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Research Article

GLUTATHIONE-S-TRANSFERASE AND CATALASE ACTIVITY IN DIFFERENT TISSUES OF MARINE CATFISH ARIUS ARIUS ON EXPOSURE TO CADMIUM

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ABSTRACT

Objective: Pollution by heavy metals is a serious problem due to its toxicity and its ability to accumulate in the biota. *In vivo* effects of Cadmium (Cd) levels of expression on antioxidant enzymes such as Glutathione-S-transferase (GST) and Catalase (CAT) were investigated in liver, kidney, gill and brain tissues of marine Catfish *Arius arius.*

Methods: The various concentrations of $CdCl_2$ such as 5.0 ppm, 10.0 ppm and 15.0 ppm exposed to a period of 24, 48, 72 and 96 hrs. GST and CAT enzyme levels were studied in the tissues of liver, kidney, gill and brain in *A. arius*. The GST enzyme levels were analyzed using the method of Habig *et al*., 1974 and measured using spectrophotometrically at 340 nm. Catalase levels were evaluated by the method of Sinha *et al., (*1972) and spectrophotometrically read at 570 nm.

Results: GST is one of the intensely investigated conjugation enzymes and is the second stage of xenobiotic detoxification. CAT is a common antioxidant enzyme which is produced naturally in almost all living organisms. The catalytic action of CAT, are important to life because it helps the body to break down hydrogen peroxide (a powerful and harmful oxidizing agent) into oxygen and water, and thus preventing the accumulation of carbon dioxide bubbles in the blood. The results showed the role of GST and CAT in antioxidant defense system to protect the animals from oxidative stress and tissue damage in the tissues of exposed fishes.

Conclusion: In conclusion, our results indicate that antioxidant enzyme assays can be used as a bioindicator for acute exposure to Cd in marine catfish *A. arius* and other fishes. Hence the GST and CAT activity can be considered as a sensitive biomarker for bioindicator of the antioxidant defense system in the aquatic organisms, contaminated with heavy metals and this may provide a useful data for future investigations.

Keywords: Glutathione-S-transferase, Catalase, Cadmium, *Arius arius*, Antioxidants.

INTRODUCTION

The aquatic environment is contaminated with innumerable organic and non-organic pollutants [1] of municipal waste, industrial, agricultural and mining industrial origin [2]. Such pollutant not only affects the integrity of the ecosystems and also affects the physiological functions of animals [1] and human, as consumers [3]. The Cd is a ubiquitous heavy metal present in aquatic environments due to natural and anthropogenic sources and is usually present in trace amounts. Cd is naturally found in the earth's crust or occurs in combination with other elements such as Zn and Cu. Cd is primarily used for electroplating with other metals and in nickeled batteries because of its relative resistance to corrosion and high electrical and thermal conductivity. These inputs may result in increased Cd levels of in the aquatic ecosystems, which can be potentially toxic to organisms such as fish. Fishes have long been used as aquatic contamination indicators for many years. Cd accumulates in drinking water and air then eventually accumulates in the body, causing a number of diseases such as Hypertension, Osteomalacia, gastric dysfunction, Central nervous system dysfunction, and endocrine disorders [4, 5] in human.

Cd causes significant metabolic alterations and injuries in biological systems at different levels [6]. Cd after entering into the organism of fishes through the gills binds to albumins and erythrocytes in the blood and is then transferred into tissues and organs where it is bound to proteins of low molecular weight producing metallothioneins by the induction of metallothionein mRNA synthesis [7]. About 75 % of the total accumulated Cd in the organism is deposited in the liver and kidney [8, 9], but it can also be deposited in the heart, gill and other tissues [10, 11]. The majority of aquatic studies has been directed toward using GST's as biomarkers of exposure to environmental chemicals [12]. One of the potential biomarkers is GST, which is a key phase II detoxification enzymes. The phase II metabolism involves the conjugation of xenobiotics with endogenous substrate, thus facilitating their excretion [13]. In 1994 GST's have been recommended by the International Council for the Exploration of the Sea as biomarker which requires additional research before monitoring applications can be undertaken [14].

Trace metals at the cellular level are often involved in oxidative stress, which results in the production of Reactive Oxygen Species (ROS). ROS include the superoxide radical (O_2-), hydrogen peroxide (H_2O_2) and the hydroxyl radical, all of which affect mainly lipids, proteins, carbohydrates, and nucleic acids [15]. The importance of antioxidant enzymes is generally emphasized in the prevention of oxidative stress by scavenging ROS [16]. Excessive ROS production in response to heavy metal pollution is the natural defense mechanism of biomolecules, leading to a cumulative damage [17]. When the antioxidant enzymes fail or are insufficient, an increase in ROS production may result in oxidative damage [18]. Recent studies have suggested that certain GST isoenzymes may be involved in the regulation of stress-activated cell signaling pathways [19, 20, 21].

Fish tissues are endowed with antioxidant defense systems consisting of CAT, GST enzyme to protect them from oxidative stress caused by metals [22]. CAT a primary antioxidant defense component, eliminates hydrogen peroxide ($2H_2O_2 \rightarrow 2H_2O+O_2$) a non-radical reactive oxygen species which can penetrate through all biological membranes and directly inactivate few enzymes. CAT activity is considered as a sensitive biomarker of oxidative stress before hazardous effects occur in fish [23, 24]. Hence, in the present study, the effect of Cd on the enzyme GST and CAT activity in a marine water catfish, *A. arius* were analyzed.

MATERIAL AND METHODS

The marine water Catfish, A. arius was collected from Chennai along the Coromandel Coast of the Bay of Bengal, Tamil Nadu, Southern India. The fishes were acclimatized in the laboratory in a stone tank (100L) at room temperature (30 ±20C) for 7 days. The CdCl2 (Merck, Mumbai, India) solution was prepared in distilled water for acute toxicity studies. The various concentrations of CdCl2 such as 0 (Control), 5.0 ppm, 10.0 ppm and 15.0 ppm exposed to a period of 24, 48, 72 and 96 hrs. Eighty fishes with similar size, length, weight about (approx. 40-60 g) were selected and divided into four groups.

Each group has 20 fishes. One group was kept as a control, the other three groups were transferred to stone tanks (100 L) containing 5.0 mg L-1, 10.0 mg L-1, and 15.0 mg L-1 of CdCl2, respectively. The test solutions were renewed daily to maintain the waterborne Cd concentration. In our biological experiments, GST and CAT enzyme levels were studied in the tissues of liver, kidney, gill and brain. The content of Cd and other heavy metals were analyzed before the start of the study and found to be below detectable levels (BDL) to rule out their role or influence in the experiments.

GST Enzyme Assay

The GST levels in response to Cd treatments were analyzed in the tissues using the method of Habig *et al*., 1974 [25]. Enzymatic assay was performed on *A. arius* liver, kidney, gill and brain. The tissues (50mg) were homogenized in 50 mM Tris–HCl buffer, pH 7.4, and containing 0.2 M sucrose and centrifuged at 16,000g for 45 min at 4°C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a volume of 3 mL contained 2.4 mL of 0.3 M potassium phosphate buffer (pH 6.9), 0.1 mL of 30 mM CDNB and 0.1 mL of 30 mM GSH, as enzyme source. The reaction was initiated by glutathione. The absorbances were read at 340 nm against the reagent blank. The results were expressed as µM/min/mg protein. The GST levels were measured using spectrophotometrically.

Catalase Enzyme Assay

Catalase levels in response to Cd treatments were evaluated by the method of Sinha *et al., (*1972) [26]. The tissues (50mg) were homogenized in 50 mM phosphate buffer, pH 7.0, and centrifuged at 16,000g for 45 min. The supernatant was used as the enzyme source. The reaction mixture contained 2 mL of phosphate buffer (pH 7.0) 0.45 mL H_2O_2, and 0.025 mL of enzyme source. The absorbance was read at 570 nm and the enzyme activity was expressed as micromoles of H_2O_2 consumed/min/ mg protein.

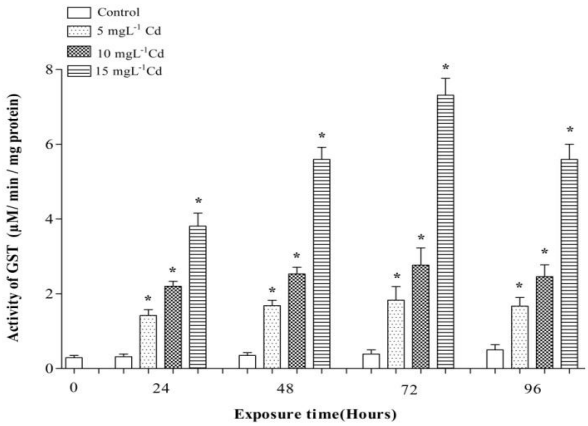
Statistical analysis

Statistical analyses of data were carried out using Graphpad Prism Version 5.0. The values are reported as mean ± SD. One-way analysis of variance was utilized to test the differences between the control, GST and CAT levels exposed for each sampling. The data of different hours of sampling were compared by ANOVA, Unifactorial analysis was used to test the differences between the control and treated groups.

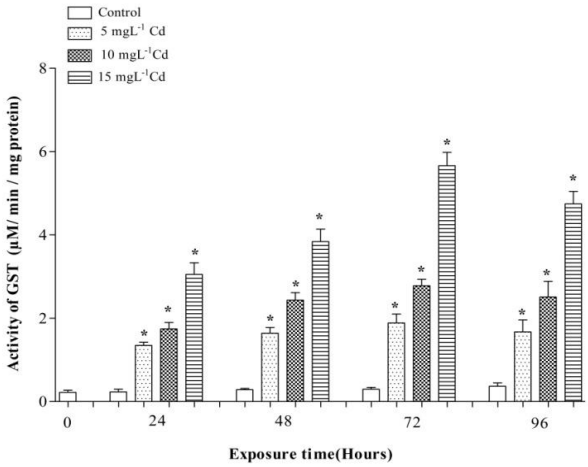
RESULTS

GST Enzyme Activity

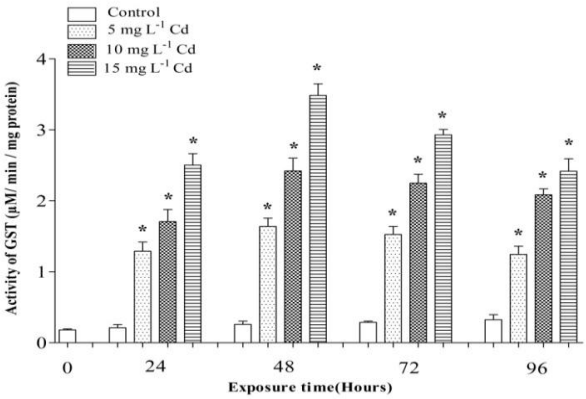
In the present study, GST activity in response to Cd treatments was analyzed in the liver, kidney, gill and brain of A. arius for the period of 96 hrs. These data were graphically represented in (fig.1). The highest levels of GST enzyme activity 7.31± 0.454 (µM/min/mg protein) was observed in the liver during 72 hrs of Cd exposure with 15.0 mg L-1, during 72 hours of Cd exposure, in the kidney GST levels were 5.661 ± 0.321 (µM/min/mg protein) when treated with 15.0 mg L-1 of CdCl2. During 48 hours of Cd exposure, in the gills GST levels were 3.485 ± 0.164 (µM/min/mg protein) when treated with 15.0 mg L-1 of CdCl2. During 96 hours of Cd exposure, the brain GST levels were 2.830 ± 0.11 (µM/min/mg protein) when treated with 15.0 mg L-1 of CdCl2. The GST levels of control tissues in the liver were 0.298 ± 0.062 (µM min/mg protein), kidney 0.219 ± 0.055 (µM/min/mg protein), gills 0.179 ± 0.016 (µM/min/mg protein) and brain 0.163 ± 0.011 (µM min/mg protein). The liver and kidney, GST enzyme levels gradually increased rapidly to reach a peak during 72 hours and then declined gradually during 96 hours. In gill, GST enzyme levels decreased after 48 hours. The brain showed the lower GST levels than all other tissues. In the brain, GST enzyme levels gradually increased at 96 hours. The data were subjected to statistical analysis of one way ANOVA and the values were found to be statistically significant at $P < 0.05$.



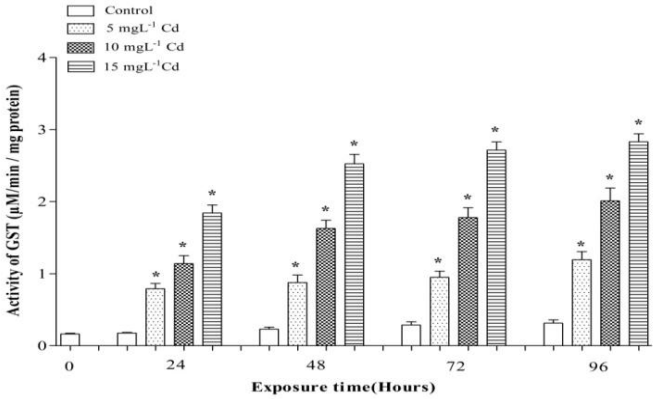
(a). Liver



(b). Kidney



(c).Gill



(d). Brain

Fig. 1: Activity of GST (µM/min/ mg protein) in *A. arius* on exposure to various concentrations of $CdCl_2$ (Control, 5.0 ppm, 10.0 ppm and 15.0 ppm) for a period of 24, 48, 72 and 96 hrs. The results were represented as Mean ± SD, n=5. Statistical comparisons were made against Control fish on each sampling day. (*Statistically significant at P < 0.05)

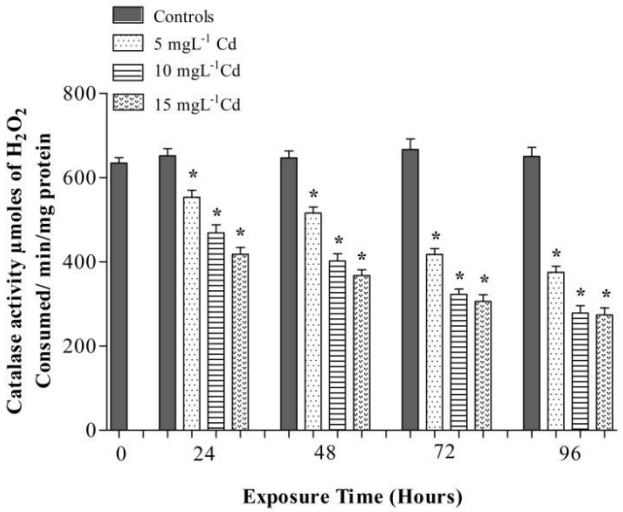
Catalase Enzyme Activity

Catalase is an important enzyme in antioxidant defense system protecting animals from oxidative stress. The effect of Control, 5 ppm, 10 ppm and 15ppm of $CdCl_2$ in the four tissues a liver, kidney, gill and brain of *A. arius* were graphically represented in (fig.2). The highest CAT enzyme activity of 553.8 ± 16.15 (µmol H_2O_2 consumed/min/mg protein) was observed in the liver during 24 hrs of Cd exposure with 5.0 mg L^{-1}, during 24 hours of exposure to Cd, the kidney CAT activities were 396.4 ± 14.36 (µmol H_2O_2 consumed/min/mg protein) when treated with 5.0 mg L^{-1} of $CdCl_2$. During 24 hours of exposure to Cd, in gills, CAT activities were 227 ± 10.49 (µmol H_2O_2 consumed/min/mg protein) when treated with 5.0 mg L^{-1} of $CdCl_2$. During 24 hours of exposure to Cd, the brain CAT activities were 167.2 ± 7.69 (µmol H_2O_2 consumed/min/mg protein) when treated with 5.0 mg L^{-1} of $CdCl_2$. The CAT activities of control tissues in the liver were showed values of 635.5 ± 12.17 (µmol H_2O_2 consumed/min/mg protein), in the kidney were showed values of 455.2 ± 10.49 (µmol H_2O_2 consumed/min/mg protein), in the gills were showed values of 252.3 ± 10.15 (µmol H_2O_2 consumed/min/mg protein) and the brain were showed values of 182.6 ± 14.98 (µmol H_2O_2 consumed/min/mg protein). CAT activities decreased on exposure to increased Cd concentration when compared to the control CAT activity. The specific activity CAT in the brain was found to be lower, when compared to all other tissues. The data's were subjected to statistical analysis of one way ANOVA and the values were found to be statistically significant at P < 0.05.

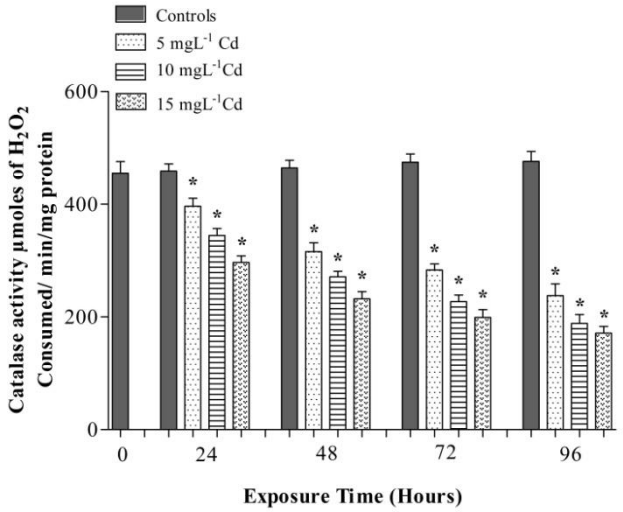
DISCUSSIONS

GST Enzyme

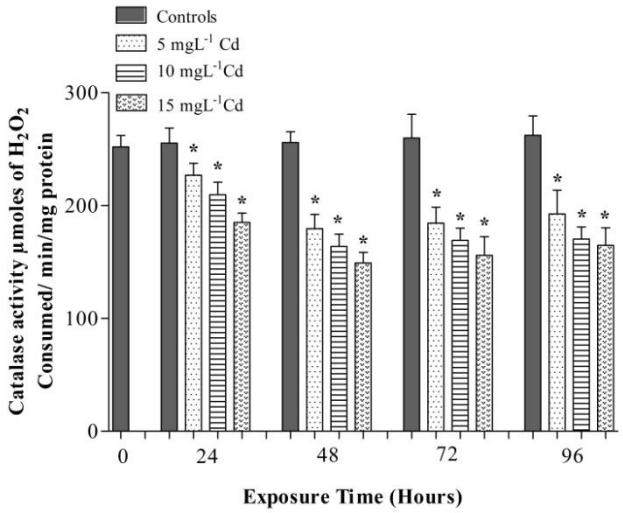
Glutathione-S-transferases are a family of multifunctional enzymes that are involved in the detoxification of both xenobiotics as well as endogenous reactive compounds of cellular metabolism. GST was shown to catalyze essential steps in the biosynthesis of prostaglandins and leukotrienes [27]. GST plays a critical role in mitigating oxidative stress in all life forms and GST activity also has been widely used as a biomarker to detect stress. As an antioxidant enzyme, a GST activity either has a significant increase or decrease with different patterns according to the exposed elements or exposure conditions. GST activity varied in different tissues and organs of aquatic animals [28]. The GST activities in the fish tissues of *A. arius* were in the following order liver > kidney > gill > brain. These results are in accordance with the works of [29] in whitefish tissues. The concentrations of Cd levels in liver and kidney were much higher than gills and brain in the experimented fishes. This might be due to the fact that the liver and kidney are the major targets for Cd distribution. Here they Cd are detoxified by binding with MT. The gills are the major entry site of heavy metals and act as a transient store for accumulation of metals [30].



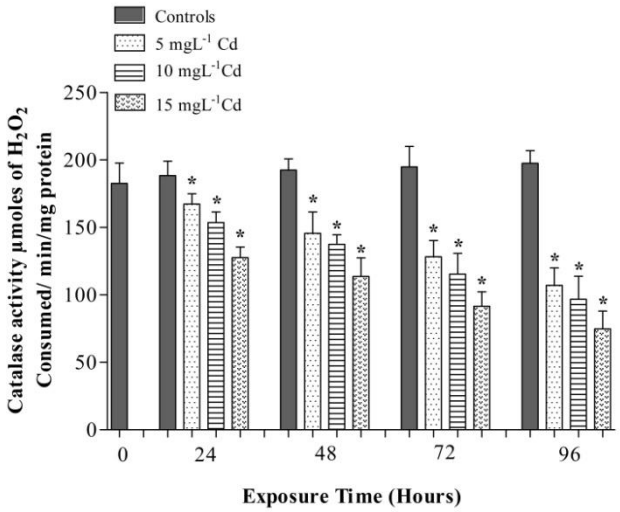
(a). Liver



(b). Kidney



(c). Gill



(d). Brain

Fig. 2: Activity of CAT (µmol H2O2 consumed/min/ mg protein) in *A. arius* on exposure to various concentrations of $CdCl_2$ (Control, 5.0 ppm, 10.0 ppm and 15.0 ppm) for a period of 24, 48, 72 and 96 hrs. The results were represented as Mean ± SD, n=5. Statistical comparisons were made against Control fish on each sampling day. (*Statistically significant at P < 0.05)

There are various modes of Cd uptake in aquatic organism, where it is most readily absorbed by organisms directly from the water in its free ionic form Cd (II). Metal ions are usually absorbed through passive diffusion or carrier mediated transport over the gills while metals associated with organic materials are ingested and absorbed by endocytosis through the intestine. It has been suggested that Cd ions enter the chloride cells in the gills through calcium channels [31]. Cd after entering into the organism of fishes through the gills, Cd binds to albumins and erythrocytes in the blood and then transferred into the tissues and organs where it is bound to proteins of low molecular weight producing metallothioneins by the induction of metallothionein mRNA synthesis [7]. The present study has also shown higher concentrations of GST activity in the liver than the gills. About 75 % of the total accumulated Cd in an organism is deposited in the liver and kidney [8, 9], but it can also be deposited in the heart, gills and other tissues [10, 11].

The role of the liver in antioxidant enzyme response as a result of its higher sensitivity to metals when compared to the kidney has been elucidated by various investigations as the liver has to overcome the oxidative stress than the other tissues because of the high antioxidant enzyme activities [32]. Liver of vertebrates exhibits a high metabolism and oxygen consumption and it is the main organ of xenobiotic detoxification. It is a particularly rich source of GST [33]. Gills uptake the heavy metals from the site and directly interact with the toxic medium [34]. But however their GST enzyme levels are low, compared to liver and kidney. The GST activity increases steadily during 24 hrs and 48 hrs of exposure and then decline slowly during 72 hrs and 96 hrs of exposure during the present study. These variations might due to the time taken to the metal to be transported to other detoxifying organs.

Catalase Enzyme

CAT being a primary antioxidant defense component, eliminates hydrogen peroxide ($2H_2O_2 \rightarrow 2H_2O+O_2$) a non-radical reactive oxygen species which can penetrate through all biological membranes and directly inactivate few enzymes. CAT is considered as a sensitive biomarker of oxidative stress before major deleterious effects occur in fish [23, 24]. CAT is an important enzyme in antioxidant defense system protecting animals from oxidative stress. The highest CAT activity was marked in liver tissue when compared to other tissues, which are in agreement with various author's [35, 24]. These data are in accordance with those reported in other fish species were CAT activity is seen in a decreasing order, as follows: liver > kidney > heart> brain > muscle [35]. In the present study, Cd decreased the CAT activity in the liver. The reduction may be associated with the direct binding of metal to –SH groups in the enzyme molecule. Liver CAT activity was found to be inhibited following both *in vivo* and *in vitro* exposure to dissolved Cd^{2+} at a concentration greater than 1 mg/L in the killifish, *Fundulus heteroclitus* and the authors suggested a direct effect of Cd^{2+} of high molecular weight compounds like CAT [36]. In previous studies, the liver was found to be stronger in the face of oxidative stress than the other tissues and a uniform organ with the highest antioxidant enzyme activities (CAT). This could be related to the fact that the liver is the site of multiple oxidative reactions and the maximal free radical generation [37].

The highest inhibition was observed in the kidney, this can be associated with the effective antioxidant system in this tissue where there is higher metal bioaccumulation and is directly related to the metal binding protein synthesis and non-enzymatic antioxidant mechanisms that has been suggested by Dautremepuits *et al*., [38]. Moreover, this can be attributed to the possible induction of stress proteins and non-enzymatic antioxidant formation [22]. A gill is first affected organ when fish are exposed to metals because it's direct contact with water medium. In the present study there was a significant change in CAT activity in the gill and this could be associated with the higher activity of GPX, which acts as a defense against the formation of H_2O_2 or effective antioxidant responses due to a higher renovation in the gill epithelium. The lowest activity of CAT was measured in the gill tissue; this was explained by the increased generation of H_2O_2, which led to a decreased CAT activity [39].

The brain is susceptible to oxidative damage by free radicals as it contains high amounts of unsaturated lipids and utilizes about 20% of total oxygen demand of the body [40]. The specific activity of brain CAT was found to be lower, which may be related to the direct binding of metal ions to –SH groups in the enzyme molecule, increased hydrogen peroxide and superoxide radical due to oxidative stress. It was indicated that rapid inactivation of CAT at high hydrogen peroxide concentration was due to the conversion of active enzyme compound to inactive compounds [41]. In general, inhibition of CAT enzyme activity in all tissues of *A. arius* may be resulted due to the direct effect of heavy metal Cd.

CONCLUSION

In conclusion, our results indicate that antioxidant enzyme assays can be used as a bio indicator for acute exposure to Cd in the marine catfish *A. arius* and other fishes. This metal stimulated rapidly the

antioxidant system as evidenced by an increase in GST activities in the detoxifying organs such as liver and kidney. GST is one of the intensely investigated conjugation enzymes and is the second stage of xenobiotic detoxification. It is very often used as a biochemical marker of aquatic environmental contamination with exogenous substances. The response of CAT activity in different tissues of *A. arius* exposed to sublethal concentrations of $CdCl_2$ solution was found to be variable depending on the tissues and duration of exposure periods. Hence the GST and 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.

Conflict of Interest Statement

There is no conflict of interests.

ACKNOWLEDGEMENT

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