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Effect of Cd^{++} On Catalase Activity on Air Breathing Fresh Water Teleost Fish *Heteropneustes fossilis* (Bloch)

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Abstract: ***The objective of this study is to determine the effect of Cadmium (a heavy metal) on Catalase activity on tissues of liver, kidney and gill of freshwater air breathing Teleost fish (Heteropneustes fossilis). Catalase, a oxido-reductase enzyme 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 for 96 hours at different concentrations 0.1 mg/itr, 0.5 mg/ltr and 1.0 mg/ltr of Cd^{++} and observed its effects on liver, kidney, and gill tissues. Cd^{++} has shown increased CAT activity in all the tissues studied. It has also been observed that highest increase of the CAT activity in liver, exposed to 1.0 mg Cd^{++}/ltr. On the other side Cd^{++} had no significant effect in gill . 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: Cd^{++} Ions, Catalase enzyme, bioindicator, Teleost fish (*Heteropneustes fossilis*

1. Introduction

Heavy metals have long been recognized as serious pollutant of the aquatic environment and cause serious damage to aquatic life [1]. 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 [2],[3. Cadmium is a nonessential 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 [4]. Even at sublethal concentration, Cadmium has a cumulative polluting effect and could cause serious disturbances in fish metabolism such as abnormal behaviour, locomotior anomalies or anorexia [5],[6]. Cadmium may also affect the blood cells [7].

Metal accumulation causes an increase in highly reactive oxygen species (ROS*) i.e.* Hydrogen peroxide (H_2O_2), super oxide radical, hydroxyl radical leading to oxidative stress in fish [8],[9]. Heavy metals can promote oxidative damage by directly increasing the cellular concentration of ROS [10]. Antioxidant enzymes contribute to the maintenance of a relatively low level of the reactive and harmful hydroxyl radical generate through the Habberer-Weiss reaction between superoxide radical and hydrogen peroxide in the presence of Cu^{2+}, CAT primary antioxidant defence component eliminates hydrogen peroxide ($2H_2O \rightarrow 2H_2O+O_2$). A non radical reactive oxygen species which can penetrate through all biological membranes & directly inactive few enzymes. Various responses of CAT activity have been observed in animals exposed to organic or metallic contaminate in both field 7 laborrtory experiments and CAT has been shown to be either induced or inhibited by metals depending on the dose, the species or the route of exposure [11].

2. Materials & Methods

2.1 Sample Collection and Maintanance

A fresh water Teleost fish *Heteropneustes fossilis* (Bloch) of 25-30 cm length and 35-40 gm body weight were collected from different locations of the river Shivnath near Durg Chhattisgarh and kept in large sand aquarium, each contain 50ltr water. Fish maintained under stander 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±2.0ºC) 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 Physico-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 Exposure with Cd^{++} Ions

In the experiment, fish was exposed to 0.1, 0.5 and 1.0 mg/L concentrations of Cd^{++} for 96 hrs water in aquariums were changed every two days to minimize metals loss just after feeding the animals to prevent contamination of the environment with food remains.

2.4 Biochemical Estimation

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 centrifuged 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.

2.4.1 Catalase Activity Assay

Catalase activity was assayed by the reference of Sinha method is based on the fact that dichromate/acetic acid is reduced to chromic acetate in presence of H_2O_2 with formation of PCA (per chromic acid), an unstable blue precipitate, chromic acetate thus produced (green), upon heating is estimated calorimetrically at 600 nm [13].

2.4.2 Protein Estimation

Protein from samples was estimated by the method of Lowry [14]. Using Folin's reagent and BSA standard.

2.4.3 Stastistical Analysis

The stastistical analysis was performed using student T- test and a value $p < 0.5$ was regarded as significant.

3. Results and Discussions

In present study determine the effect of cadmium (a heavy metal) on catalase activity on tissues of liver, kidney and gill of freshwater air breathing Teleost fish (***Heteropneustes fossilis***). The physicochemical and microbial parameters of river water sample were done and the results were compared with limits prescribed by WHO standard [15]. Temperature, pH, Dissolved Oxygen, Potassium, Sodium, Calcium, Chlorine for collected river water sample were analyzed in range of the give standard value of the WHO [Table-1].

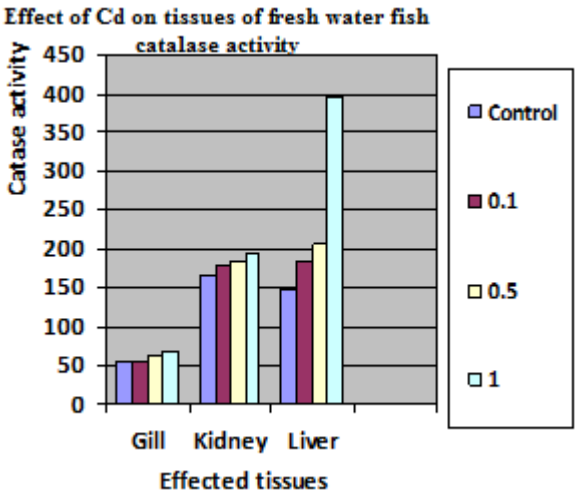
Table 1: Physico-chemical parameters of river water Shivnath near Durg Chhattisgarh

Physico-chemical parameters	*Values*	*Standard Values*
Temperature (˚C)	30 ± 2 ºC	30ºC
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})

The effect of cadmium (a heavy metal) on catalase activity on tissues of liver, kidney and gill of freshwater air breathing Teleost fish were shown different effect on different tissues, which were gradually increased with increasing concentration of doses [Table-2, Graph -1].

Table 2: Shown effect of Cd^{++} heavy metal on catalase activity of tissues of fresh water fish

Doses (in mg/ml)	Catalase activity of various tissues of fresh water fishes (in µ/mol/H_2O_2/L)		
	Gill	Kidney	Liver
Control	55	167	148
0.1	57	178	184
0.5	65	184	206
1.0	70	195	396



Graph 1: Shown effect of Cd^{++} heavy metal on catalase activity of tissues of fresh water fish.

Liver- Heavy metals exposure showed significant ($P < 0.5$) increased in CAT activity in Liver when comparing the control value (161.83± 13.84 µmole H_2O_2 /mg protein/min.). The highest increased in catalase activity (157.54%) was observed at 1 ppm Cd^{++} exposure. The highest CAT activity was determined in liver tissues compared to other tissues [16], [17]. This datum are in agreement with those reported in other fish species where CAT activity is distributed in a decreasing order as follows : liver > kidney [16]. In our study Cadmium metal increased the CAT activity in the liver. The increase of CAT activity may be associated with increased oxidative stress by this Metal (Cd^{++}). In previous studies, the liver was found to be stronger into the face of oxidative stress than the other tissues and a uniform organ with the highest anti-oxidant enzyme activities (SOD, CAT). This could be related to the fact that the liver is the site of multiple oxidative reaction and maximum free radical generation [17].

Kidney- Our experiment from Statistical Analysis showed that was no significant ($P < 0.05$) changes in CAT activity in cadmium metal exposures in the kidney as compared to control value {65.89±5.42µmole H_2O_2 /mg protein/min.). Cadmium had no effect on the CAT activity in this tissue. This can be associated with the effective antioxidant system in this tissue were the higher metal bioaccumulation and related to metal binding protein synthesis were observed [18]. The CAT activity decrease or did not change possibly because of metal binding to these proteins.

Gill- In the case of gill, results shown that there were no significant ($p< 0.05$) changes in CAT activity in the gill tissue exposed when compared to that of control (164±10.4µ mole H_2O_2 /mg protein/min). The lowest activity of CAT was observed in the gill tissue; this was explained by the increased generation of H_2O_2 which led to a decreased CAT activity.

4. 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 concluded 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 $CdCl_2$ 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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