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Research Article

Sub-Lethal Effect of Waterborne Cadmium Exposure on Glutathione S-Transferase and Total Protein Contents in Liver of Carnivorous Fish, *Wallago attu*

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Abstract: This research work was performed to evaluate the glutathione S-transferase activity and total protein contents in liver of carnivorous fish, *Wallago attu* exposed to sub-lethal concentrations (1/3rd, 1/4th and 1/5th) of cadmium for14days. Fish was sampled after 7 days interval. Spectrophotometric method was used to analyze the GST activity and protein contents. Results showed that the exposure of waterborne cadmium significantly ($p<0.05$) increased the GST activity in liver of fish in relation to control. However, protein contents were significantly ($p<0.05$) decreased due to metal exposure. It was also concluded that concentration and duration of exposure greatly influenced GST activity and total protein contents in fish. Regression analyses showed that GST activity had significantly positive relation with cadmium concentrations while protein showed significantly negative relationship with concentration.

Keywords: heavy metal, carnivorous fish, Biochemical parameters

1. INTRODUCTION

Today, human beings have faced a major problem of environmental pollution including land, water and air pollution. Among these, water pollution is difficult to be measured as compared to air and land and air pollution [1]. The behavior of toxicants in water envisages biological response of an aquatic ecosystem. Different toxicants act through their toxicity, fate and specific nature while the response of biological system involves adaptations, defense, stress response and recuperation [2]. A number of organic and inorganic substances include hazardous wastes, textile dyes, phenol, petroleum and explosive products and heavy metals are the major sources of pollution.

Heavy metals are the chief constituent of inorganic pollutants in aquatic system [2-4].Some metals are indispensable; even that metals are lethal at higher concentrations. Metals can produce oxidative stress by stimulating the formation of reactive oxygen species (ROS), and can substitute crucial metals in enzymes or pigments disrupting their function [2].Contamination of heavy metals may have negative impact on aquatic environment and also on the variety of water life [5-7].

Cadmium (Cd) is a dispensable heavy metal, known as one of the major pollutants in natural water [8]. According to Hayat et al. [9] *Wallago attu* showed decreased growth after sub-lethal exposure to waterborne Cd for long duration (20-day). At higher concentration Cd rapidly causes deficiency of calcium and low blood hemoglobin in fish [10] and can also cause internal injuries in fish. Several studies also reported that Cd may be linked with oxidative damage for the generation of ROS [11-12]. Generally, antioxidant defense system contains superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase. These enzymes are present in all body organs of vertebrates, but liver showed higher activity, a major organ for detoxification of xenobiotic [13]. The glutathione *S*-transferases (GSTs) belongs to phase II detoxification enzyme, which can

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minimize the toxicity of a variety of endogenous and exogenous compounds by facilitating nucleophilic attack by reduced glutathione (GSH) [14]. The GSTs enzymes are now increasingly evaluated in most of aquatic studies as sensitive biomarkers of exposure to environmental toxicants [15].Protein modification as a consequence of free radicals is also an important parameter for evaluating the oxidative stress [16-17].

Wallago attu commonly known as "helicopter" is a freshwater carnivorous fish belongs to family Siluridae. It is locally called Mulli**.** It is experiencing population decline when migrate through urban and agricultural water courses have persistent water pollutants such as pesticides and metals. Therefore in the present study glutathione S-transferase activity and total protein contents in the liver of fish *Wallago attu* under cadmium exposure was evaluated.

2. MATERIALS AND METHODS

2.1 Experimental Design

Fingerlings of carnivorous fish, *Wallago attu* were selected for this research work. The freshwater fish, *W. attu* were collected from their natural breeding ground and transported to the laboratory of University of Agriculture, Faisalabad. Prior to experiment *W. attu* fingerlings were kept in cemented tank to acclimatized laboratory conditions for two weeks. After acclimatization period, fish were transferred to 70-L glass aquarium. Each aquarium contained a group (n=10) fishes. The total hardness (225mgL^{-1}), pH (7.25) and temperature (30°C) of water were kept constant throughout study period. However, calcium, magnesium, sodium, potassium, total ammonia, carbondioxide and electrical conductivity were also measured on daily basis by following the method described in A.P.H.A. [18]. Continuous air was supplied to all the test and control medium with an air pump through capillary system. The chemically pure chloride salt of cadmium was used to prepare stock solution. The 96-$LC_{50}$of cadmium for *W. attu* was reported as 32.96 mgL^{-1} by Batool et al. [19].Fish were exposed to sub-lethal concentrations viz. 1/3rd, 1/4thand 1/5th of LC_{50} for 14 days and fish sampling was done after 7 and 14 days.

2.2. Preparation of Homogenate

The enzyme glutathione S-transferase was isolated from the liver of *Wallago attu.* The organ was weighed i.e. liver. To remove the RBCs the dissected organ was rinsed with 50m M Tris HCL buffer of PH 7.4 and containing 0.2 M sucrose 4 times greater than the weight of organ, 1.4 and homogenized for 15 minutes in cold buffer (1:4 w/v) using a pestle and mortar. After homogenization, organ homogenates were centrifuged for 15 minutes at 10,000 rpm and 4^{0}C. After centrifugation process, clear supernatants were stored at -80^{0}C for enzyme assay while residue was discarded.

2.3 GST Assay

Activity of GST was measured by following the method of Habig and Jakoby [20] at 340nm against the reagent blank on spectrophotometer after interval of 1-minute.

2.4 Total Protein Contents

To estimate total protein content of samples Biuret method [21] was used.

2.5 Statistical Analysis

After the calculation of enzyme activity, obtained data were subjected to statistical analyses by using the Factorial experiments with three replicates. The value of $p<0.05$ was considered statistically significant. Regression analyses were also performed to find-out possible relationships between Peroxidase activity and exposure duration.

3. RESULTS

Results showed that the exposure of cadmium significantly increased the GST activity in the liver of *W. attu* in relation to control. Comparison among different concentrations of cadmium showed that maximum GST activity was observed under 1/3rd concentration followed by that of 1/4th and 1/5th (Table 1). Total protein contents were decreased in liver of exposed fish than that of control (Table 2). It was observed that protein contents were decreased in following order:1/3rd>1/4th>1/5th. Regression equation showed that GST activity had significantly positive relation with cadmium

Table 1. GST (U/mL) activity in liver of *W. attu* exposed to sub-lethal concentrations of cadmium

Duration of Exposure	Control	Treated		
		1/5th of LC_{50}	1/4th of LC_{50}	1/3rd of LC_{50}
7 days	550.33±1.41d	575.67±0.86c	599.88±0.83b	644.92±0.82a
14 days	550.67±13.01d	592.37±0.77c	650.33±0.50b	756.66±0.50a

Means with similar letters in a single row are statistically similar at $p<0.05$.

Table 2. Total protein contents in liver of *W. attu* exposed to sub-lethal concentrations of cadmium

Duration of Exposure	Control	Treated		
		1/5th of LC_{50}	1/4th of LC_{50}	1/3rd of LC_{50}
7 days	8.67±0.77a	7.88±0.94b	6.61±0.76c	5.78±0.29cd
14 days	8.64±0.67a	6.42±0.50b	6.03±0.68c	4.19±0.67d

Means with similar letters in a single row are statistically similar at $p<0.05$.

Table 3. Relationship between biochemical parameters of *W. attu* and lead concentrations

Biochemical Parameters	Regression Equation	SE	r	R^2
GST Activity	407 + 26.7 *Concentration	0.7226	0.999	0.999
Total Protein	10.4 - 0.491 **Concentration	0.004679	0.999	0.999

SE=Standard Error; r= Multiple Regression Coefficient; Coefficient of Determination;
**=Highly significant at $p<0.01$, *= Significant at $p<0.05$.

concentrations while protein showed significantly negative relationship with concentration (Table 3).

4. DISCUSSION

Oxidative stress occur when there is an inequality between the generation of reactive oxygen species (ROS) and the ability of cell to diminish ROS. This inequality may be due to elevated level of ROS, a decline in enzymatic activities or both. Numerous ecological pollutants like heavy metals are recognized as inducer of oxidative stress [22]. Oxidative stress biomarker such as biochemical parameters include change in antioxidant enzyme activity and protein contents as indicated by *W. attu* exposed to cadmium.

Glutathione S-transferase (GST) enzyme is a constituent of antioxidant enzyme performing a key function in xenobiotic detoxification in the cell. In current work, GST activity was significantly increased in liver of cadmium exposed fish in relation to control.

Our results are supported by Espinoza et al [23] who reported the significant induction in GST expression of *Ictalurus melas* liver after exposure to heavy metal cadmium. Significant elevation in hepatic GST activity of *Oreochromis niloticus* exposed to cadmium was observed by Zirong and Shijun [24]. Exposure of cadmium significantly increased the GST activity in puffer fish (25).Lopes et al [26] investigated augmented GST in the liver of freshwater fish, *Leuciscus alburnoides* captured from the cadmium polluted area. Bozcaarmutlu et al [27] determined the higher GST activity in the liver of fish captured from polluted site than the reference site. Sub-lethal concentration of cadmium caused induction in liver GST activity of *Oreochromis mossambicus* [28]. Vinodhini and Narayanan [29] also reported the significant increase in liver GST activity of common carp exposed to heavy metal solutions. Elevated level of GST in *Oreochromis niloticus* under sub-lethal levels of cadmium was reported by EL-Gazzaret al [30].

Protein is an important structural and functional part of the cells performed various crucial functions such as they may serve as a major energy source under sub-lethal stress and also key

sourcesof nitrogen metabolism [31].Our findings are also supported by Faheem et al [32]reported the decreased protein contents in liver of cadmium exposed fish *Oreochromis niloticus*. Tantarpale [33] reported a decline in total protein contents of fish *Channa striata* exposed to toxicants (pesticides), *C. Fasciatus* (34), *Tor putitora* [35], Nile tilapia [36], *Labeo rohita* [37].

5. CONCLUSION

The findings of this study demonstrated that exposure of heavy metal cadmium at sub-lethal concentration can change the biochemical parameters like antioxidant enzyme and protein contents of fish. Furthermore, these biochemical parameters will be helpful in determine the toxic effect of metals on fish.

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