



Chromium Induced Elevated Glutathione S-transferase Activity in *Cirrhina mrigala*

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Abstract: In aquatic bodies, heavy metals contamination is a most important issue all over the world. Heavy metals are persistence in nature and can store in aquatic environment. These metals have ability to induce oxidative stress and altered the enzymes activities. Therefore, in current investigation the activity of glutathione S-transferase (GST) in hepatic, renal and cardiac tissues of fish, *Cirrhina mrigala* under various sub-lethal concentrations (1/3rd, 1/4th and 1/5th of LC50) of chromium (Cr) was evaluated. The fish was exposed to chromium for 21-day and sampling was done on weekly basis. Results demonstrated that the GST activity was significantly augmented in hepatic, renal and cardiac tissues of exposed fish in relation to control. Comparison among organs revealed that the maximum GST activity was observed in hepatic tissues of fish followed by renal and cardiac tissues. The GST activity gradually augmented with increasing the concentration and duration of exposure. In conclusion, the use of GST enzyme as biomarkers is not a new approach but can be used successfully to assess the toxicity associated with metals in aquatic animals.

Keywords: Fish, Chronic exposure, Antioxidant enzyme, Organs.

1. INTRODUCTION

Pollution of aquatic ecosystems has been increased with the rapid expansion of industries and anthropogenic activities [1]. Among these pollutants, heavy metals are unique because they cause adverse effects on aquatic plants and animals even at lower concentration [2]. Metals are non-biodegradable, have ability to a amass in aquatic animals, especially fish, [3] become harmful and at the end pass to the other living organism like humans who eat these aquatic animals as food [4]. Toxicity of metals in aquatic environment influenced by many factors includes solubility, type, and complexation. The metals interaction can alter their toxicity to aquatic life either negatively and positively [5].

Chromium in the aquatic bodies enter through waste released from textiles, leather tanneries, metal finishing, photographic and pharmaceutical industries, electroplating, mining, ceramic, dyeing

and printing industries etc. [6-7]. The lethal effects of chromium are contact dermatitis, organ system-toxicity, allergy and alterations in the histology of various parts [8].

Heavy metals have ability to produce oxidative stress by stimulating the formation of reactive oxygen species (ROS), like superoxide radical, hydroxyl radical and hydrogen peroxide, via Haber–Weiss and Fenton reactions resulted in oxidation of macromolecules such as lipids, proteins and nucleic acids, often leading to damage the structure of cell [9-10]. Aquatic animals have antioxidant defense scheme to overcome the injurious effect of ROS. This system comprises enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione S-transferase (GST) and glutathione reductase (GR) [11-12]. Glutathione S-transferase (GST) belongs to the family of detoxifying enzymes, plays a significant role in metabolism of xenobiotics, catalyzing reactions of binding xenobiotics with GSH [13]. GST is

a Phase II system enzyme which decreases the harmful effects of endo- and exogenous substances. Other than this GST performed many function in metabolism includes degradation of tyrosine, biosynthesis of hormone, dehydro-ascorbate reduction and peroxide breakdown [14].

Biochemical parameters are an important approach to assess the harmful effect in exposed fish. Oxidative stress biomarkers are now rapidly used for environmental monitoring program in the field of ecotoxicology [15]. Therefore, this experiment was performed to elucidate the sub-lethal effects of chromium on glutathione S-transferase activity in *Cirrhina mrigala*.

2. MATERIALS AND METHODS

2.1 Experimental Trail

This experiment was performed at Fisheries Research Farm department of Zoology, Wildlife and Fisheries, Universities of Agriculture, Faisalabad. Freshwater fish, *C. mrigala* were selected for this experiment. The juveniles of *C. mrigala* were obtained from Fish Seed Hatchery Faisalabad and immediately shifted to Fisheries Research at University of Agriculture, Faisalabad. Prior to experiment, *C. mrigala* fingerlings were kept in cemented tank for two weeks to acclimatize the laboratory condition. As the acclimatization period completed, fish were transferred to 75-litre glass aquarium. Each aquarium contained ten fishes. Metal solution was prepared by using chemically pure chloride compounds of chromium. The 96-LC₅₀ for *C. mrigala* was calculated as 68.22 mgL⁻¹ reported by Batool and Javed [16]. The lethal value was divided by 3, 4 and 5, and got sub-lethal concentration for *C. mrigala* about 22.74 (1/3rd), 17.06 (1/4th) and 13.64 (1/5th) mgL⁻¹. Fish, were exposed to sub-lethal concentrations for 21 days and sampling was done on weekly basis.

2.2 Physico-Chemical Parameters of Water

During the whole experiment, total hardness, pH and temperature of water were kept stable as 225 mg L⁻¹, 7.25 and 30°C. However, other variables (Ca, Na, Mg, CO₂, EC, NH₃ and K) were also calculated and maintained on daily basis [17].

2.3 Estimation of GST Activity

2.3.1. Organ Homogenate

The enzyme GST was isolated from the hepatic, renal and cardiac tissues of *C. mrigala*. The organs were weighted and add cold 50mM Tris HCL buffer (pH 7.4) containing 0.2 M sucrose, 4 times greater than the weight of organ (1:4 w/v). The organs were homogenized for 15 minutes using a pestle and mortar. After that, organ homogenates were centrifuged for 15 minutes at 10,000 rpm and 4°C. After centrifugation process, clear supernatants were stored at -80°C for enzyme assay while residue was discarded.

2.3.2. Enzyme assay

The reaction mixture of 3ml was contained 2.4 ml of 0.3 M potassium phosphate buffer (pH 6.9), 0.1ml of 30m M CDNB, 0.1ml of 30mM GSH and 0.4ml of enzyme supernatant. Absorbance was read at 340nm against the reagent blank on spectrophotometer minute [18].

2.4. STATISTICAL ANALYSIS

Data obtained from this study were analyzed by applying analysis of variance to get statistical differences among variables. The value of $p > 0.05$ were consider as statistically non-significant. MS excel was used to draw graphs.

3. RESULTS AND DISCUSSION

The level of GST in hepatic, renal and cardiac tissues of chromium exposed fish was significantly augmented as compared to control (Figure 1-3). Tissue specific response showed that maximum GST level was observed in hepatic tissue (Figure 4). Maximum GST activity was observed due to 1/3rd of LC₅₀ concentration of chromium followed by the order: <1/4th <1/5th <control. The GST activity was gradually augmented as the duration of exposure increased. The GST activity was maximum after 21-day of exposure to metal followed by 14- and 7-day. Farombi et al [19] also determined the increased GST activity in kidney, heart and liver of barbell catfish exposed to heavy metals (Cd and Cu). Yuan et al. [20] noted the Cr induced GST in whole body of *Gobiocypris raru*. Abdullah et al [21] reported the higher level of GST in liver and kidney of *Channa striata* due to lead+nikel

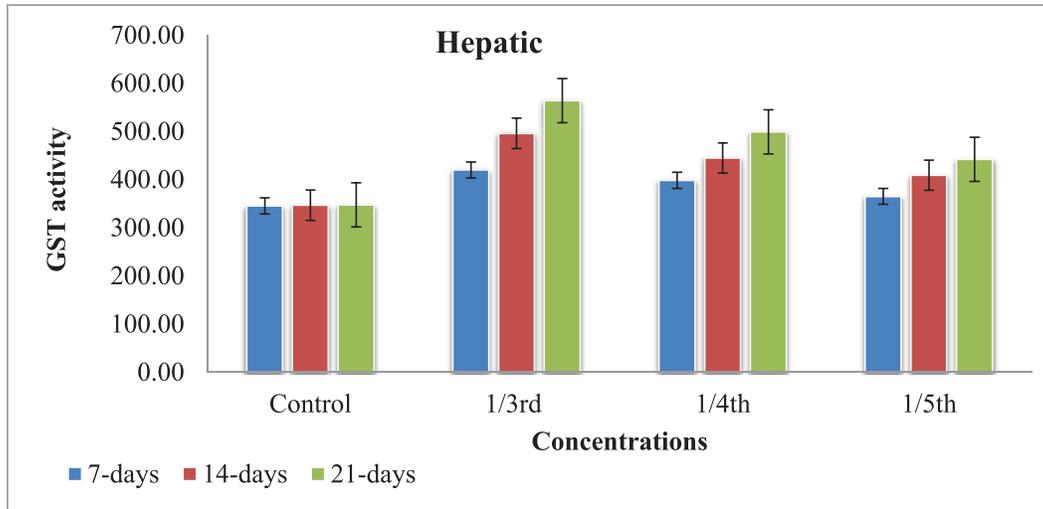


Fig. 1. GST activity (U/ml) in hepatic tissue of *C. mrigala* exposed to sub-lethal concentrations of chromium

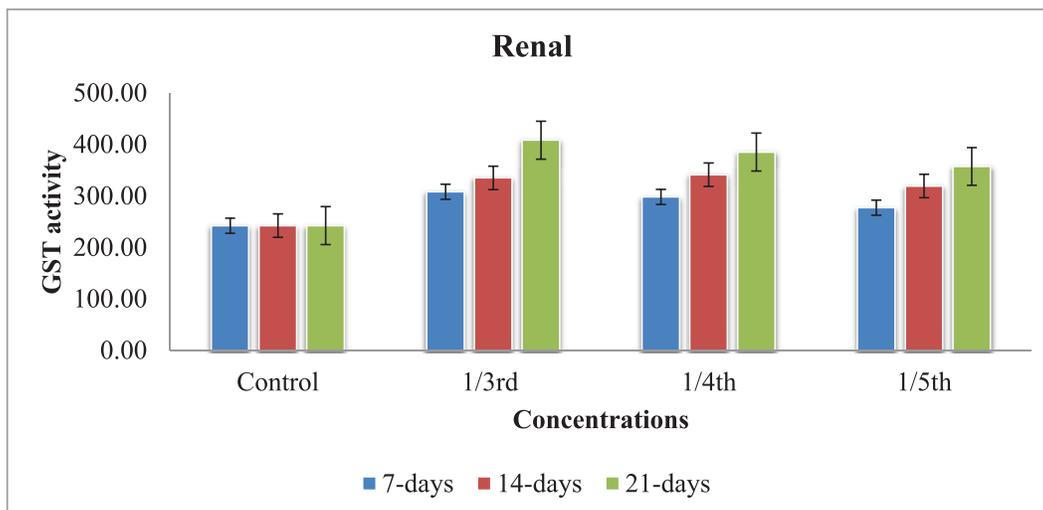


Fig. 2. GST activity (U/ml) in renal tissue of *C. mrigala* exposed to sub-lethal concentration of chromium

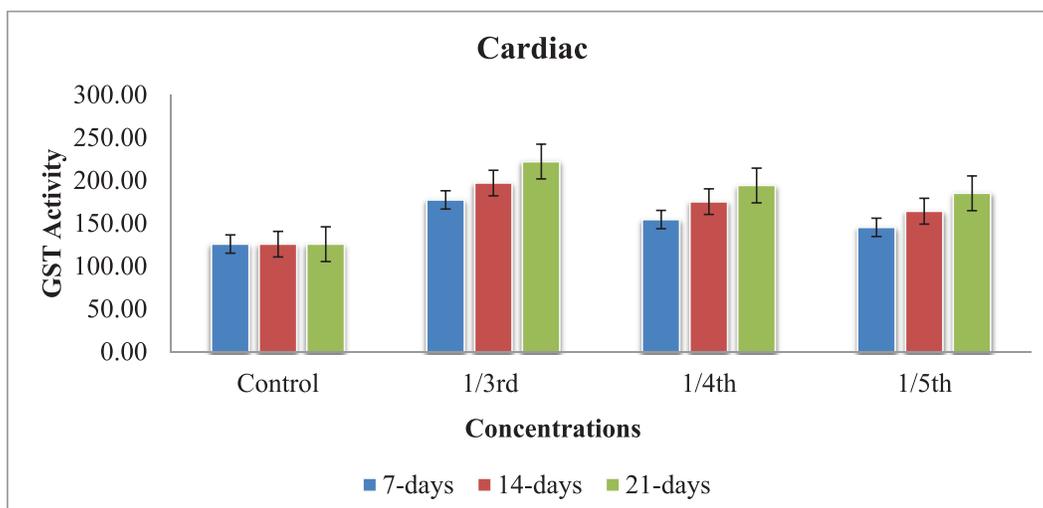


Fig. 3. GST activity (U/ml) in cardiac tissue of *C. mrigala* exposed to sub-lethal concentration of chromium

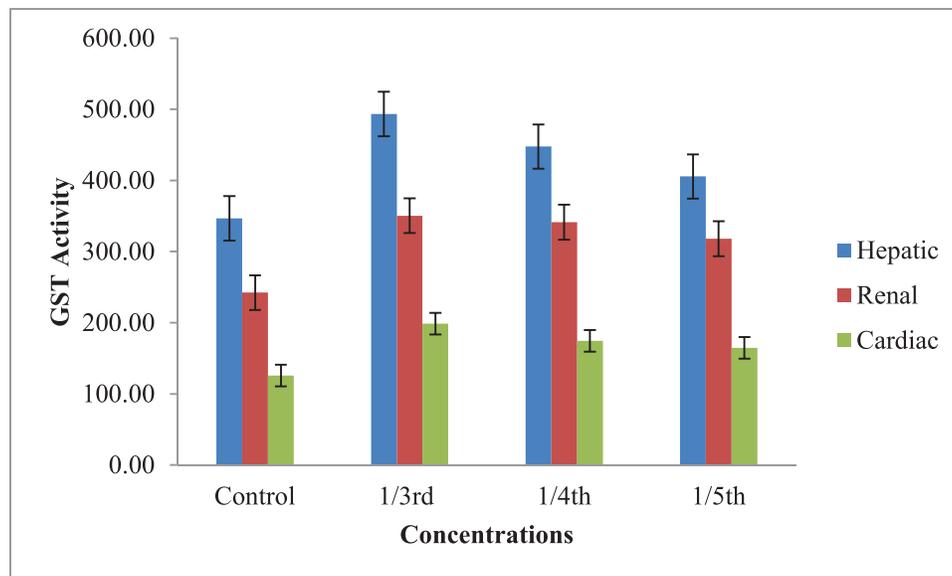


Fig. 4. Comparison among organs for GST activity in *C. mrigala* exposed to different sub-lethal concentrations

exposure.

Vinodhini and Narayanan [2] also support this study who observed the increased GST activity in common carp exposed to various concentrations of heavy metals (Cd, Ni, Pb and Cr) for 32-day. These results suggest that increased activity of enzyme may prevent the fish from damage of free radical mechanism. GST is a Phase II detoxifying enzyme, transferred the xenobiotic to harmless substances. However, antioxidant enzymes eliminate the ROS from the cell [22-23]. Previous report mentioned that GST significantly increased in the presence of heavy metals [24] mainly depends on the type of tissue and species.

According to Saliu and Bawa-Allah [25] exposure of zinc chloride ($ZnCl_2$) significantly accelerated the hepatic GST activity of *Clarias gariepinus*. Batool et al. [26] noted the significant induction of GST in liver of *Wallago attu* in a duration and concentration dependent manner. Eleyele et al [27] noted the elevated GST level in liver of *Clarias gariepinus* exposed to effluent contained heavy metals discharged from a pharmaceutical industry. According to Arafa et al [28] heavy metals (lead, cadmium, copper, zinc, iron, nickel and chromium) contamination in Ismailia channel water significantly induced the GST activity in liver of the fish *Clarias gariepinu*. Exposure of Cadmium elevated the GST activity in hepatic and renal tissues of *Arius arius* [29]. According to Aladesanmi et al

[30] higher concentration of Pb and Cr accelerated the GST activity in the fish, *Clarias gariepinus* tissue (liver, gills, fins and muscle). GST was significantly higher in liver and kidney of *Channa punctatus* inhabiting heavy metals (Mn, Fe, Zn, Co, Ni, Cu and Cr) loaded waste water [31].

4. CONCLUSION

The current study concluded that the sub-lethal exposure of chromium at various concentrations can alter the activity of glutathione S-transferase enzyme. Among tissues, hepatic tissue showed maximum activity due to its role in detoxification of toxicants. Results of this work also suggest that GST can be used as an important biomarker for evaluating the metal toxicity in aquatic ecosystems. It was also concluded that the chromium concentration above threshold level could be lethal not only to aquatic life as well as human health. Therefore, preventive measures should be taken to minimize the concentration of metals contamination in aquatic bodies.

5. REFERENCES

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