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Effects of Mercury Chloride on Oxidative Stress Biomarkers of Some Tissues of the African Catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract

The present study evaluates in *Clarias gariepinus* the oxidative damage associated with two sub-chronic exposures to mercury chloride. The destructive effects of mercury chloride on the African Catfish, *Clarias gariepinus* was revealed in terms of protein carbonyl (PC), lipid peroxidation (LPO), DNA damage and nitric oxide (NO) as oxidative stress biomarkers. Super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), glutathione reductase (GR), glutathione-s-Transferase (GST), glutathione (GSH) and total antioxidant (TAO) in the gills, kidney and liver can be used as biomarkers to identify possible environmental contamination in fish. This study aimed to investigate the impact of $HgCl_2$ (0.04 and 0.12 ppm) for 14 and 28 days of the activity of the selected parameters in different tissues of *Clarias gariepinus.*

The activity of SOD, CAT, Gpx and TAO dropped when compared to the control groups without mercury chloride exposure in all tissues under investigation. The pattern of variations in GST, GR and GSH activity in mercury-induced groups were significantly increased than that of the control group. Also, NO, CP, LPO and DNA damage, were recorded with a pattern of a significant increase toward exposure period in all tissues under investigation.

Keywords: Mercury; Antioxidant; Oxidative stress; Catfish

Introduction

The impact of heavy metals on biomarkers of oxidative stress is bio-indicators of aquatic pollution in *Clarias gariepinus* [1]. It is known that Hg has a high affinity to thiol-containing molecules such as glutathione (GSH) [2], which are required for Hg transport and detoxification in biological systems [3]. Environmental exposure to Hg can interfere with physiological as well as biochemical activities through the oxidative stress [4]. This stress is generated in cells through an increase in the production of reactive oxygen species (ROS) as superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH), or decrease in the antioxidant that modifies ROS to the less reactive intermediate and prevent diseases by scavenging free radicals [5].

Oxidative stress may produce DNA damage, protein oxidation, nitric oxide formation and peroxidation of cell constituents, especially lipid peroxidation when antioxidant defenses are impaired or overcome [6]. Enzymatic antioxidant defense mechanism includes superoxide dismutases (SOD), catalase (CAT) and glutathione peroxidase (GPX), as well as the enzymes involved in the recycling of GSH, and generation of NADPH [7]. The GSH and other thiols depletion will render cells more susceptible to oxidative damage, while elevated antioxidant enzymes activity will counteract it to a certain extent [8]. Glutathione, specifically bind with mercury, forms a complex that prevents Hg from binding to cellular proteins and causing damage to tissue [9-12]. Both GSH and cellular antioxidant enzymes play an important role in $HgCI_2$ induced tissues injury [8,13,14].

Fish, as biomonitor species, plays an increasingly important role in the monitoring of aquatic environment, due to its great sensitivity to environmental changes. Thus, the aim of this study was to determine oxidative stress biomarkers and antioxidant parameters as a response to Hg exposure at low and moderate doses in three organs of *Clarias gariepinus* after 14 and 28 days of exposures.

Materials and Methods

Fish

Forty eight adult African catfish *Clarias gariepinus* were caught from the fish farm of Faculty of Agriculture, Assiut University, Egypt. Fish immediately were transported to the fish laboratory in the Department of Zoology, Faculty of Science, Assiut University. The fish (225-250 g) were fed on a commercial pellet diet (2% of body weight per day) and kept in 180 L rectangular tanks containing tap water (conductivity 2000 ls/cm; pH 7.4; oxygen 90–95% saturation; temperature 2°C; photoperiod 12:12 light: dark). After 2 week acclimation, fishes were classified into three groups (16 fish per each): control and two Hg-treated groups (0.04 and 0.12 ppm, Ibrahim, 2011) for 14 and 28 days of exposure.

Organs collection and preparations

Gills, kidney and liver were carefully excised, surface dried with filter paper, thoroughly washed with 50 mM phosphate buffer pH 7.4 and homogenized with 50 mM phosphate buffer pH 7.4 containing, 1 mM EDTA, 1 mM DTT, 0.15 M KCl, 0.01% PMSF. Homogenization was carried out at about 4°C using 12–15 strokes of a motor driven Teflon Potter homogenizer and centrifuged at 10,000 rpm for 20 min

at 4°C and the obtained supernatants were used for determination of antioxidants and oxidative stress biomarkers.

Antioxidants and oxidative stress biomarkers

Total protein contents were determined by Lowry method [15]. Lipid peroxidation was measured according to the method of Buege and Aust [16]. DNA fragmentation was determined by the procedure of Kurita-Ochiai et al. [17]. Carbonyl protein was determined by the method of Levine et al. [18]. The NO concentration was determined by Griess reaction as described by Sessa et al. [19]. Total SOD activity was measured as described by McCord and Fridovich [20]. Catalase activity was measured as described by Aebi [21]. GPX activity was estimated using the method of Flohe and Gunzler [22]. Glutathione S-transferase (GST) was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate as described by Habig et al. [23] and adapted to a microplate reader by Stephensen et al. [24]. The GSH level was measured following the methods described by Cohn and Lyle [25]. GR activity was assayed according the method described previously by Styblo and Thomas [26]. The TAO was measured using a colorimetric assay kit (Randox Laboratories, Crumlin, U.K.) and values are expressed as mmol/L.

Statistical analyses

Data are expressed as mean ± Std. Err. Statistical significance was evaluated by ANOVA. Differences were considered significant at $P<0.05$ using the statistical software SPSS version 16.

Ethical statement

All experiments were carried out in accordance with the Egyptian laws and University guidelines for the care of experimental animals. All procedures of the current experiment have been approved by the Committee of the Faculty of Science of Assiut University, Egypt.

Results

The biomarkers of oxidative stress and antioxidant analyzed showed significant variation ($p<0.05$), ($p<0.01$) and ($p<0.001$) when compared with control. As indexes of antioxidant status, the levels of LPO (assessed by MDA content), DNA fragmentation, CP, and NO TAO,GST, GR, SOD, CAT, GSH and GPx activities were measured for evaluating the presence of oxidative stress in adult *Clarias gariepinus* catfish by exposure to (0.04 and 0.12 ppm) of $HgCl_2$ for 14 and 28 days. The oxidative stress biomarkers; MDA, CP, DNA fragmentation and NO levels were increased after the exposure doses dependent manner with an upward trend (Figure 1A-1D).

The effects of sub lethal doses (0.04 and 0.12 ppm) of mercury chloride on gills, kidney and liver of *C. gariepinus* after 14 and 28 days of exposure are shown in Figure 1.

The oxidative stress biomarkers; LPO, CP, DNA fragmentation and NO showed highly significant increases in the gills, kidney and liver ($P<0.001$) after exposed to 0.12 ppm HgCl2 for 28 days and a significant ($P<0.05$) increase in tissues under investigation after exposed to 0.04 ppm mercury for 14 days comparing with the control group (Figure 1A-1D). Also, except for TAO non enzymatic antioxidant (GSH, GR and GST) showed a significant increase ($p<0.05$) in 0.04 ppm mercury chloride and significant increase ($P<0.01$) and ($P<0.001$) in 0.12 ppm mercury exposed group when compared with the control one (Figure 2A-2C).

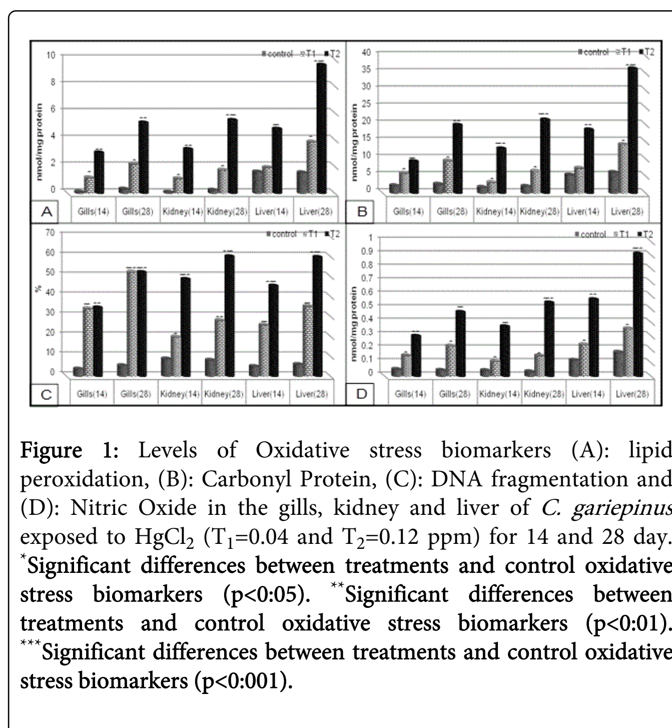

Figure 1: Levels of Oxidative stress biomarkers (A): lipid peroxidation, (B): Carbonyl Protein, (C): DNA fragmentation and (D): Nitric Oxide in the gills, kidney and liver of *C. gariepinus* exposed to $HgCl_2$ (T_1=0.04 and T_2=0.12 ppm) for 14 and 28 day. ***Significant differences between treatments and control oxidative stress biomarkers (p<0:05). **Significant differences between treatments and control oxidative stress biomarkers (p<0:01). ***Significant differences between treatments and control oxidative stress biomarkers (p<0:001).**

However, TAO exhibited a significant decrease ($p<0.05$) in 0.04 ppm mercury chloride and significant decrease ($P<0.01$) and ($P<0.001$) in 0.12 ppm mercury exposed group when compared with the control one (Figure 2D). The results of enzymatic antioxidant (CAT and SOD) in different tissues of *C. gariepinus* showed a significant decrease ($p<0.05$) in 0.04 ppm mercury chloride and significant decrease ($P<0.01$) and ($P<0.001$) in 0.12 ppm mercury exposed group when compared with the control group (Figure 3A and 3B). Also, GPx exhibited a significant decrease ($p<0.05$) in 0.04 ppm mercury chloride and a significant decreases ($P<0.01$) in 0.12 ppm mercury exposed group when compared with the control one (Figure 3C).

Discussion

The excess production of ROS by Hg may be explained by its ability to produce alteration in mitochondria by blocking the permeability transition pore. Hg^{2+} reacts with thiol groups (-SH), thus depleting intracellular thiol, especially GSH and causing cellular oxidative stress Gstraunthaler et al. [27]. Scavenging enzymes at lower concentration fish makes them vulnerable to oxidative damage when attacked by ROS [28]. Removal of H_2O_2 is an important strategy of aquatic organisms against oxidative stress [29]. The present study showed a significant increase in LPO after the exposure of $HgCl_2$. Similar results was obtained by Mahboob et al. and Park [30,31]. Moreover, It has been demonstrated that Hg decreases the antioxidant systems and produces oxidative damages via H_2O_2 generation thereby leading to LPO [13,14]. Therefore, an increase in LPO by $HgCl_2$ may induce membrane biochemical and functional alterations [9,32].

Mercury causes cell membrane damage leads to the imbalance between synthesis and degradation of enzyme protein [33]. The present study showed a significant increase of carbonyl protein (CP) in all tissues of fishes exposed to $HgCl_2$.

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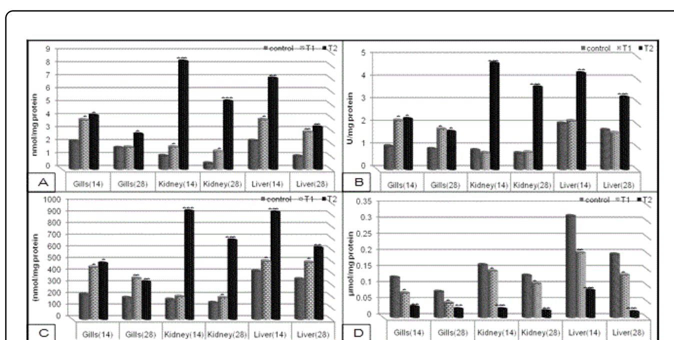

Figure 2: Activities of non-enzymatic antioxidant (A): Glutathione, (B): Glutathione reductase, (C): Glutathione-S-Tranferase and (D): Total Antioxidant in the gills, kidney and liver of C. gariepinus exposed to $HgCl_2$ (T1=0.04 and T2=0.12 ppm) for 14 and 28 day. *Significant differences between treatments and control non enzymatic antioxidant activities (p<0:05). **Significant differences between treatments and control non enzymatic antioxidant activities (p<0:01). ***Significant differences between treatments and control non enzymatic antioxidant activities (p<0:001).

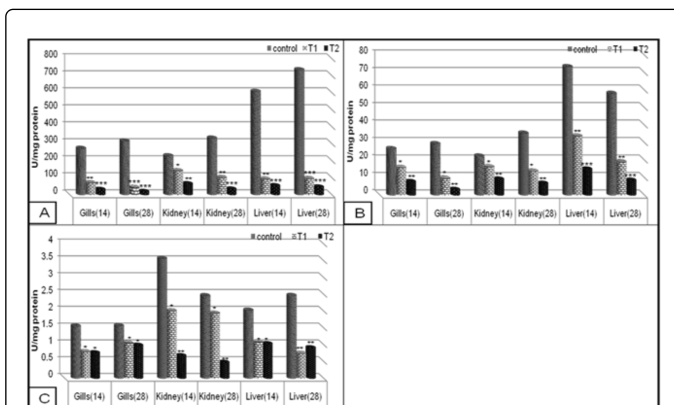

Figure 3: Activities of enzymatic antioxidant (A): Catalase, (B): Super Oxide Dismutase and (C): Glutathione Peroxidase in the gills, kidney and liver of *C. gariepinus* exposed to $HgCl_2$ (T_1=0.04 and T_2=0.12 ppm) for 14 and 28 day. *Significant differences between treatments and control enzymatic antioxidant activities (p<0:05). **Significant differences between treatments and control enzymatic antioxidant activities (p<0:01). ***Significant differences between treatments and control enzymatic antioxidant activities (p<0:001).

This increase in CP agrees with the observations of Labieniec and Parveza, Verlecar et al. [4,34,35] they found that fishes exposed to Hg^{2+} showed an increase in CP content because mercuric ions are known to induce oxidative stress by triggering ROS through mitochondrial electron transport chain in the animals [36,37]. Carbonyl compounds are toxic due to their carcinogenic properties. Their presence in the environment has a great concern as regard to their adverse effects to health [38]. Similar results were obtained by Perluigi et al. who described the elevated level of CP was not surprising considering that because of the function of the redox sensitive protein depending on the elevation of ROS [39].

Nitric oxide level showed an increase in all tissues under investigation after 14 and 28 days of $HgCl_2$ treatment. It is known that Hg^{2+} accumulation paralleled by the formation of NO. Excess NO can rapidly react with O2• to form a potent and powerful long-lived oxidant, peroxynitrite which can interact with nucleic acids [40,41]. Accordingly, the present study indicates that fish exposed to $HgCl_2$, in high and low doses, showed a significant increase in DNA fragmentation percentage in comparison with the control group. This results were in agreement with the study of Takenaka et al. who found high level of DNA damaged in rat tissues that exposed to Hg [42]. DNA damage in Hg^{2+} exposed individuals suggests that Hg overload induces an imbalance in the redox cycle [43]. Ariza et al. and Grotto et al. showed that Hg is a reactive metal that bind to DNA, leading to alterations in its structure, even at low levels [44,45]. Moreover, concludes that Hg induced genotoxicity by production of ROS, which react with DNA forming Hg species-DNA adducts; inhibition of the DNA repair systems, and inhibition of mitotic spindle formation and chromosome segregation [46].

The enzymatic antioxidant systems, which includes SOD, CAT, GPx, GR, GST as well as TAO plays a coordinated role in the prevention of oxidative damage by ROS [47]. The present study showed a significant decrease in tissues CAT, SOD and GPx activities in $HgCl_2$ exposure fishes compared to control group. The SOD removes superoxide by converting it into H_2O_2, which is rapidly converted to water by CAT. Therefore, any alteration in the activity of these two enzymes may result in several deleterious effects due to accumulation of superoxide radicals and H_2O_2 [43]. Moreover, GPx catalyzes the oxidation of GSH to GSSG, this oxidation reaction occurs at the expense of H_2O_2. The scavenger role of antioxidant enzymes in removing toxic electrophiles helps the cell to maintain its internal environment to a limited extent at lower concentration of Hg. The decrease in activities of SOD, CAT and GPx in different tissues of $HgCI_2$ treated fish may be due to the inhibition at these enzymes by H_2O_2, because it is known that H_2O_2 involved in Hg induced acute renal injury [32,48]. Moreover, inhibition of SOD activity may be related to a covalent attachment of Hg^{2+} to its reactive cystine residues that are involved in the detoxification of metals. In addition, SOD inhibition might also been consequence of excess of residue that affect enzyme structure [14,49]. The decrease in the level of CAT and GPx activities occurred probably as a defense response against H_2O_2 generated by $HgCl_2$ [9,11,49] Alternatively, SOD catalyzes superoxide anion radical dismutation into H_2O_2 accordingly, SOD activity inhibition could contribute to the enhanced oxidation observed in Hg-treated fish. Since LPO induced by $HgCl_2$ seems to be caused by increased levels of $O^{2•}$ [32,42,49].

The present study, showed a significant increase in GST, GR and GSH. Similarly, **Vinodhini and Narayanan** found that GST exhibited a significant increase in the GST activity in aquatic organisms exposed to Hg^{2+} [50] and **Bharathi et al.** for GR in rat [49]. Elevation of GST activity could indicate a defense of fish against oxidative stress damage and increase in water soluble metabolites that produced by $HgCl_2$ treatment [51]. Metal contamination of coast of Spain caused increases in GST activity and glutathione-related enzymes in tissues of *Mytilus galloprovincialis* [52]. Mercury has a high affinity to GSH and can bind and cause irreversible excretion of GSH and an increase in LPO [53]. It has been reported that Hg^{2+} at low concentrations depletes mitochondrial GSH and enhances H_2O_2 formation in rat kidney mitochondria under conditions of impaired respiratory chain electron transport [36]. The increase in GSH may indicate a faster rate of GSH utilization or degradation, which could be responsible for the observed

lower GSH content. Moreover, increase of GSH content may be related to prevention of oxidative challenge [54]. Aquatic organisms maintain high content of GSH in tissues and increased content has the function of protection that could provide the first line of defense against the influence of toxic heavy metals [51,55].

From the present results we conclude that exposure of fishes to different doses of $HgCl_2$ for different duration times caused oxidative stress in liver, kidneys and gills as shown by increased LPO, NO, DNA fragmentation and decreases in the non-enzymatic and enzymatic antioxidants levels.

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