Effect of Lead on Enzyme Activity in the Liver of Tilapia (Oreochromis niloticus)

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Abstract

Worldwide heavy metals belong to one of the most important groups of pollutants. Most of them including cadmium, copper, lead, and zinc are considered extremely dangerous in the eco-toxicological aspect. Recently, the use of biological markers or biomarkers measured at the molecular or cellular level has been proposed as a sensitive tool to evaluate oxidative stress in fish. The study aimed to investigate the potential of lead (Pb) in water to affect growth performance, weight gain, feed utilization, and its potential to induce oxidative stress and enzyme activity disruptions. Five treatments were designed including a control and Pb contaminated water at 0.01, 0.05, 0.25 and 1.25 mg/L concentrations. Results from the present study showed that fish exposed to Pb contaminated water at the highest tested concentration resulted in significantly high ($P < 0.05$) lipid peroxidation levels in tilapia when compared to all other groups and the control. Furthermore, a contaminant in water resulted in a significant increase of glutathione (GSH) activity in the liver of fish compared to control. However, no significant changes in catalase (CAT), glutathione-S-transferase (GST), and superoxide dismutase (SOD) activities were observed when compared among groups and with the control.

1. Introduction

Fast developing agriculture and industry in developing countries greatly improved the production rate and net yield. But lack of control in environmental security and discharge of untreated wastes contribute to the contamination of freshwater systems and cause adverse effects on aquatic biota.

Since fish can metabolize, concentrate, and store water-borne pollutants they are excellent subjects for the study of various effects of contaminants present in water samples. Several studies report about disturbing levels of heavy metals in rivers (Thorpe et al. 2011; Törnqvist et al. 2011). Heavy metals have the ability to accumulate in the environment and cannot be destroyed by biological degradation, it makes them toxic to the aquatic environment and consequently to people who consume aquatic animals. Heavy metal accumulated in the tissues of fish may catalyze reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress (Doherty et al. 2010).

Generally, reactive oxygen species are kept at physiologically optimal levels under normal conditions by antioxidant defense systems. Markedly, it is proved that for animals, the impeccable resistance system eliminating excessive ROS includes several antioxidant enzymes, such as superoxide dismutases (SODs), catalases (CATs), glutathione S-transferases (GSTs), etc. (Banaee et al. 2013). These enzymes are found virtually in all tissues of vertebrates but show in general, high activity in the liver, a major organ for the enzymatic transformation of ROS (Lopes et al. 2001; Adeniyi et al. 2005), this study, therefore, aimed to assess the hypothesis that Pb exposure causes oxidative stress and study enzyme activities such as catalase (CAT), glutathione (GHS), glutathione S-transferase (GST), malondialdehyde (MDA), superoxide dismutase (SOD) in the liver of tilapia (O. niloticus).

2. Materials And Methods

2.1 Experimental fish and management

In August 2016, 550 healthy 15-40g tilapias (O. niloticus) were brought from the tilapia breeding center of Freshwater Fisheries Research Center (FFRC) and transported to the laboratory in Wuxi, China. The fish were transported in tanks with oxygenated freshwater. Then they were acclimatized for 10 days in partially closed 200L aquarium tanks filled with 150L de-chlorinated aerated freshwater. Water quality was kept within standard physiological ranges; pH 7.2 ± 0.2, temperature 29 ± 2℃, dissolved oxygen (DO) 6.14 ± 0.46 mg/L (YSI 650 MDS multi-probe system, YSI inc. USA) and with 14h:10h light/dark photoperiod. Fish were fed thrice daily (08:00; 12:00; 17:00) with a commercial fish feed diet (No. 5271, 35% crude protein, Ningbo Tech-Bank co., ltd, Yuyao city, China) during the adaptation period until apparent satiation. The replacement of water in tanks was carried out every 24hours with de-chlorinated freshwater of similar temperature to maintain the water quality and reduce the build-up of metabolic wastes.

2.2 Preparation of Pb solutions and experimental layout

Fish with an average weight of 30 ± 2.85 g/fish were divided into 5 triplicate groups at a stocking density of 20 fish/tank into partially closed 200 L cylindrical white plastic tanks filled with 150L oxygenated and constantly aerated with pumps water to maintain constant dissolved oxygen. Fish were divided into groups, with the first group serving as control, and other groups were exposed to 0.01 mg/L Pb (Group 2), to 0.05 mg/L Pb (Group 3), to 0.25 mg/L Pb (Group 4), and 1.25 mg/L Pb (Group 5) for 42 days. Pb nitrate (3.9976 g) was dissolved in 100 ml of deionized water in order to get solution A with the concentration of 25 g/L, then 10 ml of solution A solved in 100 ml of deionized water to get solution B with the concentration of 2.5 g/L. 7.5 and 1.5 ml of solution A have been poured into water, so the concentration of Pb is 1.25 and 0.25 mg/L, 3 and 0.6 ml of solution B have been poured into water, so the concentration of Pb is 0.05 and 0.01 mg/L. Concentrations of Pb used in this study were designed based on the content of Pb in natural water sources reported in previous researches (Ayrault et al. 2013; Uzairu et al. 2014).

During exposure fish were fed thrice daily (08:00; 12:00; 17:00) with a commercial fish diet at the manufacturer's recommended rate (5% of their body weight daily). Uneaten food was removed at 30 minutes after feeding from all tanks and the feces were removed before feeding daily. The replacement of the water and test solution was prepared prior to use and carried out every 24 hours in order to keep concentrations of Pb close to the nominal levels.

2.3 Sampling procedure and preparation

Sampling procedure has been done after 1 week, 3, and 6 weeks on Friday. Two fish from each plastic tank were pooled, sedated (200 mg /L MS-222), dissected and the liver was gently removed. The liver was collected, placed in plastic tubes, and stored at -80°C for enzyme activity analysis.

Liver homogenate preparation has been done according to enzyme assay kit instructions. Animal tissue has been weighted accurately, homogenized in ice-cold physiological saline at a 1:9 ratio using a

homogenizer (KEMA KEUR PRO-200 Homogenizer). The homogenates were centrifuged at 3000 rpm, 3℃ for 10 min. The supernatants were stored at -80℃ as enzyme extracts without further purification.

2.4 Enzyme analysis

All procedures for enzyme analyses were done according to instructions from the assay kits (Nanjing Jiancheng Technology Co. Ltd http://www.njjcbio.com/).

CAT analysis

Ammonium molybdate can pause H_2O_2 decomposing reaction catalyzed by catalase (CAT) immediately, residual H_2O_2 can react with ammonium molybdate to produce a yellowish complex. It is able to calculate CAT activity by measuring optical density (OD) value at 405nm.

GSH analysis

Dithio-dinitrobenzoic acid (DTNB) would react with sulfhydryl compounds to generate a yellow compound, whose absorption peaks at 405nm. GSH concentration is determined based on the yellow compound's concentration its absorption.

GST analysis

GST is able to catalyze reduced glutathione (GSH) combined with 1-chloro-2,4-dinitrobenzene (CDNB), in a certain reaction period, its activity appears linear correlation with substrate concentration variance before and after the reaction. The kit used GSH concentration to reflect GST activity, the large GSH concentration reduce, the higher GST activity.

MDA analysis

Lipid hydroperoxide decomposition products can condensate with thiobarbituric acid (TBA method) to produce red compounds which have an absorption peak at 532nm.

SOD analysis

SOD analysis was done using the A001-3 SOD Assay Kit (WST-1 method).

2.5 Statistical analysis

The data were statistically analyzed using the SPSS package. One-way ANOVA and LSD multiple comparisons were used to determine average means - ± SE. Duncan's multiple range test was used to determine significant differences between groups ($p < 0.05$). The values of all biochemical parameters were expressed as mean ± SE.

3. Results

3.1 CAT

Catalase activity in the liver of tilapia (*O. niloticus)* exposed to Pb was analyzed and presented in Fig. 3 – 1. The highest catalase activity was observed at end of the first week of exposure in treatment which had 0.05 mg/L Pb and was recorded at 37.26 ± 4.42 (u/mgprot). While the lowest activity was registered in 0.25 mg/L Pb concentration on the sixth week of exposure registered at 12.41 ± 0.40 (u/mgprot).

One-way ANOVA test showed that there were no significant differences ($P > 0.05$) in CAT activity between all treatments in the liver of tilapia after 1 week of exposure. Similar to the first week, there were no significant differences ($P > 0.05$) in CAT activity on the third week of exposure between all Pb concentrations except 0.01 mg/L Pb concentration, which was significantly higher ($P < 0.05$) as compared to other groups. However, after 6 weeks of exposure catalase activity in the 0.25 mg/L group was significantly lower ($P < 0.05$) compared to control and treatment 0.01 mg/L, while treatment 0.01 mg/L were significantly higher ($P < 0.05$) as compared to all groups.

According to Fig. 3 – 2, the trend in catalase activity showed a decreasing trend in all groups. All the groups had a slight but decreasing trend except treatment 0.05 mg/L which displayed a sharp decreasing trend.

3.2 GSH

Glutathione activity in the liver of tilapia (*O. niloticus)* exposed to Pb was analyzed and presented in Fig. <link rid="fig1">3</link>–3. The highest glutathione activity was observed in treatment which had 1.25 mg/L Pb concentration on the sixth week at 42.04 ± 8.37 (u/mgprot), while the lowest activity was registered in 0.25 mg/L Pb concentration on the third week registered at 12.37 ± 5.12 (u/mgprot).

After 1 week of exposure GSH activity in the liver exhibited significantly low values ($P < 0.05$) at treatments with 0.05 and 0.25 mg/L metal concentrations compared to control.

In the third week, GSH activity in tilapia exposed to 0.25 mg/L Pb concentration was significantly lower ($P < 0.05$) as compared to control and treatment 1.25 mg/L. On the sixth week, GSH activity of treatments 0.05, 0.25, and 1.25 mg/L were significantly higher ($P < 0.05$) as compared to treatment control.

According to Fig. 3–4, the trend in catalase activity showed a moderate increasing trend in all treatments except control, which displayed decreasing trend.

3.3 GST

Glutathione-S-transferase activity in the liver of tilapia (*O. niloticus)* exposed to Pb were analyzed and presented in Fig. 3–5. The highest GST activity was observed in the control after 1 week of exposure and was recorded at 15.43 ± 3.61 (u/mgprot), while the lowest activity was registered in 0.05 mg/L Pb concentration on the first week of exposure and registered at 4.39 ± 1 (u/mgprot).

One-way ANOVA tests showed that after 1 week of exposure there was a significant difference ($P < 0.05$) in CAT activity of treatments 0.05 and 1.25 mg/L compared to control. Whereas, there were no significant differences ($P > 0.05$) between groups after 3 weeks of exposure. On the sixth week of exposure, group 0.25 mg/L showed a significant difference ($P < 0.05$) in GST activity as compared to all groups except treatment 1.25 mg/L.

According to Fig. 3–6, the trend in catalase activity showed a moderate increasing trend in treatment 0.05 mg/L, while the group exposed to 0.25 mg/L Pb concentration displayed no changes in trend. Treatment 0.01 and 1.25 mg/L Pb indicated a slightly increasing trend.

3.4 MDA

Malondialdehyde activity in the liver of tilapia (*O. niloticus)* exposed to Pb were analyzed and presented in Fig. 3–7. The highest glutathione activity was observed in treatment which had 1.25 mg/L Pb concentration on the sixth week at 106.69 ± 22.47 (u/mgprot), while the lowest activity was registered in the control group after 1 week of exposure and registered at 18.04 ± 4.14 (u/mgprot).

On the first week of exposure MDA activity in the liver of treatment 0.05 mg/L were significantly different ($P < 0.01$) compared to control. While treatment 1.25 mg/L Pb concentration was significantly different ($P < 0.05$) compared to all groups except treatment 0.25 mg/L. In the third week, there were no significant differences ($P > 0.05$) between treatments except 1.25 mg/L Pb concentration which were significantly increased compared to 0.01, 0.05, and 0.25 mg/L treatments. After six weeks of exposure, MDA activities in the liver of treatment exposed to 1.25 mg/L Pb showed a significant difference compared to all other treatments.

According to Fig. 3–8, the trend in catalase activity showed a moderate increasing trend in control and treatments 0.01 and 0.25 mg/L, while the group exposed to 0.05 mg/L Pb concentration displayed a slightly decreasing trend. However, treatment 1.25 mg/L showed a high increasing trend.

3.5 SOD

Superoxide dismutase activity in the liver of tilapia (*O. niloticus)* exposed to Pb was analyzed and presented in Figs. 3–9. The highest superoxide dismutase activity was detected in treatment which had 0.01 mg/L Pb concentration on the second week at 213.37 ± 45.92 (u/mgprot). While the lowest activity was recorded in 0.25 mg/L Pb concentration on the first week of exposure registered at 101.49 ± 6.15 (u/mgprot).

No significant differences ($P > 0.05$) were observed in SOD activities in the liver after one week of exposure between treatments except group exposed to 0.25 mg/L Pb concentration. Similar to the first week, after 3 weeks of exposure no significant differences ($P > 0.05$) were observed in SOD activities between groups compared to control except treatment 0.01 mg/L which was significantly different ($P < 0.05$) compared to groups exposed to 0.05 and 0.25 Pb concentrations.

After 6 weeks of exposure SOD activity in the liver of group 0.05 mg/L was significantly different ($P < 0.05$) compared to all other treatments except 0.01 mg/L. Similarly, treatment exposed to 1.25 mg/l Pb concentration was significantly ($P < 0.05$) different from all groups except control.

According to Fig. 3–10, the trend in catalase activity showed a slightly decreasing trend in control and treatments 0.01 and 0.05 mg/L, while the group exposed to 0.05 and 1.25 mg/L Pb concentration displayed a slightly increasing trend.

3.6 Total protein content

Total protein content in the liver of tilapia (*O. niloticus)* exposed to Pb was analyzed and presented in Figs. 3–11. The highest total protein content was identified in treatment which had 0.25 mg/L Pb concentration at 89.71 ± 2.55 (mg/ml). While the lowest value was documented in the control group and registered at 75.63 ± 3.14 (mg/ml).

One-way ANOVA test showed a significant increase in TP content ($P > 0.05$) between treatments 0.25 and 1.25 mg/L compared to control and group exposed to 0.05 mg/L Pb concentration (Fig. 2–11).

4. Discussion

The levels of specific biomarkers of oxidative stress (enzymes) were evaluated in tilapia (*O. Niloticus*). These results revealed a significant elevation of lipid peroxidation in fish in the highest metal concentration treatment. The evident increase in lipid peroxidation may be related to the accumulation of heavy metals in the flesh of the fish. Metal catalyzed formation of ROS that may damage DNA, protein, and lipids are well documented (Olaifa et al. 2004), also the level of certain biomarkers of oxidative stress was elevated in *Clarias gariepinus* from the Ogun River (Zhang et al. 2004). Catalase is one of the prime enzymes for the detoxification of ROS in all organisms and catalyzes toxic H_2O_2 to harmless H_2O and O_2. However, CAT activity analysis in the liver showed that Pb mostly had no significant effect on enzyme activity except in the group exposed to 0.25 mg/L Pb concentration after the sixth week. Atli et al. (2006) researched the response of CAT activity to Ag^{+}, Cd^{2+}, Cr^{6+}, Cu^{2+}, and Zn^{2+}. They reported that CAT activity either did not change or decreased possibly because of metal binding to these proteins. This study recorded that Ag^{+}, Cr^{6+}, and Cu^{2+} decreased the activity of CAT in vivo possibly because of metal binding to these proteins, while Cd^{2+} and Zn^{2+} had no effect. This points out that not all heavy metal pollutants affect the CAT activity in the liver of fish. According to our study, Pb may fall under the second group that does not affect CAT activity.

In contrast, levels of GSH activity in the liver of fish were significantly elevated. A noticeable increase in GSH levels in the organs suggests a protective and adaptive function of this biomolecule against oxidative stress induced by heavy metals. An increase in the production of antioxidant enzymes reflects an adaptive response that allows a partial overcoming of the oxidative stress caused by exposure to a polluted environment (Helmy, 2012). Koss et al. (1991) also reported that GSH levels can increase as an

adaptive process by means of an increased synthesis during moderate oxidative stress. Hence, we could suggest that tilapia which was in direct contact with Pb contaminated water had been exposed to moderate oxidative stress, which lead to an increased synthesis of GSH as a protective mechanism to this stressful situation.

This is in line with the research findings of Koss et al. (1991); Hamed et al. (2003); Li et al. (2003) and Pandey et al. (2003) who reported the increase of GSH levels in fish in a polluted area.

Glutathione-S-transferase (GST) is an important enzyme of phase II biotransformation. It protects the cell against toxic substances by catalyzing the conjugation of a wide variety of electrophilic substrates of the xenobiotic agents (Samanta et al. 2008; Ballesteros et al. 2009). Additionally, a decrease in GST activity during the exposure period may suggest a failure of the detoxification process, but at the same time, the induction of GST in fish tissues is regarded as beneficial for managing a stress condition. The high differences in GST activity on the first week can be explained if fish hadn't yet acclimatized completely, but on the third week, you can see the stabilization of GST activity in the liver. Then again temperature/weather changes may result in an average increase of GST activity levels on the sixth week. Overall, no significant differences between groups were recorded during the experiment except in the group exposed to 0.25mg/L Pb concentration after the sixth week. Although Ballesteros et al. (2009) observed decreased GST in the liver of *Jenynsia multidentata* exposed to endosulfan and de Menezes et al. (2011) found decreased GST in the liver of *Rhamdia quelen* exposed to Roundup, it should be understood that different pollutants affect an organism in many different ways.

Our results reveal that the highest concentration group had significantly increased MDA activity in the liver of tilapia during the entire experiment duration. The typical reaction during ROS-induced damage involves the peroxidation of unsaturated fatty acids increasing tissue MDA levels. Lipid peroxidation produced after exposure to Pb contaminated water is considered a valuable indicator of oxidative damage of cellular components (Helmy 2012). Therefore, our results indicate that tilapia has suffered a noticeable lipid peroxidation recognized from an increase in liver MDA levels, together with the increase of GSH levels in the liver.

These results agree with the findings of Upasani and Balaraman (2003) who reported that Pb exposure leads to a significant increase in lipid peroxide level and a decrease in the levels of endogenous antioxidants in the liver and kidney. Identically, Patra et al. (2001) noted that there is a significant increase in the lipid peroxidation in the liver and brain in animals exposed to Pb. In fact, several research findings suggest that Pb might produce a toxic action due to its ability to generate reactive oxygen species (ROS) which inflict oxidative damage in different tissues by enhancing lipid peroxidation through the Fenton reaction (El-Sokkary et al. 2003; Sahar et al. 2008dár et al. 2005).

Nevertheless, Pb pollution in the water did not affect considerably SOD activity during the experiment. Although SOD catalytically scavenges superoxide radicals, which may act as an important agent of toxicity of oxygen (Crestani et al. 2006), Pb did not cause SOD inhibition or induction. It suggests that each heavy metal may impact the fish in different ways.

The increased protein level in the liver after Pb exposure may indicate that organism is accumulating protein since it's being used in the anabolism process for meeting the high energy demand for augmentation of the defense mechanism of an organism against oxidative stress as a compensatory response. Similar results were also reported by Crestani et al. (2006) who observed increased protein levels in the liver of *Rhamdia quelen* exposed to clomazone. On the contrary, Begum et al. (2004) and David et al. (2004) pointed decrease in protein levels as a response to herbicides.

It is notable that CAT, SOD, and GST activities were not significantly affected during the experiment, whereas significant increases of MDA and GSH activities in the liver of fish were recorded. It may be accounted to the fact that the liver is more stable in the face of oxidative stress and a uniform organ with the highest antioxidant enzyme activities compared to other tissues (SOD, CAT) and it is the site of multiple oxidative reactions and maximal free radical generation (Avci et al. 2005; Oropesa et al. 2009).

Notably, lower concentrations of Pb did not show a significant effect, compared to the highest concentration, however, several studies report that a lot of rivers water bodies have the same and sometimes higher pollution levels as the highest concentration of Pb in the experiment (Uzairu et al. 2014; Ayrault et al. 2014).

5. Conclusion

The present study demonstrated that Pb contamination in water can result in lipid peroxidation and increased GSH activity in the liver of tilapia (*O. niloticus*). The enzyme activities can be acknowledged as a sensitive biomarker for monitoring the aquatic environment before the adverse effects occur for aquatic species. Despite the fact that several studies showed that heavy metal contamination catalyzes oxidative reactions causing the production of ROS, not all oxidative stress biomarkers used in this research responded to Pb contamination. To put it another way, GSH and MDA activity levels were significantly elevated in groups with a high concentration of Pb in water, while other enzyme activities (CAT, SOD, GST) were not affected significantly during the experiment. These findings show that Pb contamination doesn't affect all oxidative stress response systems in the organism, but have more precise targeted action. We suggest that more comprehensive researches need to be done to understand how exactly Pb influence and which defensive systems activate in the organism of fish to understand more about the impact of the mechanism of Pb contamination on aquatic organisms.

Data availability

The data used and/ or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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Author Contributions

EK and DGH written the first draft, study conception, design, material preparation, data collection and analysis. FL, CJ revised research critically for important intellectual content, served as scientific advisors and approved the version to be published.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

All Authors consent for Publication.

Conflict of interest

The authors declare no competing interests.

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Figures

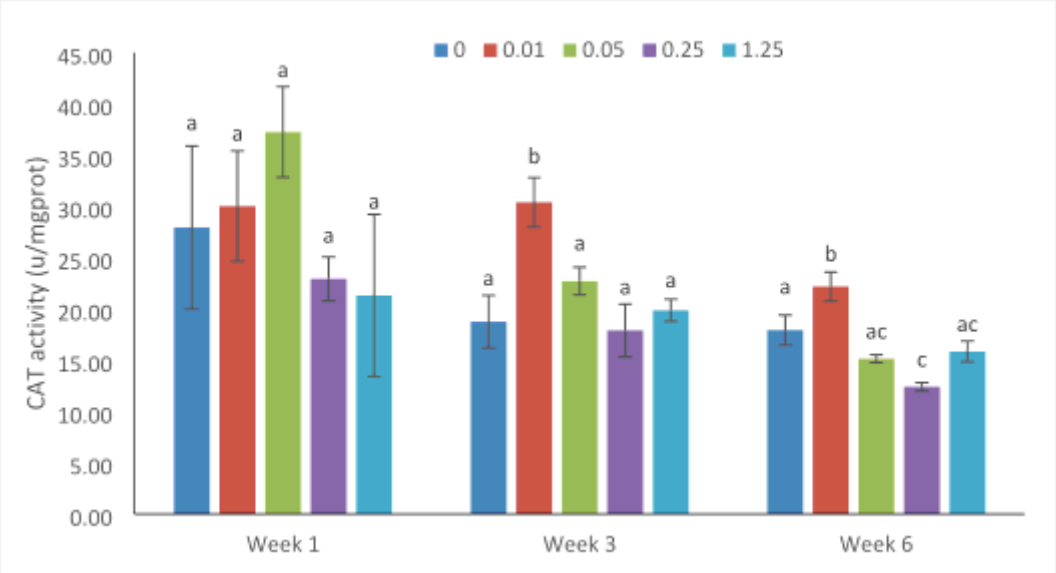

Figure 1

3-1 Catalase activity and associated standard errors by weeks in the liver of tilapia (n=6). Values with different letters (a, b, and c) in the same week are significantly different.

Figure 2

3-2 Catalase activity and associated standard errors by treatment in the liver of tilapia (n=6).

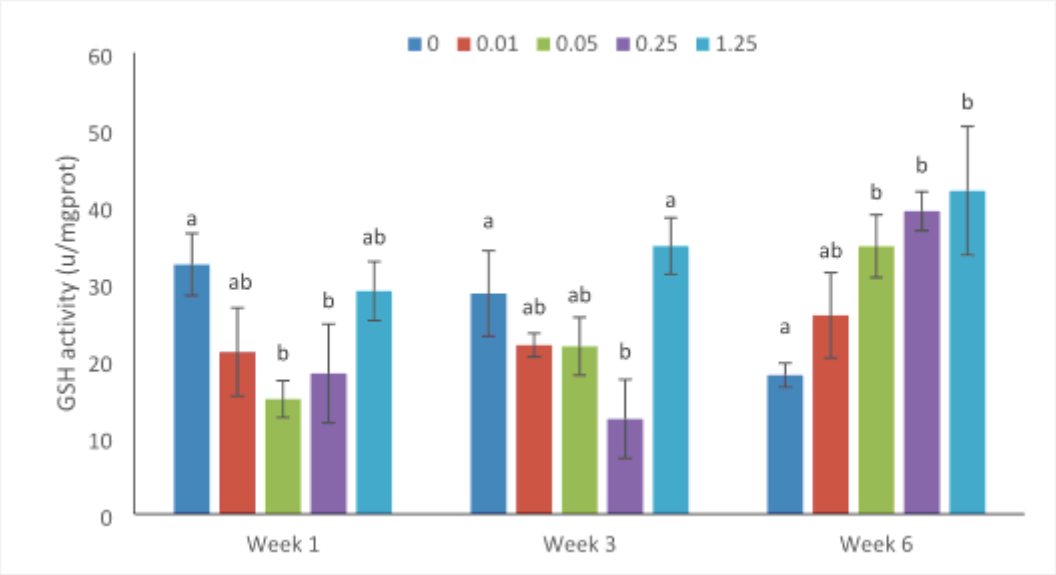

Figure 3

3-3 GSH activity and associated standard errors sorted by weeks in the liver of tilapia (n=6). Values with different letters (a, b) in the same week are significantly different.

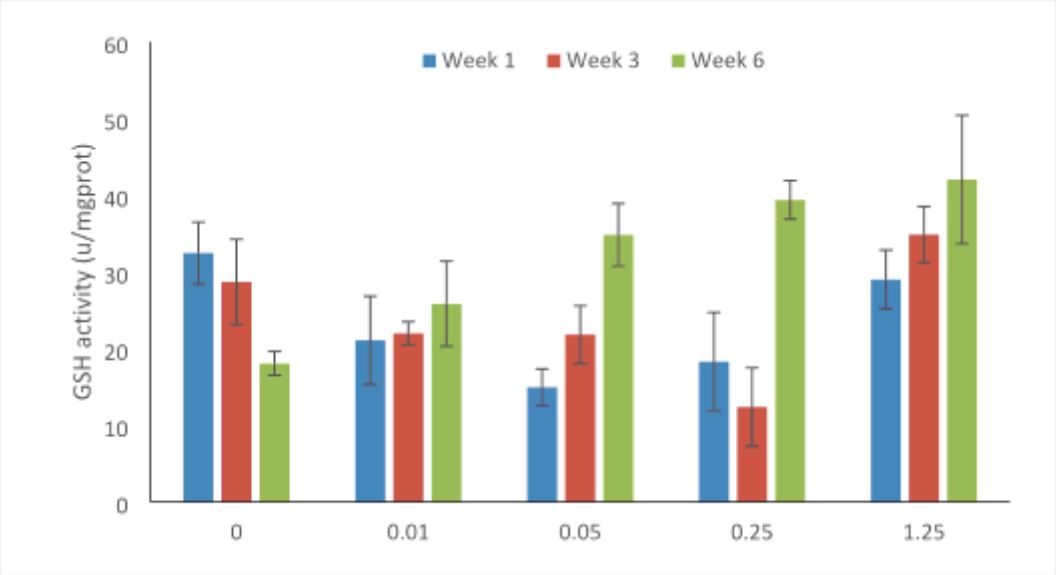

Figure 4

3-4 GSH activity and associated standard errors by treatment in the liver of tilapia (n=6).

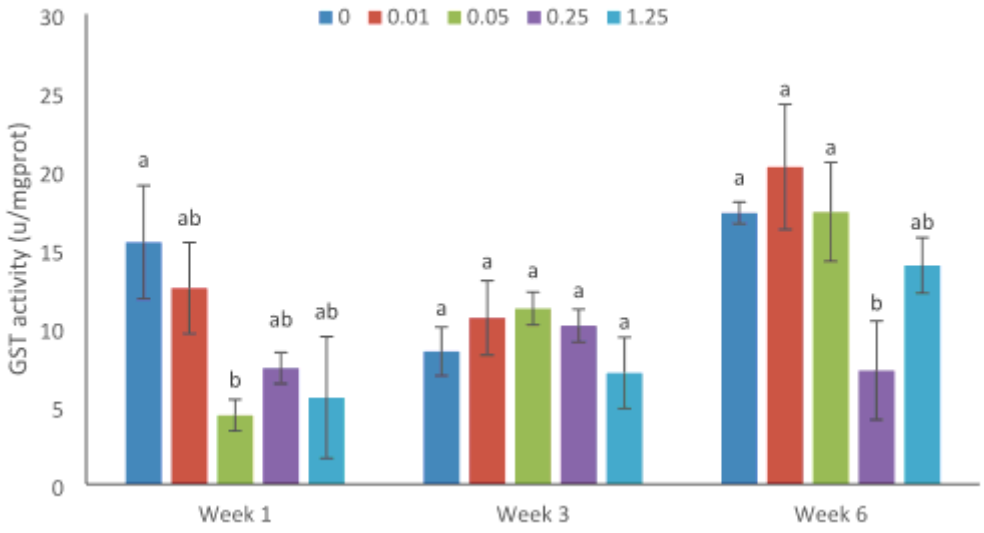

Figure 5

3-5 GST activity and associated standard errors by weeks in the liver of tilapia (n=6). Values with different letters (a, b) in the same week are significantly different.

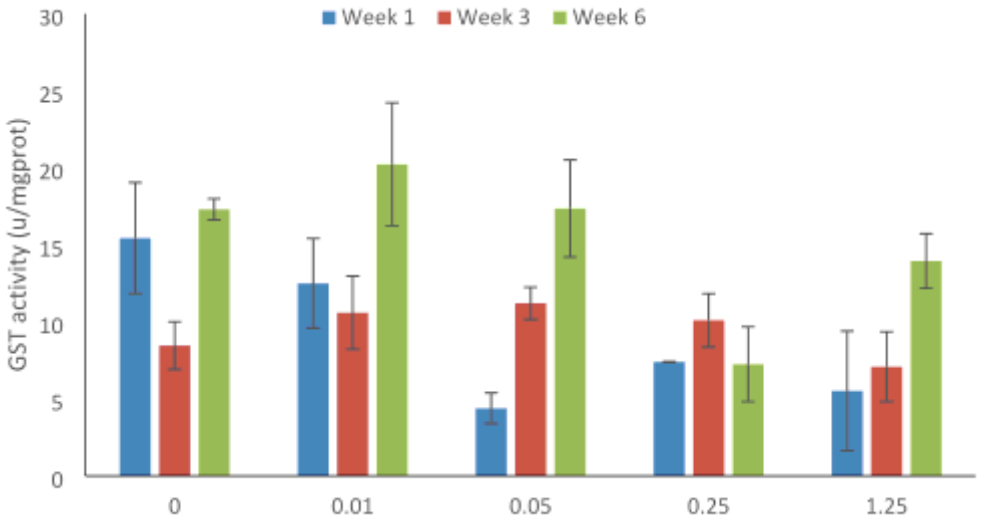

Figure 6

3-6 GST activity and associated standard errors sorted by treatment in the liver of tilapia (*O. niloticus)* measured in 1, 3, and 6 weeks (n=6).

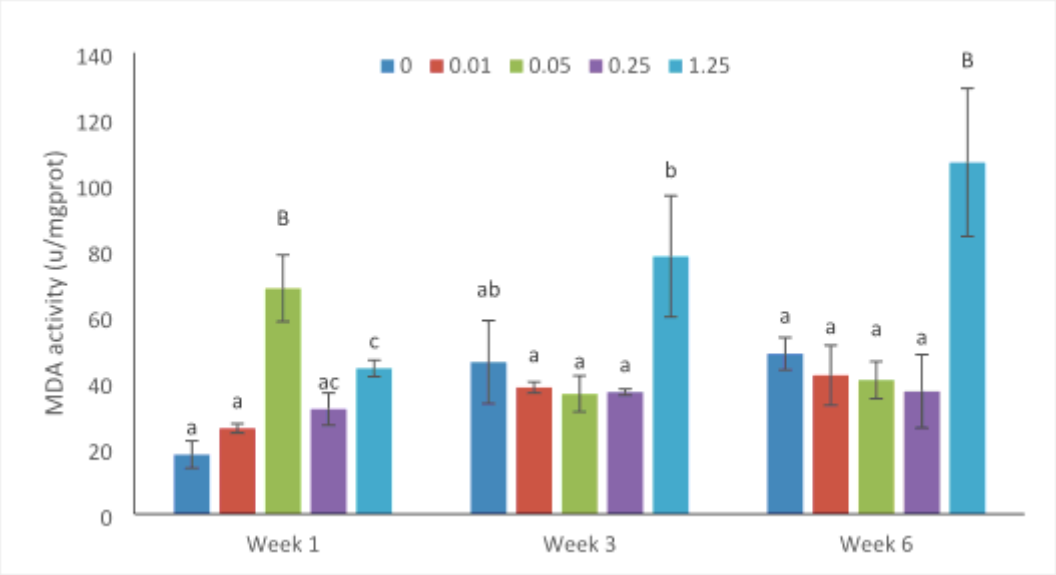

Figure 7

3-7 MDA activity and associated standard errors by weeks in the liver of (n=6). Values with different letters (a, b, and c) in the same week are significantly different.

Figure 8

3-8 MDA activity and associated standard errors by treatment in the liver of tilapia *(*n=6).

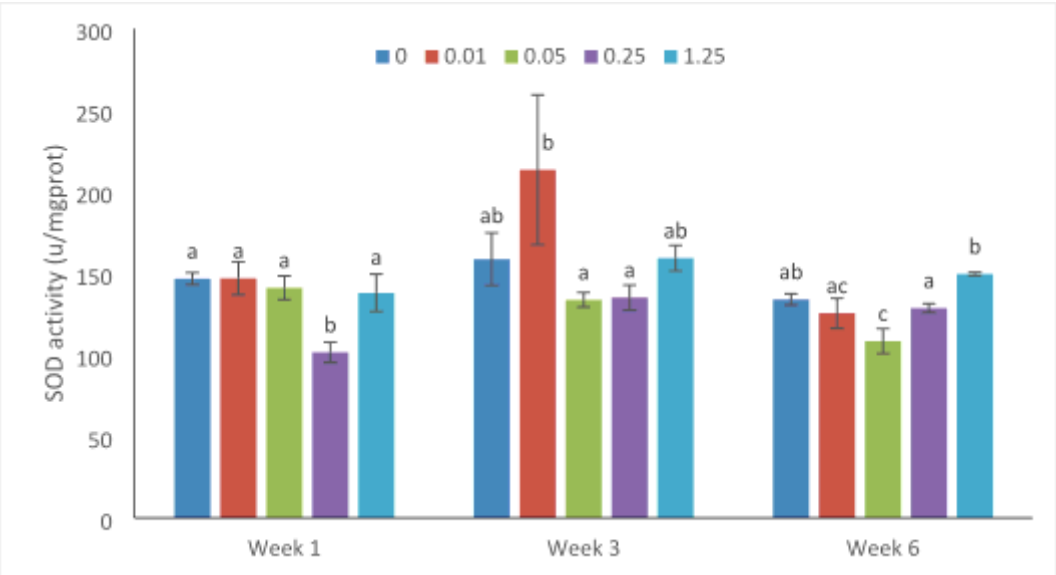

Figure 9

3-9 SOD activity and associated standard errors by weeks in the liver of tilapia (n=6). Values with different letters (a, b, and c) in the same week are significantly different.

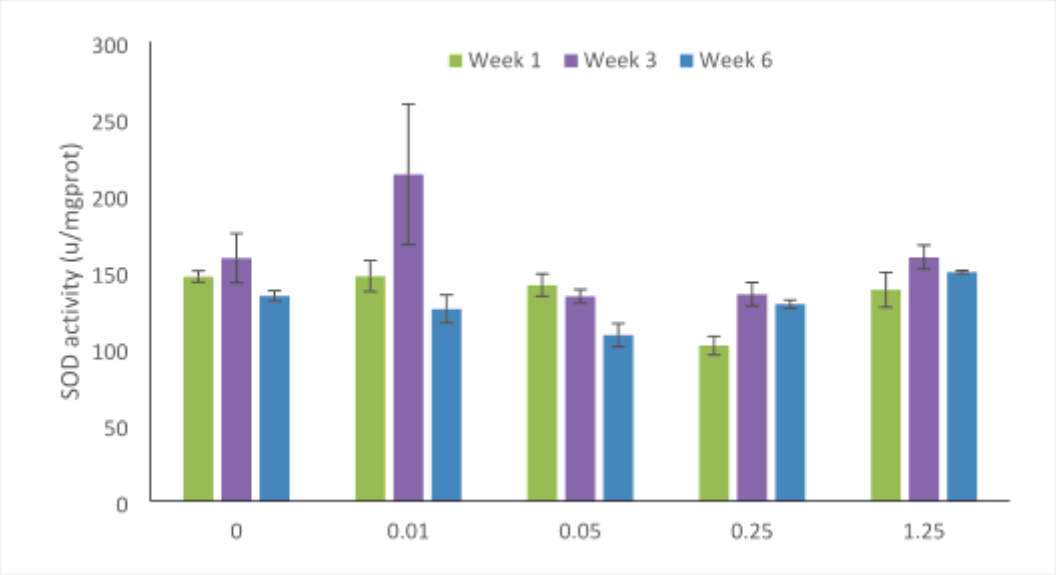

Figure 10

3-10 SOD activity and associated standard errors by treatment in the liver of tilapia (n=6).

Figure 11

3-11 Total protein content means (n=9) in tilapia (*O. niloticus*) and associated standard errors in the liver of *O. niloticus*. Groups with different superscripts (a, b) are significantly different.