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Hexavalent chromium induced oxidative, histopathological and hematological changes in snakehead fish, *Channa punctatus*

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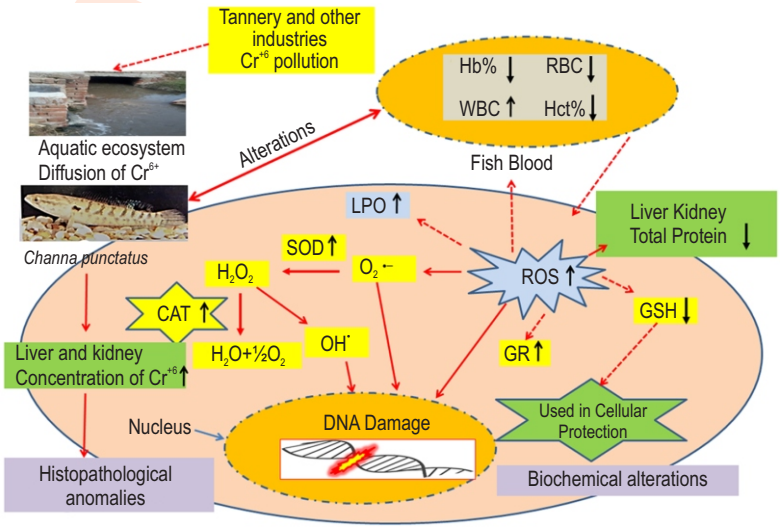
Abstract

Aim: To evaluate the effect of hexavalent chromium on biochemical, hematological and histopathological responses and to assess its concentration in fish *Channa punctatus*.

Methodology: Fishes were randomly distributed into Cr^{6+} treated groups, T_1 (2 mg l^{-1}), T_2 (4 mg l^{-1}), T_3 (6 mg l^{-1}), and one control (0 mg l^{-1}) for 30 days exposure period. Potassium dichromate ($K_2Cr_2O_7$) was used as source of Cr^{6+}. The liver, kidney and blood were sampled after 10, 20 and 30 days intervals.



Results: The significant ($p < 0.05$) increments in the concentration of hexavalent chromium and in the activities of enzymes (superoxide dismutase, catalase and glutathione reductase) as well as lipid peroxidation were recorded in the liver and kidney tissues of fish.However, the significant ($p < 0.05$) decline of reduced glutathione and total protein were noticed in both the tissues of fish. The red blood corpuscles count, hemoglobin and hematocrit significantly ($p < 0.05$) decreased, while the white blood corpuscles count increased in blood of fish. Remarkably, the histopathological anomalies, pyknosis, hypertrophy and inflammation in liver and cavity reduction in renal tubules and glomerulus degenerative changes in kidney were prominently observed in fish. Conversely, the necrosis and vacuolization were observed in liver and kidney tissues of fish.

Interpretation: The findings of the present study can be a useful tool as biomarkers for monitoring environmental pollution in aquatic ecosystem caused by hexavalent chromium.

Key words: Biochemical, Biomarkers, *Channa punctatus*, Hematological, Hexavalent chromium, Histopathological

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Introduction

Hexavalent chromium (Cr^{6+}), a stable form, is ecologically non-essential, most toxic heavy metal to the organisms. It is widely distributed in terrestrial and aquatic ecosystems through varied natural and anthropogenic sources. It is a most common contaminant of aquatic ecosystems such as surface and ground water bodies. These water bodies are the ultimate sink of industrial effluents containing heavy metals. In aquatic ecosystem, Cr^{6+} is considered as a most toxic form of chromium and it is highly capable to cross the cell membranes in living system due to its greater oxidizing, solubilizing and mobilizing nature (Oliveira, 2012). In India, the effluents from tannery (Beg and Ali, 2008) and textile industries (Gopalakrishnan and Jeyadoss, 2011) are major sources of Cr^{6+} contamination in lentic and lotic aquatic habitats. Chronically, it is concentrated and creates the gross ecological effects in aquatic fauna. Relatively, fish stand at top of the aquatic food chain (Kumar *et al.,* 2019), therefore, large amount of heavy metal is differentially concentrated in tissues of fishes.

The chronic exposure and greater concentration of Cr^{6+} pose several alterations, like molecular, physiological, biochemical including oxidative stress in fish (Bakshi and Panigrahi, 2018). In fish, its concentration engender the reactive oxygen species (ROS) or free radicals which damage the cellular molecules (Kumari *et al.,* 2014). Also, ROS breakdown proteins and enzymes consequently, it is the reason of cytotoxicity in fish (Bolognesi and Cirillo, 2014). Under stress, antioxidant defense system of organism play a key role in transforming and/or neutralizing the ROS or free radicals (Javed *et al.,* 2017). Besides biochemical parameters, the prolonged exposure of Cr^{6+} adverse by a effects the hematological components of fish. Hematological parameters are considered most important indicators of pathophysiological disturbances in fish. The analyses of hematological indices, such as haemoglobin (Hb), red blood cells (RBCs), haematocrit (Hct) and white blood cells (WBCs) are used to discern the physiological status of fish exposed to Cr^{6+} (Madhavan and Elumalai, 2016). Chronically, the biochemical and hematological changes engender the cellular and tissue grade anomalies in fish (Jerome *et al.,* 2017). These histopathological incidences associated morphological deformities amend the detoxification and biotransformation process (Jindal and Handa, 2019).

Fish are rich source of protein and widely used as an important food source for human beings. They also serve as excellent model for the study of aquatic pollution (Kumar *et al.,* 2012). Due to their higher trophic position, the heavy metals are highly concentrated in fish tissues (Annabi *et al.,* 2013). Among fishes, *Channa punctatus* is mostly used to predict the toxicity of xenobiotics, (Awasthi *et al.,* 2018). Because of its freshwater habitat, wide range of geographical distribution in south East Asian countries including India, easy availability throughout the year, low maintenance cost and easily acclimatization to laboratory conditions, it is endorsed as an excellent animal model (Talwar and Jhingran, 1991). Remarkably, liver is considered as a prime organ for detoxification of xenobiotics while kidney plays an important role in elimination of waste materials (Jaffal *et al.,* 2015). Also, the blood effortlessly illustrates the health status of fishes. To explore the deciphering amongst the eco-toxicological impairments and the concentration of Cr^{6+}, this study illustrates the generous indications for the estimation of organ-specific extent of the toxicological alterations in snakehead fish, *Channa punctatus*. Remarkably, these alterations, like biochemical, hematological and histopathological, may become the biomarkers for quick evaluation and the cost effective procedures to investigate chromium contamination in the aquatic ecosystem.

Materials and Methods

Fish, *Channa punctatus*, was collected from lentic water bodies of Lucknow, India. Fish (36 ± 4 gm; 14.20 ± 2 cm) were brought to laboratory for acclimatization under standard laboratory conditions for 10 days. After acclimatization, total 120 fishes were randomly distributed into experimental groups, each having three replicate, each replicate containing 10 fish in aquaria. The experimental groups were maintained as one control (0 mg l^{-1}) and three treated groups of Cr^{6+}, T_1 (2 mg l^{-1}), T_2 (4 mg l^{-1}) and T_3 (6 mg l^{-1}). The dose of 2 mg l^{-1} for T_1 was selected according to the maximum permissible limit of total chromium in the aquatic surface water bodies (Salf *et al.,* 2005). Therefore, for T_2 and T_3 groups, 4 mg l^{-1} (2 times of permissible limit) and 6 mg l^{-1} (3 times of permissible limit) were selected and the experiments were maintained for 10, 20 and 30 days of exposure periods. Feed was given to fish @ 2% of body weight. The food remains and excretory waste materials were eliminated from each aquarium every day. Blood samples were taken in EDTA (1.8 mg ml^{-1}) coated vials through heart puncture with heparinized syringe. For the quantification of red blood corpuscles (RBCs), the collected blood immediately mixed with Hayem's diluting fluid and counted using Neubauer hemocytometer. For enumeration of white blood corpuscles (WBCs), the blood was diluted in solution of WBC stain or WBC diluting fluid and counted using Neubauer hemocytometer.

For the evaluation of hemoglobin percentage (Hb%), the blood was taken and transferred into cleaned Sahli haemoglobinometer tube containing N/10 HCl. Constantly, N/10 HCl was added drop by drop into the tube until its color did not exactly matched with the standard color of either sides of tubes of instrument. For the estimation of hematocrit (Hct) value, the blood was taken in sterilized centrifuge tubes and EDTA was added to it. Further, the blood samples were centrifuged then RBCs separated and settled down on bottom of tubes. The settled RBCs represented the packed cell volume (PCV) which is referred as hematocrit. The hematocrit was calculated by aforesaid standard Wintrobe method. For the evaluation of histopathological changes, the liver and kidney tissues were immediately taken out from the control and treated fishes, arbitrarily. Thereafter, they were washed thoroughly and put in saline water. Further, the tissues were fixed in Bouin's fluid for 2 days and dehydrated in 70% ethanol. After that, the tissues were embedded in paraffin wax and were sectioned using rotary microtome. Firstly, the slides having tissues were stained with hematoxylin for 1 min, then, eosin dye for 2 min. Lastly, the slides were mounted in DPX

(distyrene, plasticizer and xylene) and observed under light microscope. Fish were dissected and liver and kidney tissues were collected and subjected to acid digestion. Finally, samples were dipped in glass bottles containing HNO_3 and $HClO_4$ 10:1 (v/v) ratio for 24 hrs. The digested tissues were heated at 100°C, and the concentrations of Cr^{6+} (µg g^{-1} of dry tissue weight) were estimated on a Atomic Absorption Spectrophotometer (Shimadzu AA-7000F). Total protein was estimated using Bovine serum albumin as standard (Lowry *et al.,* 1951).

Superoxide dismutase (SOD) activity in liver and kidney tissues was estimated following the method of Kakkar *et al.* (1984). Firstly, the tissue sample was taken in reaction mixture (0.052 m containing Msodium pyrophosphate, pH 8.3 + 3 M phenazine methosulphate +1 M nitroblue tetrazolium chloride. The reaction was initiated by adding 1 M nicotinamide adenine dinucleotide (NADH). Thereafter, the reaction was stopped by adding 20 M glacial acetic acid and color intensity was measured at 560 nm wavelength on a UV–VIS Spectrophotometer (LAB, UV3000 plus). The SOD activity was expressed as Units min^{-1} mg^{-1} of protein. The catalase (CAT) activity was measured following the method of Aebi, (1984). For this, 100 µl supernatant of liver or kidney tissues was added to 50 mM sodium phosphate buffer (pH 7.0) and 20 mM H_2O_2. After the completion of chemical reaction, its activity (Units min^{-1} mg^{-1} of protein) was measured at 240 nm on a UV-VIS Spectrophotometer (LAB, UV3000 plus). Glutathione reductase (GR) activity was determined by the method of Carlberg and Mannervik (1975) by measuring NADPH oxidation at 340 nm. A 100 µl of supernatant of liver and kidney tissues was separately taken in reaction buffer (0.1 M potassium phosphate + 0.5 mM EDTA + 0.1 mMKCl; pH 7.5 + 100 µl of H_2O + 100 µl of 0.1 mM NADPH) and the reaction was initiated by adding100 µl of 1 mMGSSG. The GR activity (µg mg^{-1} of protein) was determined using UV–VIS spectrophotometer (LAB, UV3000 plus). The reduced glutathione (GSH; µg mg^{-1} of protein) was evaluated by the method of Ellman (1959).

The supernatant of liver and kidney tissues were mixed with 1000 µlTrisHCl buffer and color was appeared by adding 0.01 M 2, 20-dinitro-5, 50-dithiobenzoic acid (Ellman's reagent). This reaction was completed in 10 min and its concentration was estimated by measuring absorbance at 412 nm with the help of UV–VIS spectrophotometer (LAB, UV3000 plus). The level of lipid peroxidation (LPO) in liver and kidney tissue was measured by thiobarbituric acid reactive substances (TBARS) assay (Buege and Aust, 1978). Initially, the tissues supernatant was mixed with 2 ml of TBA reagent (20% TCA + 0.5% TBA + 2.5 N HCl). After the speculated time period, the absorbance was read at 535 nm on a Spectrophotometer (LAB, UV3000 plus). LPO (nM MDA/mg protein) was calculated in terms of MDA concentration using an extinction coefficient of 1.56×105 /M/cm. Data values were expressed as mean ± standard error mean (S.E.M.). One-way ANOVA with Tukey post hoc test was employed to test the significance at $p < 0.05$. All values were analyzed using SPSS software (Version 20: IBM, Armonk, NY, USA).

Results and Discussion

The concentration of Cr^{6+} metal was significantly ($p< 0.05$) increased in liver and kidney of fish in a dose-and time-dependent manner. (Fig. 1A). On exposure period of 30 d, the highest Cr^{6+} concentration was recorded 294.38% (2 mg l^{-1}), 358.40% (4 mg l^{-1}) and 464.32% (6 mg l^{-1}) in liver and 246.04% (2 mg l^{-1}), 329.75% (4mg l^{-1}) and 431.61% (6 mg l^{-1}) in kidney over the control. The liver displayed its higher concentration than other tissues like kidney in a concentration- and time-dependent manner because of its reaction with amino groups, mercapto groups (nitrogen and sulphur) of metallothionein and oxygen carboxylate in hepatocytes (Al-Yousuf *et al.,* 2000), detoxification process leading to the tendency of concentrating metals (Handa and Jindal, 2021).

Table 1: Cr^{6+} exposure altered various hematological parameters in treated groups, T_1 (2 mg l^{-1}), T_2 (4 mg l^{-1}) and T_3 (6 mg l^{-1}) as compared to control after 10, 20 and 30 days.

Experimental groups	Exposure periods (days)	RBC (x 10^6 mm^{-3})	Hb (g%)	WBC (x 10^3 mm^{-3})	Hct (%)
Control	10	2.91±0.11	10.99±0.39	15.18±0.50	32.54±0.07
	20	2.84±0.04	10.51±0.18	16.35±0.08	33.24±0.28
	30	2.94±0.02	11.42±0.64	16.41±0.32	33.64±0.09
T_1	10	2.68±0.14(-7.88)	9.06±0.17*(-17.58)	17.32±0.07*(+14.09)	28.63± 0.11*(-12.01)
	20	2.38± 0.17(-16.06)	8.41±0.03*(-19.96)	18.52±0.13*(+13.22)	26.76± 0.35*(-19.48)
	30	1.93±0.04*(-34.2±7)	7.85±0.25*(-31.22)	20.08±0.24*(+22.35)	24.33±0.24*(-27.67)
T_2	10	2.35±0.06*(-19.31)	8.01±0.17*(-27.12)	20.66±0.19*(+36.10)	26.55±0.16*(-18.39)
	20	1.95±0.18*(-31.30)	7.75±0.11*(-26.24)	21.39±0.23*(+30.79)	24.55± 0.47*(-26.13)
	30	1.61± 0.14*(-45.13)	6.98±0.32*(38.86)	23.18±0.39*(+41.26)	21.72±0.27*(-35.43)
T_3	10	1.83± 0.09*(-37.02)	7.02±0.27*(-36.13)	23.63±0.17*(+55.70)	23.24±0.04*(-28.41)
	20	1.48±0.02*(-47.71)	6.83±0.12*(-34.99)	24.83±0.09*(+51.84)	21.96±0.30*(-33.93)
	30	1.16±0.05*(-60.40)	5.93±0.38*(-48.05)	26.73±0.08*(+62.89)	17.29±0.21*(-48.58)

Values are mean of 10 replicates ± S.E.M (n = 10) and superscript * shows the statistical significance at $p < 0.05$; Values in parenthesis illustrate the percent changes over control.

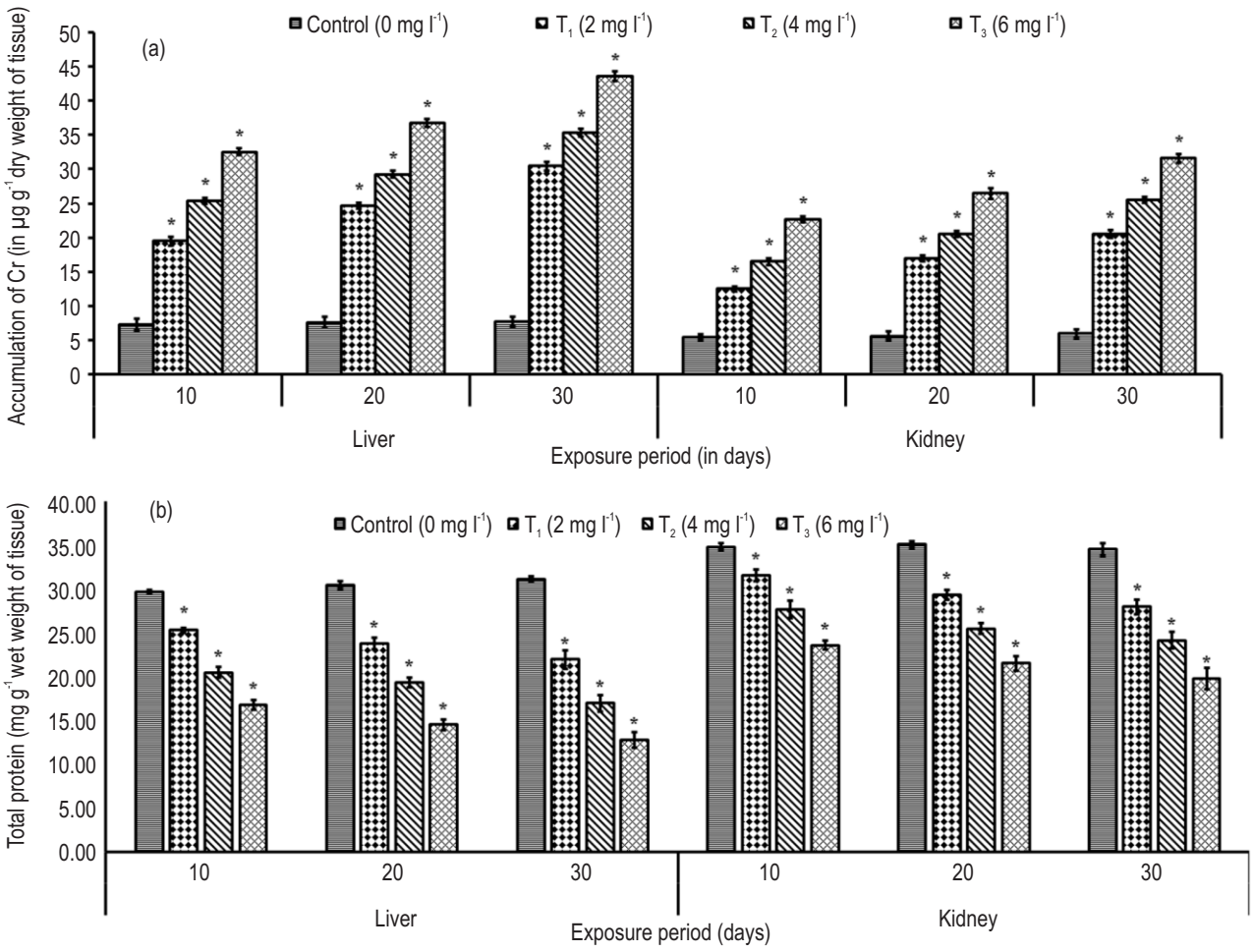


Fig. 1: The concentration of Cr^{6+} (A) and total protein (B) in liver and kidney tissues of *Channa punctatus* treated groups, T_1 (2 mg l^{-1}), T_2 (4 mg l^{-1}) and T_3 (6 mg l^{-1}) after 10, 20 and 30 days (Values are expressed as mean ± S.E.M., n = 10 fishes in each replicate, *represents significant ($p < 0.05$) values of treated groups in comparison to control).

Similarly, the concentration of Cr^{6+} was documented in the different tissues of fish *Sebastes schlegelii* (Kim and Kang, 2016).

The total protein content reduced significantly ($p < 0.05$) in liver and kidney of fish in a dose- and time-dependently manner (Fig. 1B). The highest reduction in protein level was estimated 29.55% (2 mg l^{-1}), 45.48% (4 mg l^{-1}) and 59.04% (6 mg l^{-1}) in liver and 19.07% (2 mg l^{-1}), 30.13% (4 mg l^{-1}) and 42.82% (6 mg l^{-1}) in kidney, after 30 days. Remarkably, the proteins are involved in maintaining the architecture of cell and in physiological metabolism of organisms. Thus, the depletion of protein fraction in liver and kidney tissues may due to their degradation and possible utilization for metabolic purposes (Palaniappan and Muthulingam, 2016). Its reduction was due to the breakdown of proteins in the fabrication of some amount of energy in tissues of organism (Tulasi and Rao, 2013). Also, in the chromium exposed fish, *Channa striatus*, the decline in protein level might be principally due to its utilization in the tissue repairing and organization of constituents of the cell membranes and organelles (Palaniappan and Muthulingam, 2016). GSH level significantly ($p < 0.05$) decreased in liver and kidney tissues of fish in a dose- and time-dependent manner. (Fig. 2A). The maximum decrease in GSH level was observed in liver as 30.19% (2 mg l^{-1}), 38.35% (4mg l^{-1}) and 49.08% (6 mg l^{-1}) followed by kidney as 27.82% (2 mg l^{-1}), 37.51% (4 mg l^{-1}) and 45.21% (6 mg l^{-1}) after 30 days of exposure period as compared to control. The decrease in GSH level was due to oxidation of GSH into oxidized glutathione (GSSG) during neutralization of free radicals. In fact, GSH is considered as a non-enzymatic antioxidant, which protects the cellular system as a result of detoxification of oxidizing radicals (Valko *et al.,* 2007). Similar observations associated with the reduction in GSH level were reported in metals exposed tissues of fish (Fatima *et al.,* 2015). Lipid peroxidation (LPO) level was significantly ($p < 0.05$) increased in liver and kidney of fish in a dose- and time-dependent way. (Fig. 2B). The level of lipid peroxidation was highly increased as 68.00% (2 mg l^{-1}), 93.32% (4

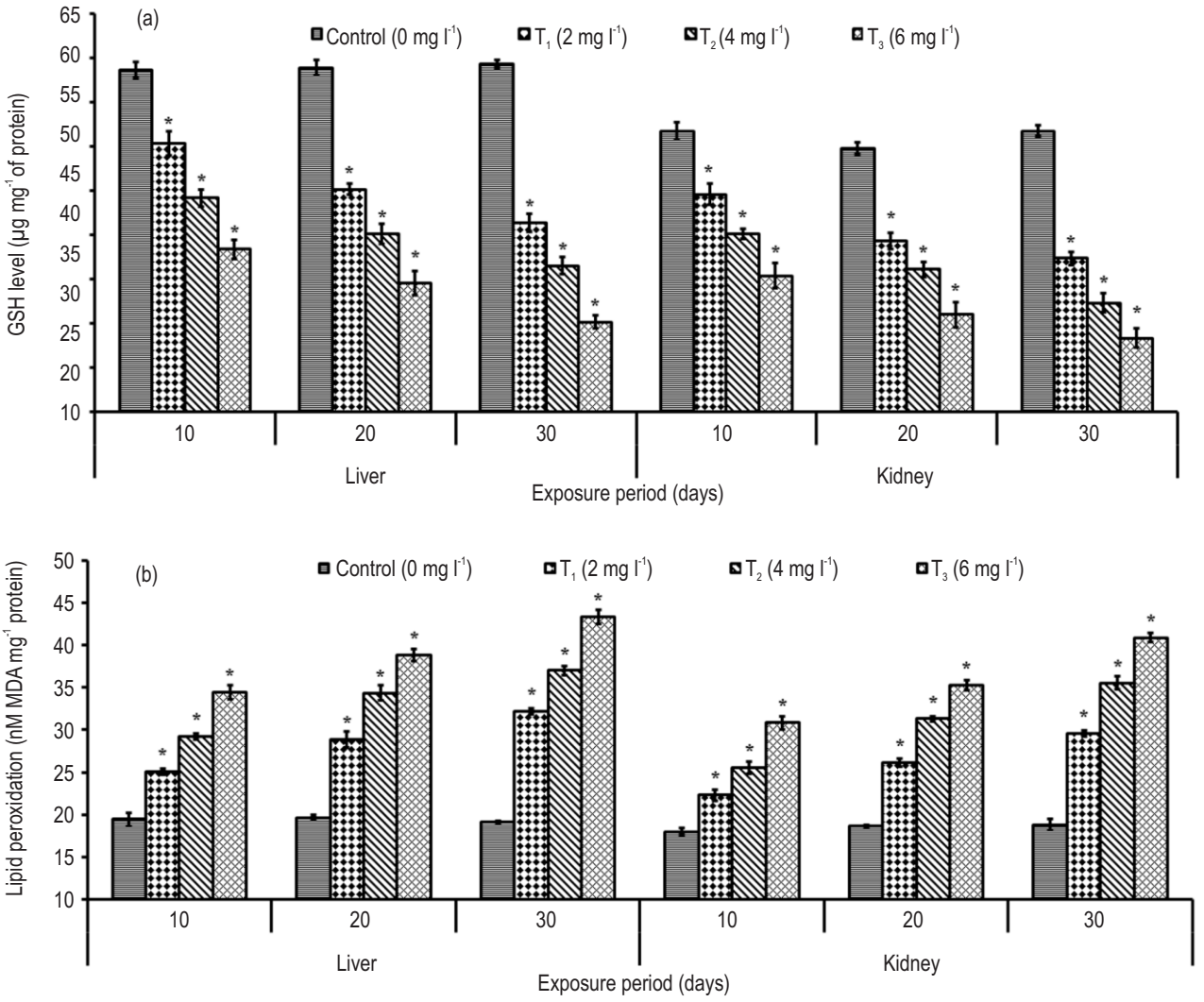


Fig. 2: Graphs showing significant ($p < 0.05$) reduction in GSH level (A) and increment in lipid peroxidation (B) in liver and kidney of fish of treated groups, T_1 (2 mg l^{-1}), T_2 (4 mg l^{-1}) and T_3 (6 mg l^{-1}), in comparison to control, after 10, 20 and 30 d (Values are expressed as mean ± S.E.M., n = 10 fishes in each replicate, *represents significant ($p < 0.05$) values of treated groups as compared to control).

mg l^{-1}) and 126.35% (6 mg l^{-1}) in liver and 56.91% (2 mg l^{-1}), 88.60% (4 mg l^{-1}) and 116.78% (6 mg l^{-1}) in kidney, after 30 d. This process mainly enhanced due to the interaction between the oxygen reactive species and polyunsaturated fatty acids (PUFA), having double bonds, which are more susceptible to the peroxidation (Javed *et al*., 2017). In fishes, lipid peroxidation event excessively occurred under the stressed environment because of having higher availability of PUFA.

The metals basically detected herein are all in redox active form, and concentrated prominently in fish tissues. Further, they contribute to the generation of ROS which are responsible for damaging lipids, proteins and DNA (Javed *et al.,* 2017). These oxidative damages of cellular components are involved in the elevation of lipid peroxidation process. Previously, the chromium induced oxidative stress, as demonstrated by the increased lipid peroxidation in liver and kidney tissues of Cr^{6+} exposed fish *Cyprinus carpio* (Yonar *et al*., 2014). The significant ($p < 0.05$) increment in the activities of SOD (Fig. 3A), CAT (Fig. 3B) and GR (Fig. 3C) was observed in Cr^{6+} exposed liver and kidney tissues of fish in a dose-and time-dependent manner. In reference to control, the highest increase in SOD activity was reported in liver as 19.50% (2 mg l^{-1}), 26.81% (4 mg l^{-1}) and 35.99% (6 mg l^{-1}), followed by kidney where it was 17.13% (2 mg l^{-1}), 24.35% (4 mg l^{-1}) and 31.24% (6 mg l^{-1}), after 30 days. Notably, this enzyme plays a key role in primary defence system of organism and the elevated level of SOD illustrated its scavenging property against the overproduction of reactive oxygen species under the stressed cellular environment of fish induced by chromium metal (Yonar *et al.,* 2014). Similar to SOD, the higher increase in CAT activity was

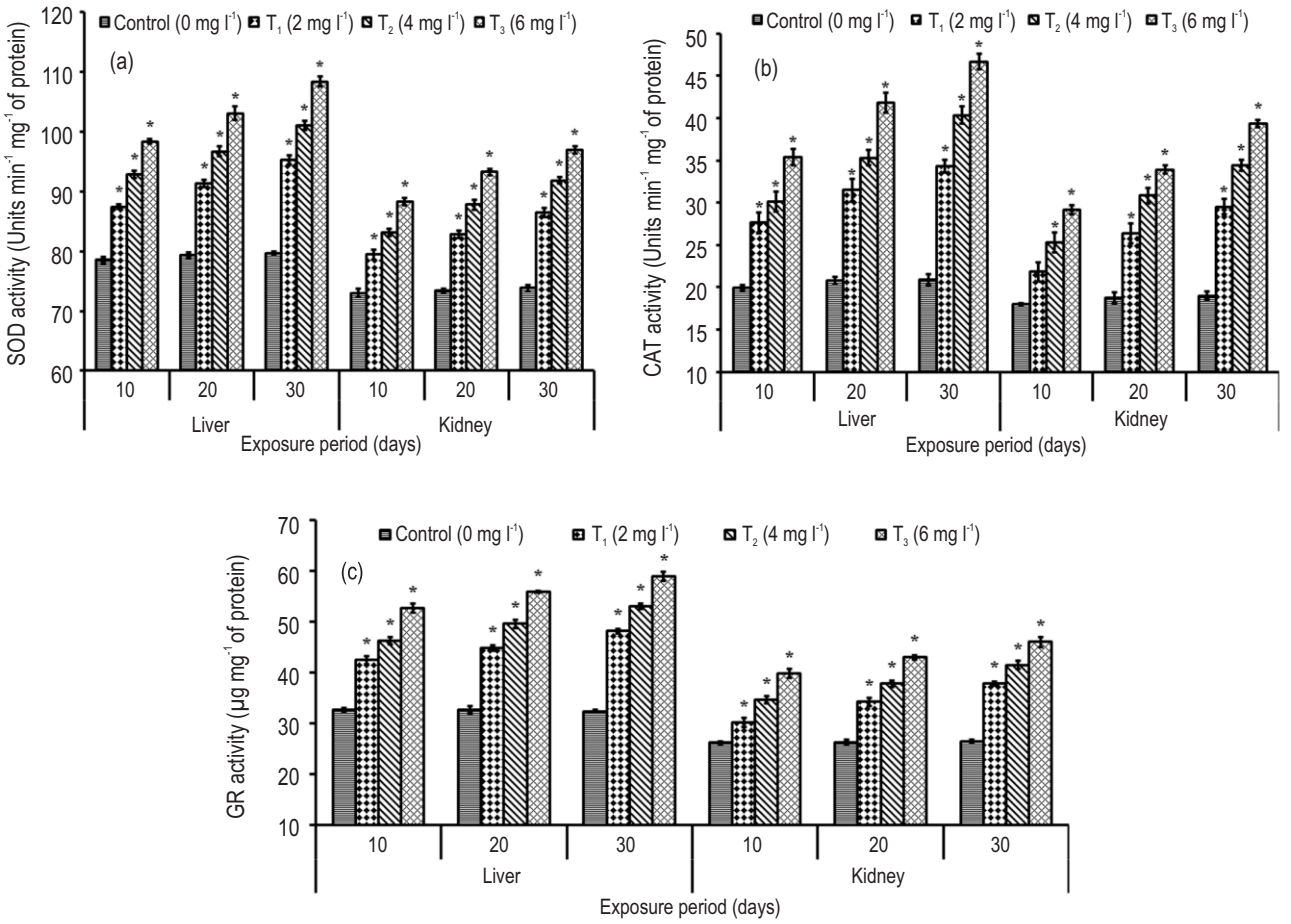
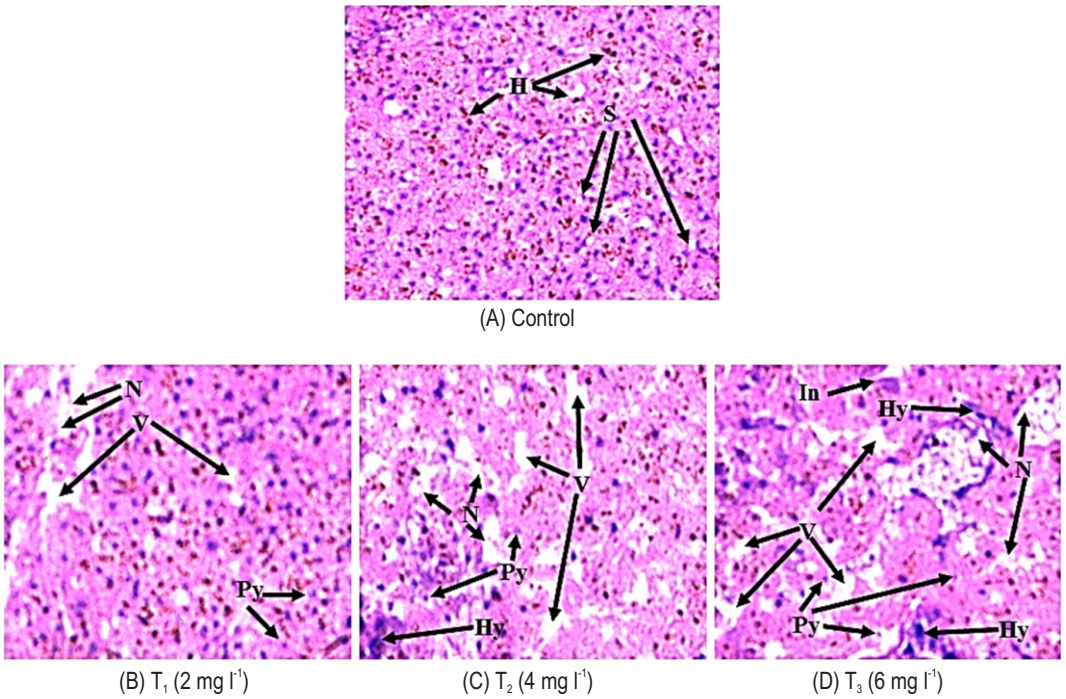


Fig. 3: Graphs showing significant ($p < 0.05$) increment in the activities of SOD (A), CAT (B), and GR (C) in liver and kidney of fish of treated groups, T_1 (2 mg l^{-1}), T_2 (4 mg l^{-1}) and T_3 (6 mg l^{-1}), in comparison to control, after 10, 20 and 30 days (Values are expressed as mean ± S.E.M., n = 10 fishes in each replicate, *represents significant ($p < 0.05$) values of treated groups as compared to control).

noticed in liver as 63.79% (2 mg l^{-1}), 92.80% (4mg l^{-1}) and 123.05% (6 mg l^{-1}) than in kidney as 55.61% (2 mg l^{-1}), 81.48% (4 mg l^{-1}) and 107.25% (6 mg l^{-1}), after 30 days. Similar findings regarding the increased activity of CAT are also well documented in tissues of fish (Ratn *et al.,* 2017). In normal course of action, SOD and CAT are considered as markers of first-line defense against oxygen toxicity (Atli *et al.,* 2006). Also, the augmented activities of SOD and CAT reveal the increased level of ROS (Awasthi *et al.,* 2019).

To maintain the appropriate level of GSH, GR transforms the oxidised glutathione (GSSH) into reduced glutathione (GSH). The maximum increase in GR activity was also noticed in liver as 48.80% (2mg l^{-1}), 63.91% (4 mg l^{-1}) and 82.11% (6 mg l^{-1}) and in kidney as 43.06% (2 mg l^{-1}), 56.79% (4 mg l^{-1}) and 74.00 % (6 mg l^{-1}) after 30 days over the control. These findings are correlated with the elevated GR activity in different fishes after exposure to metals (Li *et al.,* 2011). The decrease in RBC count, Hb and Hct values as well as increase in WBC count were recorded in treated groups in a dose- and time-dependent manner (Table 1). In comparison to control, the highest percent reduction in RBC count was 34.27% (2 mg l^{-1}), 45.13% (4 mg l^{-1}) and 60.40% (6 mg l^{-1}) after 30 days. Similarly, RBCs count, the maximum percent reduction in Hb level was 31.22% (2 mg l^{-1}), 38.86% (4 mg l^{-1}) and 48.05% (6 mg l^{-1}) after 30 days over control. The highest percent reduction in Hct was 27.67% (2 mg l^{-1}), 35.43% (4 mg l^{-1}) and 48.58% (6mg l^{-1}), as compared to control, after 30 days. The variations in different hematological parameters such as RBC count, hemoglobin and hematocrit revealed the strong physiological distributions in response to toxic substances (Dethloff *et al.,* 2001). Similar finding was reported in *Sebastes schlegelii* and *Clarias batrachus* intoxicated with Cr^{6+} (Madhavan and Elumalai, 2016). Further, the maximum percent enhancement in WBC count was 22.35% (2 mg l^{-1}), 41.26% (4 mg l^{-1}) and 62.89% (6 mg l^{-1}), as compared to control, after 30 days.

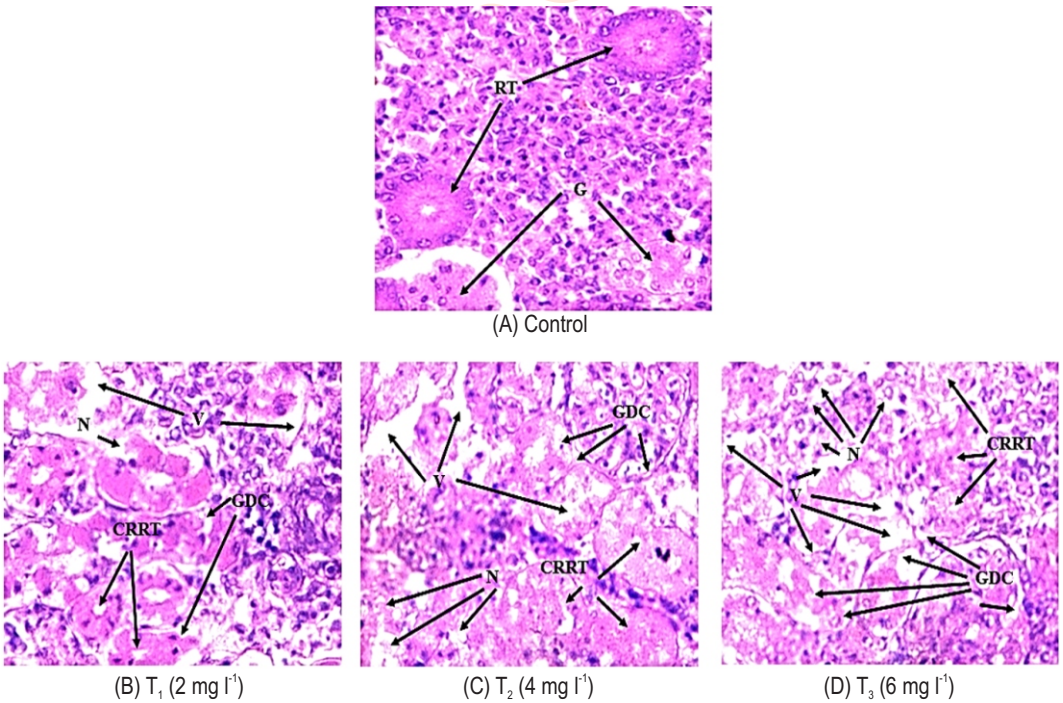
This increase in WBC count indicates stimulation of immune system to protect the freshwater fish, *Channa punctatus* under the stressed aquatic milieu generated by tannery effluent (Parveen *et al*., 2017). The microphotographs of liver tissue illustrate the histological alterations in treated groups (2, 4 and 6



(A) Control

(B) T_1 (2 mg l^{-1}) (C) T_2 (4 mg l^{-1}) (D) T_3 (6 mg l^{-1})

Fig. 4: Microphotographs of liver of fish showing compact normal hepatocytes (H) and well-spaced sinusoids (S) in control (panel A), along with necrosis (N), vacuolization (V), pyknosis (Py), hypertrophy (Hy) and inflammation (In) in treated groups, T_1 (panel B), T_2 (panel C) and T_3 (panel D) after 30 days under 40X magnification of objective lenses.



(A) Control

(B) T_1 (2 mg l^{-1}) (C) T_2 (4 mg l^{-1}) (D) T_3 (6 mg l^{-1})

Fig. 5: Microphotographs of kidney of control fish showing normal renal tubules (RT) and glomerulus (G) in control (panel A), but in the case of T_1 (panel B), mild vacuolization (V), necrosis (N), cavity reduction in renal tubules (CRRT), and glomerulus degenerative changes (GDC) were observed. These aforesaid changes were found moderately in T_2 (panel C) and strongly in T_3 (panel D) after 30 days under 100X magnification of objective lenses.

mg l^{-1}) in *Channa punctatus*, as compared to control, after 30 days of exposure (Fig. 4). The microscopic examination of liver of control exhibited a regular symmetry in mass of hepatocytes (H) along with fluid filled sinusoids (S) (Fig. 4 A). However, the liver of fish of T_1 group showed mild tissue abnormalities such as vacuolization (V), necrosis (N) and pyknosis (Py) (Fig. 4 B). The anomalies like necrosis (N), pyknosis (Py), vacuolization (V) and hypertrophy (Hy) were moderately increased in liver of fish of T_2 group (Fig. 4 C). In addition to above alterations, the major changes observed in liver tissue were necrosis (N), pyknosis (Py) vacuolization (V), hypertrophy (Hy) and inflammation (In) in fish of T_3 group after 30 days of exposure (Fig. 4 D). Notably, the alterations in liver due to metal toxicity are often associated with the degenerative necrotic condition (Mishra and Mohanty, 2008). Moreover, it was also recorded that the prolonged exposure of copper induced the histopathological changes, vacuolization and necrosis, in the liver of Nile tilapia, *Oreochromis niloticus* (Figueiredo-Fernandes *et al*., 2007).

Comparatively, the figures show the histopathological kidney anomalies in treated groups of *Channa punctatus* (2, 4 and 6 mg l^{-1}), as compared to control, after 30 days (Fig. 5). The image of kidney of fish of control group illustrated uniform functional units called normal renal tubules having adequate space and normal glomerulus structure surrounding anatomical architecture in an organized manner (Fig. 5 A). The slight cavity reduction in renal tubules (CRRT), glomerulus degenerative changes (GDC) and minor/light vacuolization (V), and necrosis (N) are shown in kidney of fish of T_1 group (Fig. 5 B). Whereas, the kidney of fish of T_2 group indicated higher necrosis, vacuolization, extensive cavity reduction in renal tubules (CRRT) and glomerulus along with degenerative changes (GDC) (Fig. 5 C). Further, the image of kidney of fish of T_3 group displayed the highest increase in necrosis, vacuolization, extensive cavity reduction in renal tubules (CRRT) and glomerulus degenerative changes (GDC) after 30 days of exposure (Fig. 5 D). Comparatively, the vacuolization, inflammation and necrosis were reported in the liver of fishes (Subburaj *et al*., 2015) and the reduction in renal tubules and their lumens was recorded in kidney of fish (Mishra and Mohanty, 2009). Moreover, the tissues specific abnormalities were also witnessed in metal exposed freshwater food fish (Awasthi *et al*., 2019; Ratn *et al*., 2018).

The prolonged chromium exposure engenders oxidative cellular stress via excessive production of free radicals in tissues of fish. This oxidative stress poses the biochemical, hematological and histopathological impairments in fish. Therefore, the present findings and their roles as sensitive biomarkers will be beneficial for the bio-monitoring of chromium contaminated aquatic regimes and also important for the well being of fish fauna.

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Authors' contribution: P. Kanaujia: Overall writing of the manuscript including plotting of graphs and tables; **A. Ratn:** Statistical analysed of biochemical data and analyses of histopathological images; **Y. Awasthi:** Analysis and quantification of hematological findings; **S. P. Trivedi:** Design the concept and experiments of this study; **M. Kumar:** Preliminary corrections and revision of the manuscript; **Y. K. Sharma:** Critical evaluation and reading of the manuscript.

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