EVALUATION OF GLUTATHIONE REDUCTASE (GR) ACTIVITY IN LIVER OF FISH, *CHANNA PUNCTATUS* (BLOCH, 1793) EXPOSED TO HEXAVALENT CHROMIUM

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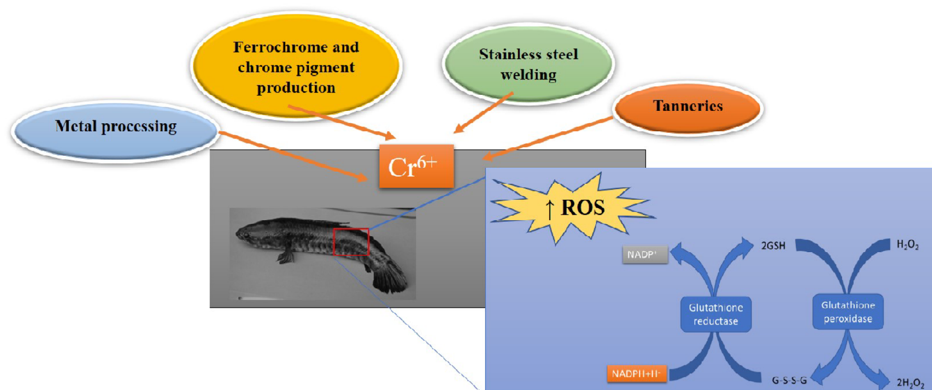
ABSTRACT: Antioxidant defence system is known to be a sensitive biomarker of metal exposure widely employed in predicting toxicity after sub-lethal exposure of Cr^{6+}in fish. The present study conducted to assess the impact of Cr^{6+}on Glutathione reductase (GR) activity in liver of fish, *Channa punctatus*. Fish were exposed to two different sub-lethal concentrations of Cr^{6+}*viz.*,3.93 mg/L (5% of 96h LC_{50}) (T1) and 7.85 mg/L (10% of 96h LC_{50}) (T2) along with one control group without any toxicant exposure, for 15, 30 and 45 d. Results showa significant (p <0.05) increase in GR activity with an increase in dose and exposure period. This study emphasized the role of GR in monitoring of metal toxicity in aquatic regimes as an early warning tool.

KEYWORDS: Cr^{6+}, *Channa punctatus,* Glutathione reductase, Liver, Antioxidant.



INTRODUCTION

Biomonitoring of metal pollution in aquatic ecosystem is important in many aspects and is very often done in sentinel organisms like fishes because they provide an insight about the manifestation of toxicant induced stress. Use of fish in this kind of toxicological studies have a reason because consumption of fish contaminated with metals harshly affects human health. Among all contaminants, heavy metals are significant environmental toxicants due to their non-biodegradable nature. Chromium mainly exists in two valence state *i.e.,* Cr^{3+} and Cr^{6+}in environment. In comparison to Cr^{3+}, $Cr^{6+}$is more toxic and a potent human carcinogen[1]. According to Environmental

Protection Agency (EPA), $Cr^{6+}$is listed as one of the priority pollutant at 129th rank. It also comes under 14 most noxious heavy metals due to its wider application in industries like electroplating, stainless steel production, tanning and textile manufacturing; consequently, Cr^{6+} gets easily discharged into aquatic ecosystem[2]. When released to land, Cr^{6+} compounds bind to soil and in water these compounds are very continual as sediments, with a high potential for accumulation in aquatic life[3]. Cr^{6+}produces cytotoxicity and detrimental impact on behaviour of fish such as hypertrophy and paraplegia in gill epithelium, uneven swimming and suspended feeding [4].

For long, fishes are being used as a genetic model to elucidate toxicological mechanisms induced by contaminants for hazard identification in humans *via* read-across. Moreover, these mechanisms are not only conserved among fish species but also between other vertebrates including mammals and even humans[5]. Fish, *Channa punctatus* is becoming increasingly important in toxicological research, particularly in South-east Asian countries, due to its availability throughout the year, easy maintenance in aquaria, a broad range of responsiveness and sensitivity against a variety of aquatic pollutants[6,7].

The toxicity of Cr^{6+} in biological systems is closely related with greater generation of reactive oxygen species (ROS)[8,9]. Superoxide (O^{-2}), one of the parental forms of intracellular ROS, is highly reactive molecule, but it can be converted to H_2O_2 that is produced by the action of superoxide dismutase (SOD)and reduced to water by catalase (CAT) and glutathione reductase (GR)[10,11]. GR catalyses the reduction of oxidized glutathione (GSSG) to glutathione (GSH) and it is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH[12]. GSH plays a critical role in protecting cells from oxidative damage, toxicity of xenobiotic electrophiles and maintaining redox homeostasis[13]. Cr^{6+} induced oxidative stress has already been reported in goldfish tissues wherein it affected the activity of antioxidant defence system and associated enzymes[14]. The response of GR activity could help to predict metal toxicity in the aquatic environments and be useful as an "early warning tool" in natural monitoring studies.

MATERIA S AND METHODS

2.1 Test organism and acclimatization:

Fish specimens (14 ± 1.0 cm ; 35 ± 3.0 g) of *Channa punctatus* (Bloch, 1793), a common pond murrel (class - Actinopterygii, order – Perciformes, family – Channidae), were procured from lentic habitats of Lucknow by the aid of fishermen. Fish were placed in aquarium for the prophylactic treatment with 0.05% $KMnO_4$ for 2-5 min to avoid dermal infections and acclimatized to laboratory condition (pH 6.8 ± 0.1, temperature 27 ± 3°C, dissolved oxygen (DO) 7.1 mg/L, total dissolved solids (TDS) 197.89 mg/L and hardness

72.90 as $CaCO_3$ mg/L) for 15 days following the standard procedure[15]. Fish were fed twice a day with fresh goat liver and commercial aquarium food pellets. The faecal matter and other waste material were removed daily from aquaria.

2.2 Test Chemical:

Chromium trioxide (Cr^{6+}), Batch no. T-8371997, manufactured by Sisco-Research Laboratories Pvt. Ltd., Navketan Industrial Area, Mumbai, India-400069 was purchased through a local dealer. All chemicals used during experiment are of analytical grade.

2.3 Determination of LC_{50} of Chromium trioxide:

Fish were exposed to different concentrations of 96 h-LC_{50} of the Cr^{6+} which was already worked out by Awasthi et al., 2018[16]. It was 78.54 mg/L with 95% lower and upper confidence limits of 68.14 and 90.53 mg/L, respectively.

2.4 Experimental design:

The acclimatized fish were randomly divided into three groups*viz.,*group 1 as control, group 2 (T1) and group 3 (T2) as treated groups, each comprising three replicates. The 96 h-LC_{50} of Cr^{6+} was assessed and used as a reference for the selection of sub-lethal concentrations of treated groups (T1 and T2). For the estimation of Glutathione reductase (GR) activity, fishes of groups (T1 and T2) were exposed to 3.93 mg/L and 7.85 mg/L corresponding to 5% and 10% of 96 h-LC_{50} of Chromium trioxide respectively for 45 days of experimental period. After the termination of exposure period, tissue sampling was done after 15, 30 and 45 d and 3 fish from each replicate were euthanized for the removal of liver tissue on each sampling day during experimentation. Fish were fed twice a day after every 7d interval with commercial fish food. Experiments were performed under semi-static conditions and the aquaria water was changed every 24 h with 7 d aged tap water containing relative chromium concentration[17].

2.5 Biochemical Analysis: Glutathione reductase (GR) activity

Dissected tissues were weighed and homogenized in homogenization buffer (HB; 0.32 M sucrose, 1 mM EDTA, 10 mM Tris-HCl and pH 7.4) in a proportion of 1:10 (w/v). After the homogenization, cell suspension was taken in 10 mL falcon tubes and centrifuged at 1600 rpm for 10 min at 4ºC followed by two times washing. Once more, HB was dissolved in pellet and centrifuged at 1600 rpm for 10 min at 4ºC. Finally, pellet was suspended in 500µL lysis buffer (0.1 mM Tris-HCl, 5 mM EDTA, 1% SDS, 1% β-mercaptoethanol and pH 8.0) to which 1 mM Phenylmethylsulfonyl fluoride (PMSF) and 1 mM Dithiothreitol (DTT) were added as 10 µL vol. of each chemical and then the whole mixture was vortexed. Subsequently, the cell suspension was incubated for 30 min at 4°C and centrifuged at 16,000 rpm for 15 min at 4°C. Eventually, post mitochondrial supernatant or cell lysate was collected for

further analyses of glutathione reductase.

The activity of Glutathione Reductase (GR) was determined by measuring NADPH oxidation at 340 nm[18]. The reaction mixture comprises of 600 µL of buffer (0.1 M potassium phosphate + 0.5 mM EDTA + 0.1 mM KCl; pH 7.5), 100 µL of 0.1 mM NADPH, 100 µL of H_2O and 100 µL of supernatant of liver tissue. After the pre-incubation for 5 min at 37°C, the reaction was initiated by adding 100 ìL of 1 mM glutathione disulphide (GSSG). The GR activity was estimated by using UV-VIS spectrophotometer (Shimadzu, UV-1800 pharma spec), and is expressed as µg mg^{-1} of protein.

2.6. Statistical Analysis:

All data were statistically analysed at $p < 0.05$. They were presented as mean ± standard error mean (S.E.M.). The variations in values of control and treated groupsdifferences were assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. All statistical analyses were carried out using SPSS software (version 20.0, Chicago, IL, USA).

RESULTS AND DISCUSSION

The present study aimed to elucidate the toxicity of Cr^{6+} in liver of fish, *Channa punctatus*. Cr^{6+} is regarded as a human carcinogen[19,20]. *In vivo,* Cr^{6+} enters into the cell by anion transporter mechanism, then reduces into different valence form[21]. This reduction process and various reduced states of Cr^{6+} lead to Reactive oxygen species (ROS) production[22]. The ROS species have been found to be implemented into various signalling processes; however, increasing intracellular levels found to be associated with activation of defence system in organism system. In usual course, the antioxidant enzymatic system protects organisms from the toxic effects of the activated oxygen species and helps to maintain cellular homeostasis by removing ROS[23]. As a matter of fact,the antioxidant defence system of an organism works at two levels, enzymatic and nonenzymatic systems. The enzymatic system comprises of superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx) and glutatione reductase (GR) while nonenzymatic system includes several low molecular weight scavengers of these reactive species. Among the enzymatic system, the first level of defence system is created by SOD and CAT while second level of defence system is created by GPx and GR. Among these two, GR plays an important role in maintaining the level of GSH whichdirectly involves in reduction process of Cr^{6+} when it enters into the cellular system of organism[24].

The activity of GR in liver of fishes of treated groups (T1 and T2) as compared to unexposed fish for defined exposure intervals were depicted in **Fig. 1** and **Table.1.** The activity of GR was found to be significantly ($p<0.05$) raised with an increase in a dose- and time-dependent manner. Compared to unexposed group, the activities of GR in liver increased from

5.04% to 32.98 % for T1 and from 14.42% to 47.49% for T2 group with an increase of exposure time after the onset of the experiment. On the basis of percentage change in GR activity, it shows its higher sensitivity against the oxidative damages in liver of fish, *Channa punctatus* intoxicated with Cr^{6+} chronically.

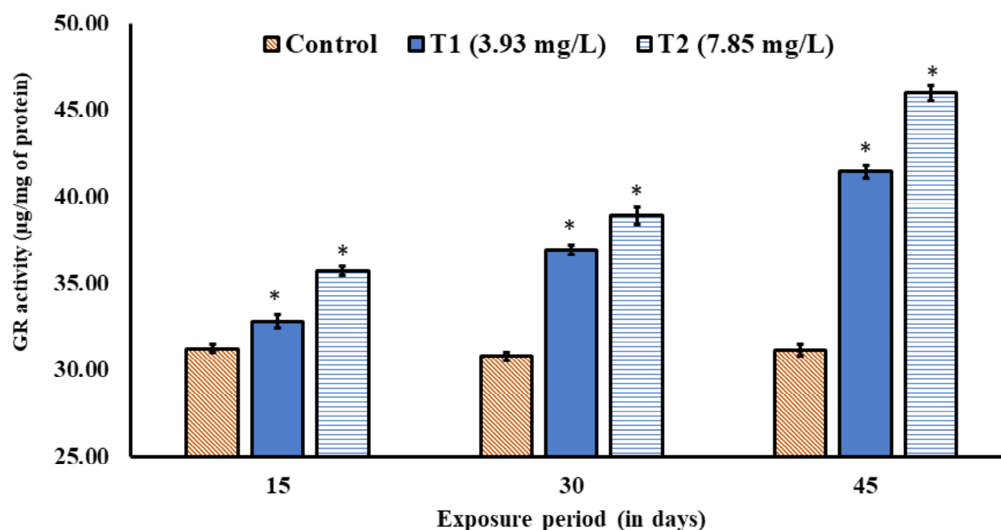


Fig. 1 The activity of GR enzyme in Cr^{6+} induced liver tissues of treated groups (T1=3.93 mg/L and T2=7.85 mg/L) and in control group (C) for 15, 30 and 45 d of exposure period. The data represent mean ± SEM (n = 3) of three replicates of each group (*$p < 0.05$ indicate significant difference as compared to respective control group (C).

Table.1 The activity of GR enzymes (in µg/mg of protein) in liver of treated groups of fishes, T1 and T2, as compared to unexposed control group after 15, 30 and 45 d of exposure.

Table.1 Activity of GR measured in liver tissue in fish, *Channapucntatus*

Oxidative stress marker	Exposed groups	Exposure periods (d)		
		15	30	45
GR activity (µg/mg of protein)	Control	31.22 ± 0.24	30.78 ± 0.23	31.16 ± 0.33
	T1 (3.93mg/L)	32.79 ± 0.39*	36.94 ± 0.25*	41.44 ± 0.36*
	T2 (7.85mg/L)	35.72 ± 0.27*	38.90 ± 0.50*	45.96 ± 0.45*

(*$p < 0.05$ represent significant difference as compare to control)

The increase in hepatic level of antioxidant enzymes is a clear indicative feature of oxidative stress. The findings of the present study revealed a significant increase in GR activity upon exposure of Cr^{6+}. Our study is in agreement with the studies of Matos et al.,(2007)[25] in which they assessed an increase in GR activity in an oxidative stress situation. Kumari et al.,(2014)[26] also reported an increment in GR activity under the exposure of Cr^{6+} in fish, *Labeorohita*. Another study on brain tissues of fish, *Oncorhynchus mykiss* also documented the similar induction in GR activities with the significant value on exposure of chromium[27]. The increment in GR activity is directly linked with the re-establishment of the GSH level that is oxidised. On other hand, decline in activity of GR leads to depletion in GSH level which results in prooxidative effect. It was also reported that depletion in GSH level causes variation in GST activity under the exposure of metal[28]. We also find ample support from the findings of Atli and Canli, (2010) [29] who have documented variation in antioxidant enzyme activity under the exposure of different metals in fish, *Oreochromis niloticus*. Thus, study indicates that Cr^{6+} induces oxidative stress as is evident by an increment in ROS production in the liver of fish, *Channa punctatus*, exposed to sub-lethal concentrations of aforesaid metal.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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