ANTIOXIDANT DEFENCE SYSTEMS, LIPID PEROXIDATION AND ACETYLCHOLINESTERASE ACTIVITY OF *OREOCHROMIS NILOTICUS* EXPOSED TO MERCURY AND MERCURY + SELENIUM

Gülbin Firidin[1,*], Ferit Kargın[2], Özge Fırat[3], Hikmet Y. Çoğun[4], Özgür Fırat[5], Beran Firidin[6], and Tüzin A. Yüzereroğlu[7]

[1] Life Science Research and Application Center, Gazi University, 06830 Ankara, Turkey
[2] Department of Biology, Faculty of Science and Letters, Cukurova University, 01330 Adana, Turkey
[3] Vocational School of Kahta, Adiyaman University, 02400 Adiyaman, Turkey
[4] Faculty of Veterinary of Ceyhan, Cukurova University, 01920 Adana, Turkey
[5] Department of Biology, Faculty of Science and Letters, Adiyaman University, 02040 Adiyaman, Turkey
[6] Faculty of Education, Gazi University, 06500 Ankara, Turkey
[7] Vocational School of İmamoğlu, Cukurova University, 01700 Adana, Turkey

ABSTRACT

The objective of the present study was to evaluate the oxidative stress potential of mercury (Hg) exposure with the modulatory effect of selenium (Se) in the brain and kidney tissues of *Oreochromis niloticus*. The fish were exposed to 0.01 and 0.1 mg/L Hg, 0.01 mg/L Hg+0.1 mg/L Se, and 0.1 mg/L Hg+1.0 mg/L Se, for 7 and 14 days. The activities of total glutathione (tGSH), malondialdehyde (MDA) and catalase (CAT), glutathione S-transferase (GST), and acetylcholinesterase (AChE) were measured from samples of the tissues. The results indicated that all concentrations of Hg caused a time-dependent increase in CAT, GST activity, whereas AChE activity and tGSH content were decreased in brain and kidney tissues. MDA levels in tissues of fish exposed to Hg+Se mixtures were lower compared to those exposed to Hg only. Selenium application in combination with mercury provided an alleviation of the toxic effects of mercury in the investigated tissues for biochemical parameters.

KEYWORDS: *Oreochromis niloticus*, mercury, selenium, antioxidant parameters, oxidative stress

1. INTRODUCTION

The aquatic environment is the last destination of pollutants distributed around the world. Hg, a non-essential metal, is a ubiquitous contaminant in the environment and comes from geological emissions and anthropogenic activities, such as fuel burning, mining, smelting operations, and the chlor-alkali industry [1]. Hg often poses a high ecotoxicological risk for aquatic organisms due to its high toxicity, bioaccumulation, and other deleterious effects on biota, including genetic effects and mutagenicity [2]. Selenium (Se) is an essential trace element, and potentially protective against the toxic effects of Hg [3]. It is also a structural component of several enzymes involved in antioxidant defence including glutathione peroxidase (GSH-Px) and thioredoxine [4]. Some investigations showed that Se may offer protective effects against Hg-induced toxicity through several mechanisms, such as sequestration of Hg, antioxidative effect, GSH synthesis, increased GSH peroxidase (GPx) activity, and high selenoprotein levels [5].

Metal toxicity causes an increase in ROS (reactive oxygen species), such as hydrogen peroxide, hydroxyl and superoxide radicals leading to oxidative stress in fish [6]. Oxidative stress occurs when the balance between oxidants and antioxidants is broken down [7], producing lipid peroxidation, DNA damage, and protein oxidation [8]. The most widely used assay for lipid peroxidation is MDA formation as a secondary lipid peroxidation product, and it is a good indicator for the extent of lipid peroxidation, induced by heavy metal pollution in aquatic organisms [9].

Antioxidant systems, such as GST and GSH, are of great importance in an oxidative stress system to cope with ROS formation leading to several defects [10]. GST represents a group of multifunctional enzymes with a predominant cytosolic defense systems responsible for the protection of cellular components against various toxic effects and oxidative stress [11], and catalyzing the nucleophilic attachment of glutathione (GSH) to molecules presenting an electro-

* Corresponding author

philic carbon, nitrogen, or sulphur atom [12]. GSH plays a central role in protecting eukaryotic cells from oxidative stress as an antioxidant [13]. CAT (EC 1.11.1.16) decomposes H_2O_2 to water and molecular oxygen, and plays a crucial role in reducing reactive oxygen-free radicals [14]. AChE (EC 3.1.1.7) plays a significant role in neurotransmission at cholinergic synapses by the rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate [15]. It was investigated that this enzyme is sensitive to environmental contaminants, such as metals, and could provide useful information about the toxic impact of Hg [16].

Biochemical constituents, like antioxidant systems, lipid peroxidation and AChE are the potential biomarkers of pollutants exposure in different organisms [17, 18]. The antioxidants investigated in the present study included CAT, GST and GSH. The oxidative damage was evaluated by determining lipid peroxidation, and was measured by malondialdehyde (MDA) content.

The aim of the present investigation was to determine the protective effect of Se supplementation against Hg toxicity in the oxidative stress capacity of kidney and brain tissues of *O. niloticus*. The changes in activities of GST, CAT, and AChE with GSH and MDA levels were determined. Hg-induced changes in the antioxidant and AChE enzymes, as well as lipid peroxidation content, were evaluated in the kidney and brain tissues.

2. MATERIALS AND METHODS

2.1 Experimental protocol

Freshwater fish *O. niloticus* (56.5±2.1 g and 15.9±1.8 cm) were obtained from Çukurova University Fish Culture Farm, and transferred to the laboratory where they were acclimatized for 2 months in 120-L glass aquariums containing dechlorinated and gently aerated tap water at 25±1 °C, the temperature of the experimental conditions. The properties of water were as follows: dissolved oxygen 7.25±0.72 mg/L, pH 8.4±0.8, temperature 23.1±0.5 °C, alkalinity 184.5 ± 4.4 mg/L as $CaCO_3$, and total hardness 278.2 ± 8.7 mg/L as $CaCO_3$ at acceptable levels. The fish were maintained under a 12-h light/12-h dark illumination period. The control and experimental fish were fed once daily with commercial food pellets (Pinar Feed, Turkey), approx. 3% of their body weight.

After the period of acclimatization, experimental fish stocks have been randomly divided into 3 groups each containing 18 fish. The first group was held in tap water as control, and the other groups were exposed to Hg and Hg+Se mixtures for 7 and 14 days. Three replicate groups of fish were used for each exposure. All the experimental solutions were completely replaced every other day by newly prepared stock solutions to reduce the ammonia content in water. There was no fish mortality during the experiments.

Prior to analysis, 6 fish were taken out from the aquaria, and fish were killed by transaction of the spinal cord (cutting nerves); then, kidney and brain tissues were dissected separately on an ice-cold plate. Tissues were then washed in ice-cold physiological saline solution, blotted dry, weighed, and stored frozen at -80 °C until analyses. All chemicals and reagents were purchased from Merck and Sigma-Aldrich Chemical Corporation.

Tissues were homogenized in ice-cold buffer containing 0.05 M phosphate buffer, 0.25 M sucrose (pH 7.4), with a ratio of 1/10 at 9500 rpm for 2-3 min. Homogenates were centrifuged at 9500 ×g for 30 min. All processes were carried out at +4 °C. The supernatants were collected for determination of CAT, GST, and AChE activities as well as total protein, GSH, and MDA levels.

2.2 Biochemical tests

GSH contents in the supernatants were determined using a recycling reaction of the GSH with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in the presence of excess GR [19]. The incubation medium contained 143 mM sodium–potassium buffer (Na-K Fosfat (containing 6.3 mM EDTA, pH 7.5), 0.3 mM NADPH, 6 mM DTNB, and 50 units of GR. DTNB formation was monitored continuously at 412 nm for 3 min.

CAT activity was determined spectrophotometrically by measuring the decomposition of H_2O_2 (10 mM solution) at 230 nm using the method of [20].

GST was measured according to Habig *et al.* [21] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The reaction mixture consisted of 100 mM tris buffer (pH 7.4), 1 mM GSH and 1 mM CDNB (1-chloro-2,4-dinitrobenzene). Enzyme activity was determined by monitoring the changes in the absorbance at 340 nm, which reflects the rate of CDNB conjugated with the GSH at 25 °C for 2 min.

Lipid peroxidation was estimated by measuring MDA contents with the method of Ohkawa *et al.* [22]. MDA levels were determined after incubation at 95 °C and pH 3.4, with thiobarbituric acid as an indicator of lipid peroxidation. MDA concentrations were analyzed by measuring the pink colour produced at 532 nm The reaction medium contained 8.1% of SDS, 20% of acetic acid, pH 3.4, 0.8% of TBA, and a mixture of *n*-butanol/pyridine (14:1).

Acetylcholinesterase activity was determined by the method of Ellman *et al.* [23] at 30 °C. Enzymatic activity was spectrophotometrically determined by measuring the increase in absorbance of the sample at 412 nm. Acetylthiocholine iodide and dithiobisnitrobenzoic acid were used as substrates.

The protein concentrations were measured by the method of Lowry *et al.* [24]. Protein content was expressed in mg/g wet weight of the tissue. Samples were treated with Folin-phenol reagent and the absorbance was measured at 750 nm. Bovine serum albumin was used to prepare the standard graphics.

2.3 Data analysis

All parameters were expressed as means ± standard error. The data were statistically analyzed by a series of Stu-

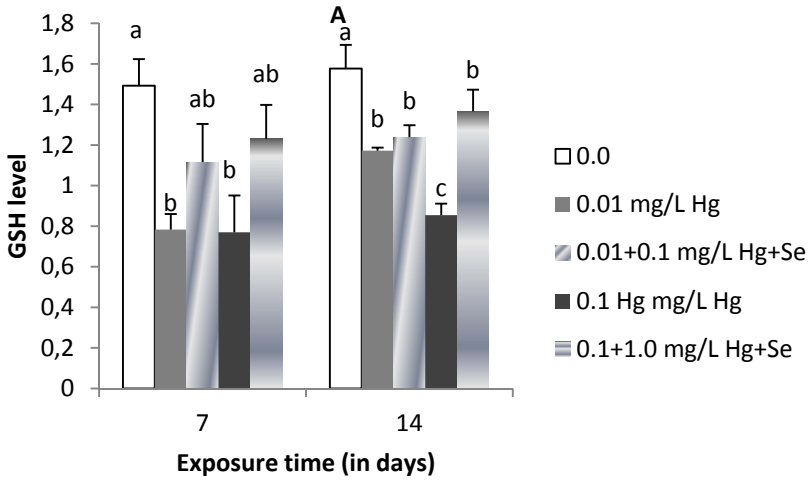
dent Newman Keul's tests and SPSS statistical package programs to identify any differences among tissues and experimental period. Data shown with different letters are significant at the $P < 0.05$ level.

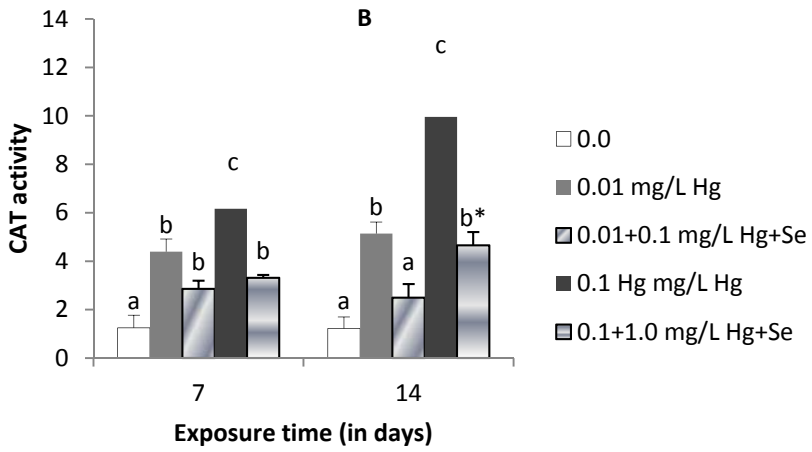
3. RESULTS

In the present investigation, there were significant ($p<0.05$) alterations in the GSH and MDA levels, CAT, GST, and AChE activities, in brain and kidney of *O. niloticus* exposed to Hg concentrations, observed for different concentrations and exposure periods.

The exposures to Hg and Hg+Se caused a decrease in GSH levels in the searched tissues, in relation to the control levels. This decline was higher for exposure to Hg alone than in the presence of Se with Hg, at the end of the period. Kidney GSH showed a significant reduction at low Hg concentration, with respect to the high Hg concentration, for a period of 14 days (Fig. 2A). Considerable differences did not occur in GSH levels of tissues between the exposure times ($P>0.05$). As shown in Figs. 1A and 2A, a significant reduction in GSH level was recorded at 0.1 mg/L Hg, 0.1 mg/L Hg+1.0 mg/L Se, and 0.01 mg/L Hg, 0.01 mg/L Hg+0.1 mg/L Se for the groups in brain (25, 21, 45, 13%) and kidney (57, 23, 59, 32%), compared to control, respectively, for a period of 14 days.

CAT and GST activities in brain and kidney tissues increased, in response to Hg exposures when compared to control during 7 and 14 days. At the end of the exposure period, elevation in these enzyme activities of fish exposed to Hg was higher when compared with Hg+Se-treated groups. There was no statistical significant difference between the exposure time in brain and kidney CAT activity, except for 0.1 mg/L Hg-exposed fish. CAT activity at high Hg and Hg+Se concentrations showed 8-4 (brain) and 2-1.5 (kidney)-fold increase after14 days, compared with control groups (Figs. 1B, 2B). Kidney GST activity, at low Hg concentration, indicated a significant increase after14 days, according to that at 7 days (Fig. 2C). GST activity was increased by 115 and 65% in brain as well as 65 and 39% in kidney, at high exposure concentrations and exposure duration when compared to control values (Figs. 1C, 2C).





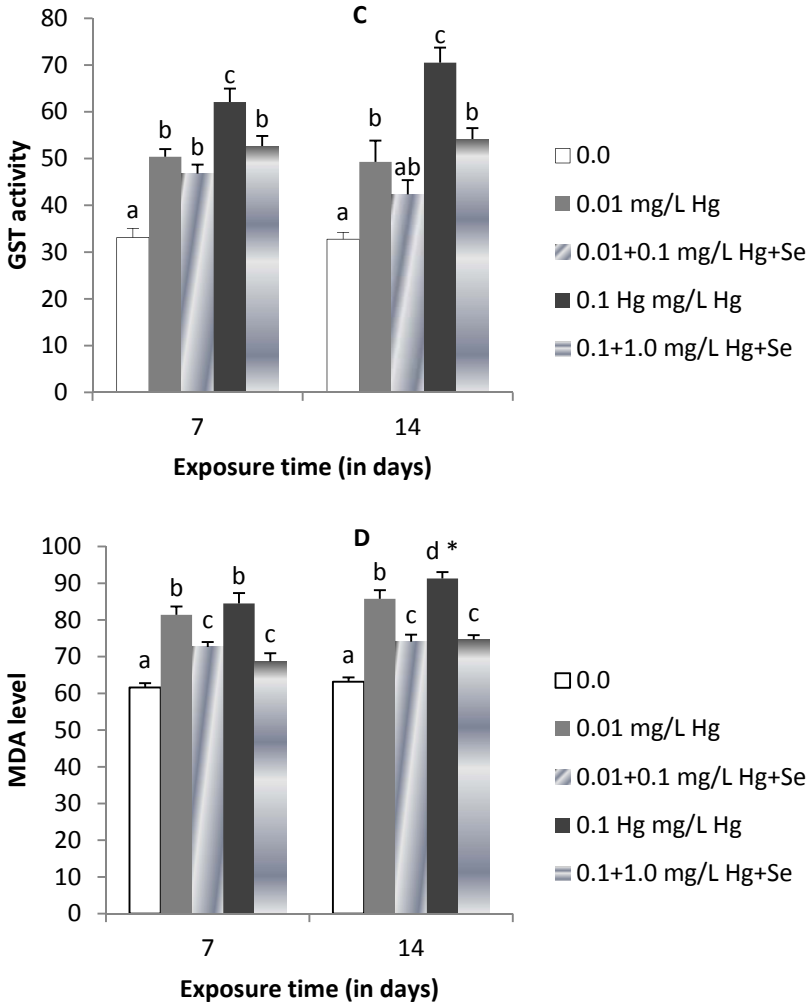
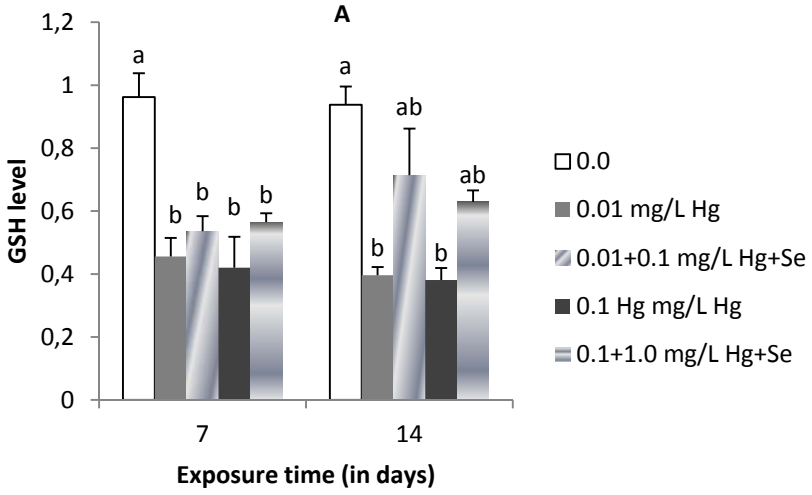
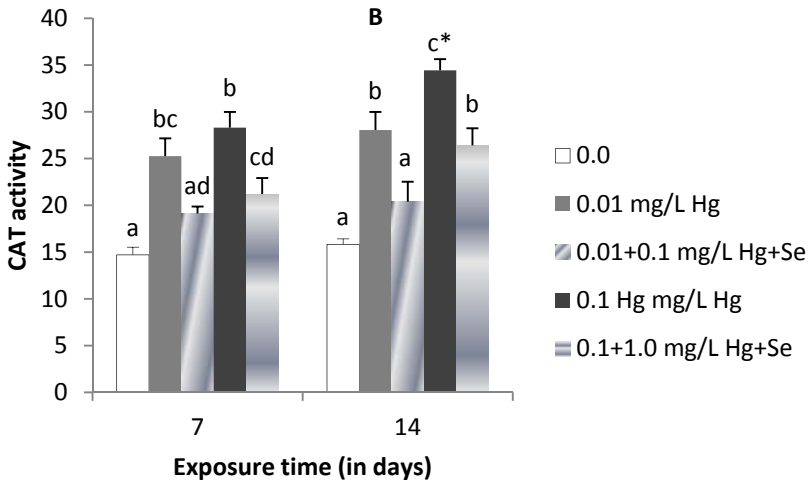
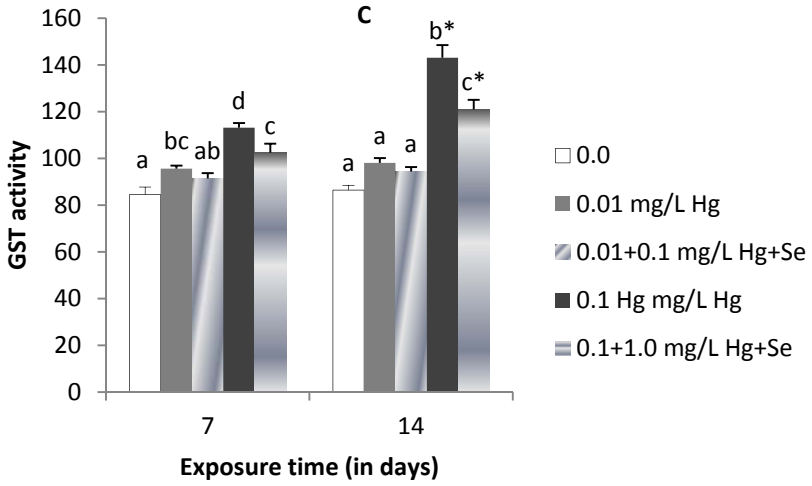


FIGURE 1 - GSH level (µM/mg protein, A), CAT activity (U mg^{-1} ofprotein, B), GST activity (U mg^{-1} of protein, C), LPO level (nmol MDA mg^{-1} of protein, D) in brain of *O. niloticus* exposed to metals for 7 and 14 days (data are expressed as means ± standard error (N=6); different letters indicate significant differences among groups at the same time (P < 0.05); * shows significant differences between times for the same exposure group (P<0.05).







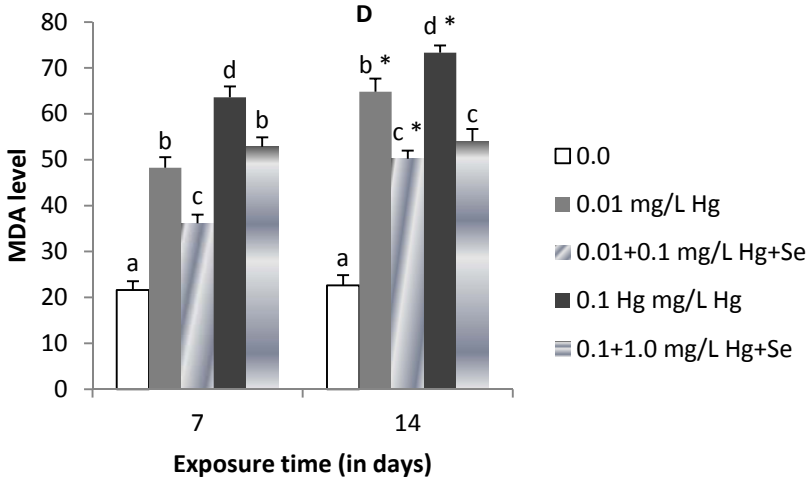


FIGURE 2 - GSH level (µM/mg protein, A), CAT activity (U/mg protein, B), GST activity (U/mg protein, C), LPO level (nmol MDA mg^{-1} of protein, D) in kidney of *O. niloticus* exposed to metals for 7 and 14 days (data are expressed as means ± standard error (N=6); different letters indicate significant differences among groups at the same time (P < 0.05), and * shows significant differences between times for the same exposure group (P<0.05).

TABLE 1 - Brain AChE activity (U/mg protein) in *O. niloticus* exposed to metals for 7 and 14 days.

Metal concentrations (mg/L)	7 days	14 days
0.0	0.576±0.022^{ax}	0.521±0.059^{ax}
0.01 Hg	0.344±0.027^{bx}	0.277±0.040^{bx}
0.01 Hg+0.1 Se	0.423±0.011^{cx}	0.347±0.012^{by}
0.0	0.576±0.022^{ax}	0.521±0.059^{ax}
0.1 Hg	0.332±0.034^{bx}	0.290±0.027^{by}
0.1 Hg+1.0 Se	0.430±0.026^{cx}	0.401±0.176^{aby}

Values are expressed as means ± standard error (N=5). Letters a, b and c indicate differences among concentrations, and letters x and y indicate statistical differences among tissues at the Pb<0.05 level.

TABLE 2 - Kidney AChE activity (U/mg protein) in *O. niloticus* exposed to metals for 7 and 14 days.

Metal concentrations (mg/L)	7 days	14 days
0.0	0.055±0.003^{ax}	0.057±0.002^{ax}
0.01 Hg	0.040±0.001^{bx}	0.035±0.003^{bx}
0.01 Hg+0.1 Se	0.042±0.003^{bx}	0.048±0.003^{cx}
0.0	0.055±0.003^{ax}	0.057±0.002^{ax}
0.1 Hg	0.034±0.006^{bx}	0.029±0.005^{bx}
0.1 Hg+1.0 Se	0.042±0.005^{abx}	0.046±0.004^{ax}

Values are expressed as means ± sStandard error (N=5). Letters a, b and c indicate differences among concentrations, and letters x and y indicate statistical differences among tissues at the P<0.05 level.

MDA contents in all tissues of fish increased significantly with increasing concentrations of Hg in the exposure medium and with long treatment durations. MDA levels in tissues represented a significant rise in all treatments when compared with control values, and this increase was much higher after14 than 7 days. Particularly higher MDA levels were observed from control, at each time of exposure during 4 and 21 days, in brain and kidney, respectively. MDA levels exhibited an increase of 32, 18, 37, and 12% in brain as well as 123, 67, 194, and 144% in kidney, in relation to the reference Se concentrations of 0.1 mg/L Hg, 0.1 mg/L Hg+1.0 mg/L Se, and 0.01 mg/L Hg, 0.01 mg/L Hg+0.1 mg/L Se, respectively, for a period of 7 days (Figs. 1D, 2D).

AChE activity was inhibited in brain and kidney under the effects of metals. The important inhibition of AChE was recorded to be 46, 33, 44, and 23% in brain as well as 39, 16, 49, and 19% in kidney, for 0.1 mg/L Hg, 0.1 mg/L Hg+1.0 mg/L Se, and 0.01 mg/L Hg, 0.01 mg/L Hg+0.1 Se concentrations, as compared to control groups (Tables 1 and 2). AChE activity in brain at 0.1 and 0.1 mg/L Hg+1.0 mg/L Se concentrations were decreased significantly after14 days, with respect to those at 7 days, showing no statistically significant differences between the exposure periods in kidneys. AChE activity observed in kidneys after 7 days and in brain after14 days was close to that of the control group at 0.1 mg/L Hg+1.0 mg/L Se concentration.

4. DISCUSSION

At all concentrations, the tested tissues of *O. niloticus* showed the effects of the treatments above in enzym activities as well as MDA and GSH levels. Se is remarkably protective against toxic effects of Hg in fish species [3-25]. This protective effect seems to be a result of the formation of Se-Hg complexes [26].

GSH is important in the antioxidant defense, and its depletion may reduce the cellular ability to scavenge free radicals [27]. In this investigation, GSH content in the tissues decreased with increasing Hg, Hg+Se concentrations, and exposure periods. Decreasing GSH may be due to direct binding of the metal to GSH through its SH group, or enhanced oxidation of this thiol [28]. Exposure to Hg caused a significant decrease in GSH levels but increase in CAT and GST activities in tissues of *Brycon amazonicus* [29].

GST catalyzes the synthetic conjugation reactions of xenobiotics to GSH, and thus, this function protects fish tissues from oxidative stress [12]. CAT is responsible for the conversion of H_2O_2 to water [14]. After Hg and Hg+Se treatments, in this study, GST and CAT activities in brain and kidney were promoted significantly, depending on Hg concentrations and exposure periods. The increase in CAT activity was probably due to continuous production of $O2^{\bullet-}$ into H_2O_2, as a result of oxidative stress.

Elia *et al.* [28] concluded that exposure to Hg resulted in increased GST activity and decreased GSH level in the liver of *Ictalurus melas*. Dabas *et al.* [30] observed that GST activity and lipid peroxidation increased in liver, kidney and gill, whereas CAT activity increased in liver but was decreased in kidney and gill of Cd-exposed *Channa punctatus.* Results might reflect that tissue GST activity could be induced to neutralize the metal toxicity, as forming part of an adaptive response to metal stress. Therefore, increasing GST activity suggested that the detoxification processes were increased.

Lipid peroxidation has been used as a biomarker of oxidative damage in aquatic organisms exposed to various compounds [31]. The outcomes of the present study indicate that contents of MDA in the tissues decreased in the presence of Se. Our results are in agreement with the findings of other investigations who found that Hg induced lipid peroxidation in tissues of fish [32, 33]. Orun *et al.* [34] demonstrated that exposure to Cd and Cr resulted in increased MDA levels in tissues; however, Se exposure with metals decreased MDA levels. On the other hand, Hg may lead to alterations in brain activity, motor development, and neuro-psychological action [35]. The results demonstrated an AChE inhibition in tissues after 7 and 14 days of exposure. Several studies also showed that AChE activity could be inhibited by Hg exposure [32-36]. In a similar study, Richetti *et al.* [37] observed that Hg significantly decreased AChE activity and antioxidant competence in zebrafish brain. However, in our study, inhibition of this enzyme was much higher in the Hg medium, compared to Hg+Se mixtures. In addition, Sen *et al.* [36] revealed that Se could activate the AChE activity which was inhibited by Hg exposure in fish brain. Inhibitions of AChE could be suggested to be an expression of Hg neuro-toxicity. This study demonstrated that AChE activity can be used as a biomarker in fish exposed to polluted environmental conditions.

5. CONCLUSION

The results revealed that tissues were significantly affected, particularly for long treatment durations and higher exposure concentrations. Exposure to Hg and Hg+Se resulted in inhibition of AChE and depletion of GSH, finally resulting in oxidative damage to lipids, accompanied with elevated of MDA levels and induction of antioxidant enzymes in brain and kidney tissues. Alterations in these parameters are presumably a response against increased ROS generation under Hg toxicity, and Se was able to antagonize the toxic effects of Hg, as a result of the antioxidant ability of Se, or the formation of a Se-Hg complex. So, we concluded that Se is protective against toxic effects of Hg.

The authors have declared no conflict of interest.

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CORRESPONDING AUTHOR

Gülbin Firidin
Life Science Research Center
Gazi University
06830 Ankara
TURKEY

Phone: +90 3124842157
Fax: +90 312 484 62 71
E-mail: gulbinfiridin@gazi.edu.tr

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