh et al., 2007). The permissible levels of Cr for drinking water recommended by the Indian Drinking Water Quality Standard (IS: 10500:2012) is 50 µg/l. Hexavalent Chromium, Cr(VI) is highly carcinogenic and may cause death to animals and humans if ingested in large doses (Zyed and Terry, 2003). This form of Chromium rarely occur naturally but is produced from anthropogenic sources and profoundly used in industry for metal planting, cooling tower, water treatment, tanning, and wood preservation (Palmer and Wittbrod, 1991). Among the various compounds, the dichromate compounds, especially $K_2Cr_2O_7$, is the most profoundly found form of Chromate in India, mainly due to its wide industrial application.

Fishes are more prone to such pollution due to their

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continuous exposure to toxicants as well as bioaccumulation potential, which makes them good biological indicators of heavy metal toxicity (Hedayati et al., 2010). The effects of toxicant in fish as studied from a haematological and histological perspective have been used as successful biomarkers for assessing the toxic effects of xenobiotics (Yancheva et al., 2015). Histological alterations in the tissues of liver, kidney, and gills of fish are sensitive biomarkers for metal pollution that provide for a better evaluation of the effects of pollution (Poleksic et al., 2010). Under the above background, a study was conducted to assess the sub-lethal concentration of Chromium ($K_2Cr_2O_7$) on the selected tissues of climbing perch, *Anabas testudineus* (Bloch, 1972), to bring out the immediate impact of Cr(VI) toxicity on this freshwater fish.

Materials and Methods

About 100 healthy adult *A, testudineus* (Bloch, 1972) weighing between 40-45 gr and length 10-15 cm were procured from Pulimugham hatchery, Thakazhi, Kerala. They were transported to the laboratory in anaesthetized condition, examined for any pathological symptoms, treated with 0.1% $KMnO_4$ solution to avoid any dermal infection and acclimatized in dechlorinated tap water for 30 days. Fishes were raised in wide-mouthed glass tanks of 600 L capacity. Stocking density was maintained at a rate of one fish per 30 L. Adequate aeration was given without vigorous bubbling of the water. Water was replaced with filtered dechlorinated water every 2 to 3 days, depending on the water quality. Fishes were adequately fed with commercially available crumble fish feed (CP Feeds), and any feed waste was siphoned out. At the end of the acclimatization phase, healthy fishes were selected and maintained in separate aquaria before actual experimentation. Water was maintained at pH 7.4±0.3; D.O. 6.6±1.2 mg/l; total alkalinity 310±52 mg/l and temperature of 28±1ºC.

A stock solution of $K_2Cr_2O_7$ was prepared, and their dilution was made according to standard guidelines (APHA, 2012). To determine the appropriate range of toxicity, a series of different concentrations was prepared (in triplicate), and the sublethal toxicity of the heavy metal was determined following Brungs et al. (1977). The fishes were not fed during the period of exposure. The water in the aquaria was replaced every 24 hr. The mortality data were used to calculate the 96 hr LC_{50} value. The LC_{50} value at 96 hr was statistically evaluated using Probit analysis method (Finney, 1952).

This was followed by the analysis of the effect of sub-lethal concentration of $K_2Cr_2O_7$ on the selected tissues of *A. testudineus.* For this based the results of LC_{50}, two sub-lethal concentrations of $K_2Cr_2O_7$ approximately $1/5^{th}$ and $1/10^{th}$ the value of the lethal concentration, i.e., 12 and 6 mg/l, respectively were selected. The experimental set-up included quadruplicate tanks for each treatment. T1 was maintained as control tanks devoid of any $K_2Cr_2O_7$, while T2 and T3 were treatments with 12 and 6 mg/l of $K_2Cr_2O_7$, respectively. The fishes in these treatments were exposed to $K_2Cr_2O_7$ for 15 days. The fishes were moderately fed during the period of exposure. The water in the aquaria was replaced every 24 hr. At the end of the 15-day exposure period, the hematological and histological changes in the gill, liver, and kidney of the *A. testudineus* were analyzed.

Haemoglobin (Hb%) was measured by Sahli's Acid Haemoglobin method (Hesser, 1960). RBC counts were taken in Neubauer's hemocytometer using Hendrick's solution as diluting fluids. Packed cell volume (PCV) was determined by the microhaematocrit method. Differential leucocyte count (DLC) was carried out by preparing a thin blood smear and staining it with Leishman's stain. Calculation of mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean cell volume (MCV) was done using standard formulae (Dacie and Lewis, 1982). Mean values were compared using one-way ANOVA and Post hoc analysis (Duncan Multiple Range Tests) following Ranjeet et al. (2013) using statistical software (SPSS 17.0 for Windows).

Results

The calculated 96 hr LC_{50} value of Potassium

Table 1. Probit analysis for *Anabas testudineus* during a 96 hr exposure to various concentration of potassium dichromate

Exposure period (hr)	LC_{50} (mg/l)	95% Fiducial Limits (mg/l)		Slope function (b)	Intercept (a)	r value
		Lower	Upper			
96	59.92	47.59	75.44	3.657	-1.501	0.924

Table 2. Effect of potassium dichromate on hematological indices of *Anabas testudineus* (Mean ± SD)

Parameter	**T1**	**T2**	**T3**
Heamoglobin (gr%)	11.77±0.85 [a]	6.70±0.10 [b]	10.17±0.25 [c]
TBC (X 10^6/cmm)	8400.00±360.56 [a]	11000.00±1000 [b]	9700.00±200 [c]
PVC (%)	32.63±2.14 [a]	20.93±0.45 [b]	32.00±1.45 [a]
MCV(fl)	123.70±10.52 [a]	73.27±0.47 [b]	100.93±1.23 [c]
MCH (pg)	42.53±3.01 [a]	23.33±0.38 [b]	33.03±1.61 [c]
MCHC (%)	35.10±1.25 [a]	31.67±0.15 [b]	33.23±1.03 [a,b]
RBC	3.30±0.26 [a]	2.47±0.40 [b]	2.82 ±0.32 [a,b]
Neutrophils (%)	17.68±0.58 [a]	12.00±1.00 [b]	21.67±0.58 [c]
Lymphocytes (%)	75.00±1.00 [a]	81.00±1.00 [b]	72.00±2.65 [a]
Eosinophils (%)	3.00±0.00 [a]	1.00±0.00 [b]	4.67±0.58 [c]
Monocyte (%)	3.00±0.00 [a]	4.66±0.57 [b]	3.00±0.00 [a]

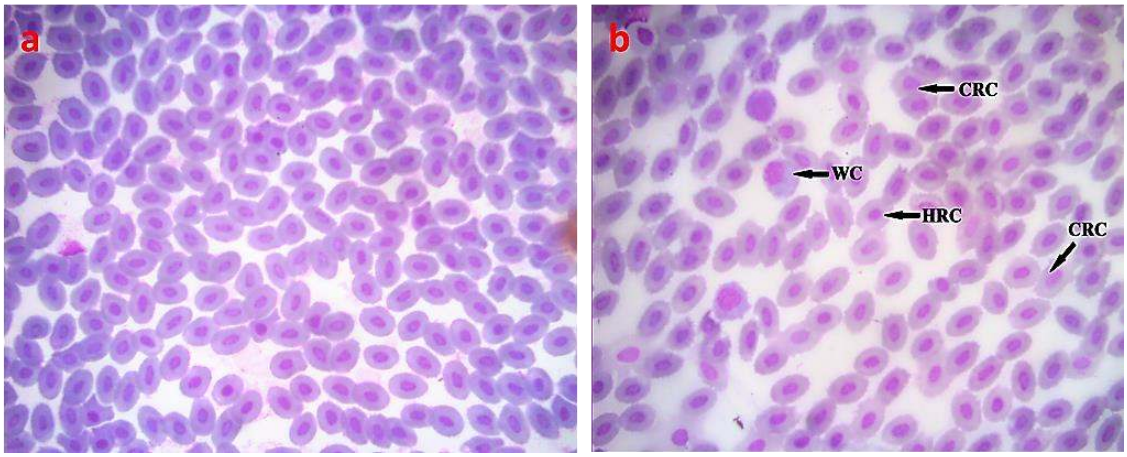

Figure 1. Morphology in the blood corpuscles of control fish (1a) and those exposed to sublethal concentration $K_2Cr_2O_7$ (1b). Presence of crenated red cell (CRC), white cell (WC) and hypochromic red cells (HRC) in exposed fishes.

dichromate for *A. testudineus* was found to be 59.92 mg/l in a static bioassay with aeration (Table 1). Acute toxicity tests of $K_2Cr_2O_7$ showed a direct relationship between mortality and concentration of the chemical, with no mortality recorded from the control groups. Table 2 shows the impact of two sub-lethal concentrations of $K_2Cr_2O_7$ on the haematological parameters. Inversely to the increasing concentration of $K_2Cr_2O_7$, a decrease in the haematological parameters such as total RBC, Hb, and PCV could be seen, while that of total WBC count increased proportionately with concentration. Duncan's Multiple Range test (DMRT) analysis revealed the differences between the three treatments. The results showed that most of the haematological variables were significantly (P<0.01) different within the two sub-lethal concentrations indicating that even a slight change in the sub-lethal concentration had a direct bearing on the haematological parameters.

Studies on the morphology of a mature erythrocytes showed that healthy *A. testudineus* had an elliptical erythrocyte and nucleus, which was centrally located (Fig. 1a). However, under sub-lethal concentrations of $K_2Cr_2O_7$ the cell wall become crenate and the cells club together. Presence of Hypochromic Red Cells (HRC) in fishes exposed to sub-lethal concentration indicates a disproportionate reduction of red cell hemoglobin, which ultimately

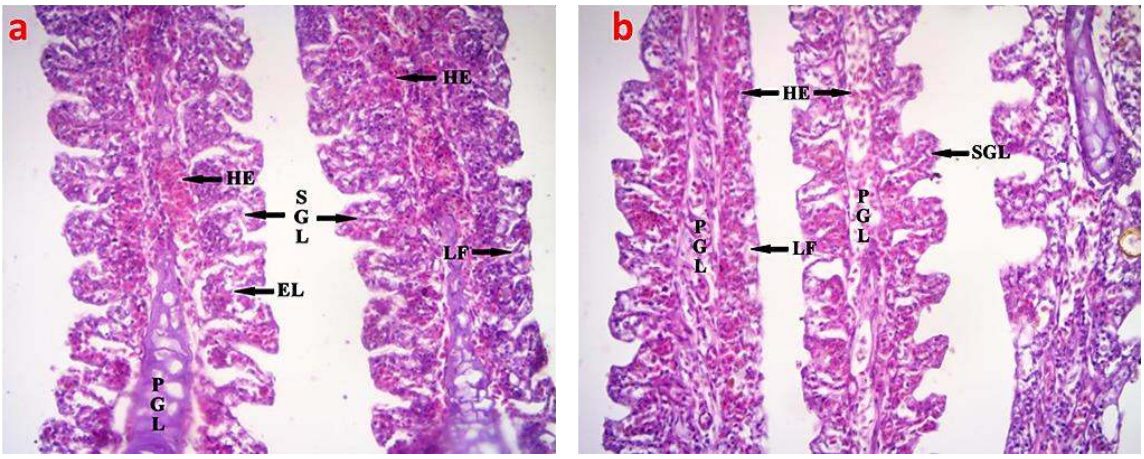

Figure 2. Histological section showing gill of exposed fish to sublethal concentration of $K_2Cr_2O_7$. Hyperplasia in lamellar epithelium leading to lamellar fusion (LF), epithelial lifting (EL) and curling of secondary lamellae (SGL) due to exposure to dichromate.

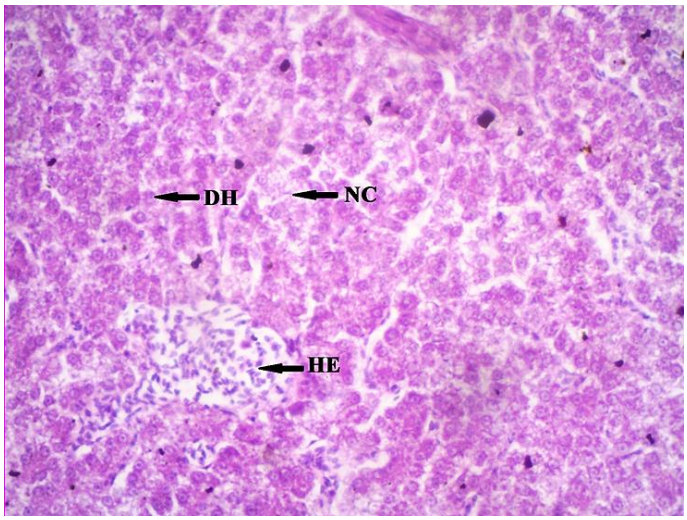

Figure 3. Histological section showing hepatic tissue of exposed sublethal concentration of $K_2Cr_2O_7$. Presence of hepatic haemorrhage (HE) necrosis (NC) and degenerated hepatocyte (DH) in hepatic tissues.

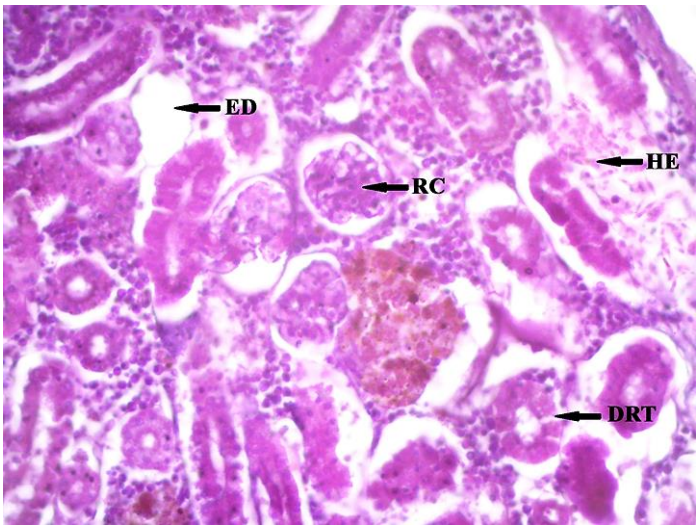

Figure 4. Histological section showing renal tissue of exposed sublethal concentration of $K_2Cr_2O_7$. Exposed fishes with degeneration of cortical tubules epithelial cell (ED), renal corpuscles (RC), renal haemorrhage (HE) and degeneration of renal tubules (DRT).

affects the fish physiology (Fig. 1b).

The results of histopathological studies suggested that once exposed to $1/5^{th}$ of the sublethal concentration of Potassium dichromate, remarkable morphological alterations in the ultrastructure of gill, liver and kidney tissues could be noticed. The histological analysis in the treated fish showed abnormalities in secondary gill lamellae (SGL); lamellar fusion (LF), epithelial lifting (EL) and haemorrhage (HE) (Fig. 2a, b). Microscopic examination of the treated liver showed degenerated hepatocytes (Fig. 3). The hepatic tissue of fish exhibited additional structural alteration such as dilated sinusoids, a modest collection of blood in the liver parenchyma (HE), partial necrosis (NC). Similarly, the renal tissue also showed marked alteration such as edema, interstitial haemorrhage, and degeneration of renal tubules (DRT) (Fig. 4). Therefore, the results illustrate the severe impact of sub-lethal concentration of $K_2Cr_2O_7$ on the blood and vital organs of *A. testudineus* clearly.

Discussions

It is evident from the present study that the lethal effect of $K_2Cr_2O_7$ in *A. testudineus* could be directly attributed to reduced Hb levels. The reduction of

haemoglobin affects the oxygen binding capacity (Mini, 2014) and also manifest anaemic condition in fish, which may be due to the stress related hemolysis (Mallesh et al., 2004). Cr(VI) is highly water soluble, easily penetrable through the biological membranes and causes cellular damage by inducing oxidative stress (Irwin et al., 1997; Begum et al., 2006). The study also shows various abnormalities in the morphology of erythrocytes which is related to the capacity of Cr(VI) to disintegrate erythrocytes and leaving the nuclei free in the blood film, as well as cell fragments (Smith, 1968). The reduction in hematocrit values is an indication of anaemia or oligohemia (Wepener et al., 1992). The lowered MCV and MCH values were observed among the 12 mg/l concentration treatments (T2) indicating the possibility of microcytic anaemia. Changes in MCH may be due to increased lysis of RBCs and reduction in cellular blood iron, which reflects a decline in Hb content due to metal toxicity (Sharma and Langer, 2014). In the present study, leucocytes count in fish of T2 was high compared to control (T1), and this indicates protective response during Cr(VI) exposure (Bhatkar, 2011). The changes observed in the blood cells were corresponding to the concentration of the toxicant and duration of the exposure time.

The Cr(VI) exposure exhibited marked degenerative changes in the histology of gills, kidney and liver tissues. In the present findings, fusion of gill lamellae was observed, and it seems to be resulting from changing and/or coagulation of mucus through altering the composition of glycoprotein as reported by Khalesi et al. (2016). The present study also revealed that there is a strong link between liver damage and chromium toxicity, which results in extensive hepatocyte necrosis with chronic inflammation. The kidney is yet another organ susceptible to sub-lethal doses of $K_2Cr_2O_7$. In the present study, degeneration of tubular epithelial cells and tubular necrosis was observed at higher concentrations of Cr(VI) exposures, which This may be due to the accumulation of inflammatory cells associated with Cr toxicity (Kurtović et al., 2008).

Conclusion

The present study is an *in situ* analysis of the effect of Cr(VI) that enter the aquatic ecosystem through effluents discharged from various industries. Fishes are highly prone to chromium toxicity as they assimilate the metal by ingestion or through gill uptake and accumulate it in hepatic and renal tissues. The overall toxic impact on organs like gill, kidney, and liver may seriously affect the metabolic, physiologic activities and could impair the growth and behaviour of fish. The present study brings out the impact of potassium dichromate in freshwater air-breathing fish *A. testudineus*. Exposure to the sublethal concentrations of this metal resulted in significant changes in hematological and histopathological parameters.

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