

Catalase Activity in Different Tissues of Fresh Water Teleost *Heteropneustes Fossilis* on Exposure to Cadmium

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Abstract: The objective of this study is to determine the effect of Cadmium (a heavy metal) on Catalase activity in tissues of liver, kidney and gill of freshwater air breathing Teleost fish (*Heteropneustes fossilis*). Catalase enzyme (CAT) play an important role in anti-oxidant defence System which protect animal from oxidative stress. *Heteropneustes fossilis* (Bloch), a medicinally important fresh water fish were exposed with 0.7 ppm dose of Cd⁺⁺ for different periods (1, 2, 3, 7 & 14 day) and observed its effects on liver , kidney , and gill tissues. Cd⁺⁺ has shown increased CAT activity in all the tissues studied on first day and observed decreased activities from the next following days . The result of these studies in fish tissues may prove that CAT enzyme could be used as a sensitive bioindicator of the antioxidant defense system.

Keywords: Bioindicator Cd⁺⁺ Ions, Catalase enzyme, , Teleost fish (*Heteropneustes fossilis*).

I. Introduction

Heavy metals have long been recognized as serious pollutant of the aquatic environment and cause serious damage to aquatic life (Karbassi et al, 2006; Al Masri 2002). A large part of these elements exert their toxic effect by generating reactive oxygen species (ROS), causing oxidative stress. Most of the heavy metal ions are toxic or carcinogenic in nature & pose a threat to human health and the environment (Darnien et al, 2004; Farombi et al 2007). Cadmium is a non-essential heavy metal; however it is considered as one of the most toxic water contaminants and could cause toxicity at each level in organism , from population and communities to cell elements (Rashed, 2001) . Even at sublethal concentration , cadmium has a cumulative polluting effect and could cause serious disturbances in fish metabolism such as abnormal behaviour , locomotion anomalies or anorexia (Woo et al 1994; Cicik and Engin 2005). Cadmium may also affect the blood cells (Witeska 1998).

CAT primary antioxidant defense component eliminates hydrogen peroxide ($2\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{O} + \text{O}_2$). A non radical reactive oxygen species which can penetrate through all biological membranes & directly inactivate few enzymes. Various responses of CAT activity have been observed in animals exposed to organic or metallic contaminants in both field laboratory experiments & CAT has been shown to be either induced or inhibited by metals depending on the dose , the species or the route of exposure (Romeo et al).

II. Material and Method

2.1 Sample collection and method- A fresh water Teleost fish *Heteropneustes fossilis* (Bloch) of 25-30 cm length and 35-40 gm body weight were collected locally & kept in large sand aquarium , each contain 50 litre water. Fish maintained under standard fish maintenance procedure. Fish were acclimatized 15 days prior to experiment. They were supplied daily with commercial fish feed at a 2-5% of body weight and temperature was maintained at ambient laboratory temperature ($30 \pm 2.0^\circ\text{C}$) and pH 7.8. Fishes are transferred to a fresh volume of water every 24 hrs to minimize contamination from metabolic wastes of fishes. Feeding was stopped 24 hrs prior to experiment.

2.2 Physio-Chemical analysis of River water -Physiochemical analysis (Temperature, pH, Dissolved Oxygen, Potassium, Sodium, Calcium and Chlorine) were done for collected river water sample according to APHA standard methods [12].

2.3 Chemicals- Glacial Acetic acid, Bovine Serum Albumin, Follin's Regent. Na-K Tartarate, K₂Cr₂O₇, CuSO₄, NaOH , NaHPO₄ H₂O₂ were purchased from Sigma Chemicals. Cadmium-chloride Salt was used as metal sources. This chemical was obtained from Merck India , of analytical grade were prepared in double distilled water

2.4 Experiment-In the experiment, fish were exposed to 0.5mg/ltr concentrations of cadmium chloride Salt for 1, 2, 3, 7 and 14 days in water aquariums, were changed every two days to minimize metal loss just after feeding the animals to prevent contamination of the environment with food remains

2.5 Biochemical Estimation:

2.5.1 Post-Mitochondrial Supernatant Preparation- The specimens scarified : the liver, kidney and gill, were quickly removed, cleaned and washed with cold fish saline. The tissues were homogenized in chilled phosphate buffer 0.1 in pH 7.4 containing KCl(1.17%), using homogenizer. The supernatant was centrifused at 1000 g in Eltek Refrigerated Centrifuged (RC-4100) for 30 min at 4°C to obtained supernatant, which was used for further biochemical analysis.

2.5.2 Catalase Activity Assay- Catalase activity was assayed by the reference of Sinha method(1972) is based on the fact that dichromate/acetic acid is reduced to chromic acetate in presence of H₂O₂ with formation of PCA (per chromic acid), an unstable blue precipitate, chromic acetate thus produced (green), upon heating is estimated calorimetrically at 600 nm.

2.5.3 Protein Estimation- Protein from samples were estimated by the method of Lowery et al(1951) using Folin’s reagent and BSA standard.

2.6 Statistical Analysis- The statistical analysis was performed using student ‘t’ test and a value p<0.01 was regarded as significant.

III. Result and Discussions

The present study determines the effect of cadmium on catalase activity on tissues of liver, kidney and gill of freshwater air breathing teleost fish (*Heteropneustes fossilis*). The physicochemical parameters of river water sample were taken and the results were compared with limits prescribed by WHO standard [W.H.O.]. Temperature, pH, Dissolved Oxygen, Potassium, Sodium, Calcium, Chlorine for collected river water sample were analyzed in range of the given standard value of the WHO [Table-1].

Table 1: Physio-chemical parameters of river water of Gomati in Jaunpur

Physio-chemical parameters	Values	Standard Values
Temperature (°C)	30 ± 2 °C	30°C
pH	7.8 ± 0.32	6.5-8.5
Dissolved Oxygen (mg/l)	6.8 ± 0.4	5.0(mgl-1)
Potassium (ppm)	0.012 ± 0.008	
Sodium (ppm)	0.015 ± 0.008	
Calcium (ppm)	0.018 ± 0.005	75(mgl-1)
Chlorine (ppm)	1.44 ± 0.050	250(mgl-1)

3.1 Liver-Cadmium exposure resulted in a significant (p<0.01) induction in the activity of catalase, when compared with control group of fish. The induction in the activity in liver was found 172% after 24 hours’ treatment of cadmium and 129% of activity remained after 7 days’ treatment subsequently no further significant change was reported till 14 days (table 2 graph 1).

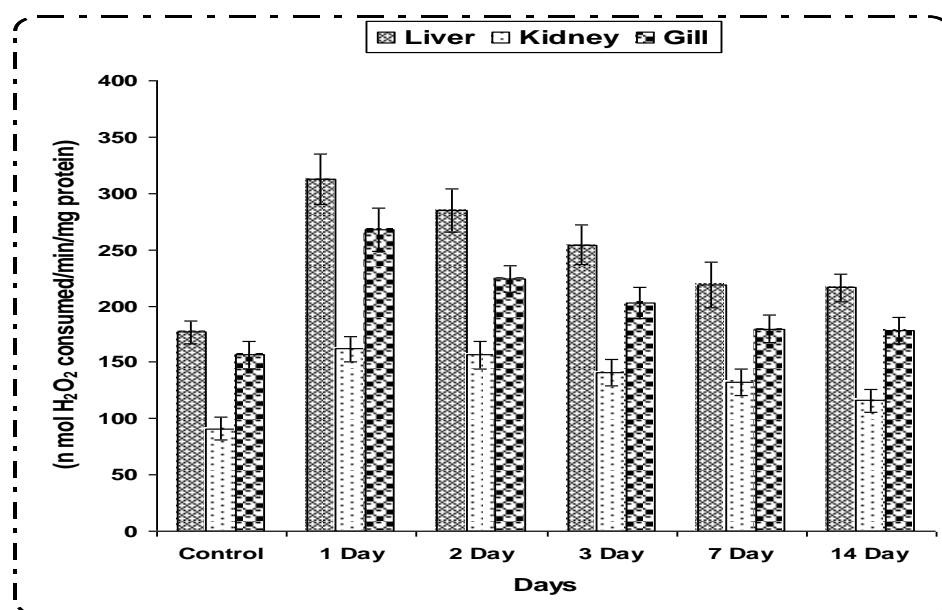
3.2 Kidney –Cadmium exposure for 14 days, increased the activity of catalase in the kidney of experimental fish. The induction in the activity of catalase was found 195% after one day cadmium exposure, thereafter an increase of activity was found 130% after 14 days exposure (table 2 graph 1).

3.3 Gill--Cadmium exposure resulted in a significant increases (p<0.01) in catalase activity when compared the corresponding control values. A maximum induction in the activity of catalase was observed 152% after one day of cadmium exposure. After 14 days of cadmium exposure, it remained 113% in the of activity of catalase of treated fish (table 2, graph 1).

Table 2: Significance of Cadmium exposure

Metal ions exposure	Liver	Kidney	Gill
Control	161.50±13.83	68.26±7.81	143.50±12.43
1 Day	278.48±20.12	133.11±11.42	218.16±18.12
2 Day	263.18±22.62	127.68±13.04	203.63±11.62
3 Day	248.12±19.13	103.12±9.05	199.12±13.82
7 Day	208.63±2.46	98.19±7.04	169.63±16.12
14 Day	209.18±11.83	89.84±3.16	162.83±11.63

GRAPH-1



The Effect of Cadmium Metal Ions on the Activity of Catalase (n mol H₂O₂ consumed/min/mg protein) from the Liver, Kidney and Gill of Freshwater Teleosts *Heteropneustes fossilis* (Bloch.)

IV. Conclusion

The effect of cadmium (a heavy metal) on catalase activity on tissues of liver, kidney and gill of freshwater air breathing Teleost fish results that antioxidant enzyme assays can be used as a bioindicator for acute exposure to Cd⁺⁺ in the fresh water teleost fish *H. Fossilis*. This metal stimulated rapidly the antioxidant system as evidenced by an increase in CAT activity. The responses of CAT activity in different tissue of *H. Fossilis* exposed to sub lethal concentrations of CdCl₂ solution was found to be variable depending on the tissues and duration of exposure periods. Hence the CAT activity can be considered as a sensitive biomarker for biomonitoring the aquatic environment, contaminated with heavy metals and this may provide a useful data for future investigations.

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