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Effects of dietary selenium of organic form against lead toxicity on the antioxidant system in *Cyprinus carpio*

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Abstract In this study was evaluated potential protective effect of organic selenium (Se) on heavy metal stress induced by lead (Pb) in *Cyprinus carpio*. For this reason, *C. carpio* was exposed to sublethal concentration of Pb (1.5 mg/L $Pb(NO_3)_2$) for 14 days. The fish were fed a basal (control; measured 0.55 mg/kg Se) diet or a basal diet supplemented with 2.50 mg/kg (measured 2.92 mg/kg Se) organic Se (Sel-Plex®) during the experiment period. The variations in glutathione peroxidase (GSH-Px), glutathione S-transferase (GST) activities, and levels of reduced glutathione (GSH) with malondialdehyde (MDA) in liver and brain tissues of *C. carpio* were investigated in experimental groups. GSH levels in liver and brain tissues were significantly decreased by exposure to Pb. GST activity was significantly increased ($p < 0.05$) in liver tissue, but decreased in brain of treated fish by exposure to Pb. Also, GSH-Px activity was significantly increased in liver tissue, but decreased in brain of Pb-treated fish. Levels of MDA were increased in liver and brain of Pb-treated fish. The organic Se treatment for Pb-intoxicated animals improved activities of GSH-Px, GST and levels of MDA within normal limits. Supplemented Se could be able to improve Pb-induced oxidative stress by decreasing lipid peroxidation and regulating antioxidant defense system in tissues.

Keywords *Cyprinus carpio* · Selenium yeast · Lead · Antioxidant enzyme

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Introduction

Lead (Pb) is a common, nonessential heavy metal, which enters the ecosystem from natural as well as anthropogenic sources (industrial effluents, agricultural runoffs) and has a toxic effect on freshwater fish (Ramesh et al. 2009). Many investigations have indicated that lead exposure could induce a wide range of biochemical and physiological dysfunctions in humans and laboratory animals (Adonaylo and Oteiza 1999; Flora et al. 2003). It has also been reported that lead can induce oxidative stress by generating free radicals and reactive oxygen species (ROS) (Ates et al. 2008). ROS can induce oxidative damage and may be a mechanism of toxicity for aquatic organisms living in environments receiving water-borne contaminants (Valavanidis et al. 2006; Lushchak 2011). An excess of ROS results in oxidative stress and may finally cause cell death. ROS levels

within cells and in extracellular body fluids are controlled by action of enzymatic and nonenzymatic antioxidants (Steinbrenner and Sies 2009).

Mechanisms of antioxidant defenses in fish include the enzyme system and low molecular weight antioxidants, similar to those in mammals (Di Giulio and Meyer 2008). The antioxidant defense system contains enzymes such as glutathione peroxidase (GSH-Px; 1.11.1.9), glutathione S-transferase (GST, EC 2.5.1.18) and reduced glutathione (GSH), which can be used as biomarkers of oxidative stress (Pacini et al. 2012). Lipid peroxidation can be increased in the tissues of lead-treated fish, as indicated by an increased production of malondialdehyde (MDA). The most widely used test for oxidative stress is the measurement of MDA, a product of lipid peroxidation, by the thiobarbituric acid (TBA)-reacting substances assay (Valavanidis et al. 2006).

Cooperative interaction between endogenous and dietary antioxidants is important in mainly defense against the harmful effects of oxidative stress (Jacob 1995). It was explained that treatment with dietary antioxidants can result in reversal or amelioration of oxidative stress after exposure to oxidative pollutants (Flora et al. 2003).

Selenium (Se) is an essential element in almost all biological systems and is well-established antioxidants (Klotz et al. 2003) and can prevent or decrease the harmful effects of metal on the antioxidant system in different tissues. The protective effect of selenium against the different heavy metal toxicity in biological systems appears to have been studied (Orun et al. 2008; Ates et al. 2008).

Selenium in foods and biological materials can exist in both organic and inorganic chemical forms (Dumont et al. 2006). In general, the organic forms, such as selenomethionine, are more bioavailable than the inorganic selenites or selenates. Also, it is reported that in fish, organic Se sources have been found to have greater potency in terms of bioavailability and effects on health than inorganic Se (Rider et al. 2009). In feedstuffs, Se is naturally present organically bound in Se-containing proteins. Se-enriched yeast (yeast Se, YS) is a common form of Se used to supplement the dietary intake of this important trace mineral. YS is the product of the aerobic fermentation of *Saccharomyces cerevisiae* in a Se-enriched medium. In YS, the most are present in the selenomethionine form. The advantage of YS over inorganic Se has been widely reported (Baowei et al. 2011). Selenomethionine is the major selenocompound in Se-enriched yeast (Sel-Plex®, Alltech, USA), which is used as a natural form of Se for dietary supplementation. YS is increasingly used in animal nutrition; its use is FDA-approved for several animal species (Schrauzer 2006).

Normally, the feed additives may be able to provide optimum amounts of minerals. Se is one of those essential mineral elements able to preserve from health damage. Nevertheless, Se can become very toxic when it is elevated above a threshold concentration (Hamilton 2004), and the difference between nutritional requirement and toxic level can be very small (Dörr et al. 2013). High levels of Se in the diet have toxic effects, resulting in reduced growth, feed efficiency and increased mortality (Watanabe et al. 1997). It was reported that levels above 13–15 mg/kg dry feed for trout can be toxic (Hilton et al. 1980; Gatlin and Wilson 1984). In addition, it was reported that the highest dietary concentration (5 mg/kg Se) did not result in any negative production-related impact (growth and survival) on gibel carp (*Carassius auratus* gibelio) (Han et al. 2011). The growth and feed efficiency in *Oreochromis niloticus* were enhanced with supplemented 5.54 mg/kg Se in diet (Abdel-Tawwab and Wafeek 2008). The authors found that the requirement of Se was 0.38 mg/kg diet for *Salmo gairdneri* (Hilton et al. 1980); 0.77 mg/kg diet for *Epinephelus malabaricus* (Lin and Shiau 2005); 1.18 mg Se/kg diet for *C. auratus gibelio* (Han et al. 2011). The Se requirement may be different between fish species (Lorentzen et al. 1994), and the various forms of Se have different bioavailability for fish (Wang and Lovell 1997). In farmed fish, requirements for Se may be elevated due to the low availability of Se from diets and the effects of various physical and environmental stressors (Han et al. 2011). Se reserves are available for the maintenance of a healthy immune system as well as for antioxidant defense.

The liver is the primary organ site for xenobiotic metabolism and is sensitive to peroxidative damage because they are rich in oxidizable substrates (Mudipalli 2007). The brain is more susceptible to oxidative stress than most other organs due to its high oxygen consumption. In particular, high quantities of hydrogen peroxide and organic hydroperoxides are continuously generated in the brain (Steinbrenner and Sies 2009). Both of tissues have a high potential for ROS production. The liver and brain are especially sensitive to exposure to lead (Patrick 2006).

Biochemical parameters can be accepted as sensitive biomarkers for biomonitoring the aquatic environment. It has a great potential to serve as sensitive indicators, signaling exposure and understanding the toxic mechanisms of stressors in aquatic ecosystems (Valavanidis et al. 2006). Fish are often subjected to prooxidant effects of pollutants present in the aquatic environment. To a certain extent, environmental conditions have led to the development of defense mechanisms that protect fish against ROS (Jovanovic et al. 1997). Dietary modifications are among the most preferable and practical methods of improving the effects of environmental stressors and farming methods on the growth of fish. Common carp is one of the most common species for aquaculture and has economic value throughout the world. Also, it has an adaptive response in a polluted aquatic environment (Vinodhini and Narayanan 2008). In this study, the test organism chosen was carp (*Cyprinus carpio*, L., 1758), because it is a widespread and strong fish species and resists polluted waters. For this reason, we have investigated the response of activities of GSH-Px, GST and levels of GSH and MDA (for LPO) in liver and brain tissues of *C. carpio* following 14-day Pb exposure. In addition, we investigated the potential protective effect of organic Se (yeast Se) as an antioxidant in lead-induced oxidative stress in liver and brain tissues of *C. carpio*.

Materials and methods

Chemicals

Organic Se as Sel-Plex® obtained from Alltech Inc., Nicholasville, KY, USA. All other chemicals were purchased from Sigma–Aldrich Chemical Corporation (USA).

Test animals and treatment

C. carpio (mean weight 42.40 ± 2.30 g) were obtained from Çukurova University, Fisheries Faculty, Aquaculture Department and transferred to laboratory where the temperature was kept at 23 ± 1 °C (12:12 L:D). Throughout the experiments, dechlorinated tap water with pH value of 7.5, an alkalinity of 340 mg/L $CaCO_3$, and oxygen concentration of 6.20 mg/L was used. The fish were allowed to acclimatize to these conditions for 4 weeks. The fish were fed at a rate of 2 % body weight/day with a commercial pellet diet during the acclimation period. Commercial fish diet, Çamlı Yem/Bioaqua, Turkey (44 % crude protein, 18 % crude fat, 12 % moisture, 12 % ash and 3 % fiber), was used as the basal control diet. The pellet was ground, and Se yeast (Sel-Plex®) was added at 2.5 mg per kg to a commercial fish growing diet. Se yeast was blended in a kitchen robot for homogenous mix, and sufficient water (400 g/kg) was added to the mix to form soft dough. The resultant dough was passed through a mincer with 3 mm diameter die. The pellets were air-dried at 40 °C in an oven and stored at 4 °C during the experiment. Each diet was given to carp by hand at 2 % of fish body weight (at 09:00 and 16:00 h) for 14 days.

During this experiment, fish were fed unsuplemented diet (Control) or suplemented diet with organic Se. The measured Se concentrations were 0.55 and 2.92 mg/kg (wet weight) in the basal (control) and supplemented diet, respectively. Se concentration was determined by hydride generation atomic absorption spectrophotometer (AA6501, Shimadzu Ltd, Japan) according to the method described by Tinggi (1999). It was used as a mixture of nitric, sulfuric and perchloric acids to digest the samples and then to reduce the tetravalent Se to H_2Se in the acidic medium. The generated H_2Se was transported into a heated quartz tube by carrier gas for atomization, and the determination of Se was performed by using atomic absorption spectrometry.

The proximate compositions of the diets were analyzed according to Association of Analytical Communities (AOAC 1995) procedures as follows: moisture was determined by oven drying at 105 °C for 24 h, crude protein (N × 6.25) by the Kjeldahl method and crude ash by combustion in a muffle furnace at 550 °C for 16 h. The total lipid concentration was determined by extract with the chloroform–methanol method, described by Bligh and Dyer (1959).

Experiments were conducted in glass aquaria containing 100 L test solution. Fish were exposed to 1.5 mg/L sublethal concentrations of lead nitrate ($Pb(NO_3)_2$) for 14 days. Sublethal concentration of lead was chosen due to the earlier studies (Ramesh et al. 2009). The water was refreshed every 2 days to compensate for the metal (Pb) lost in the exposure medium. Thirty-two fish were divided into four groups

($n = 8$ in each group). The control group (Cont) was exposed to tap water (the absence of Pb) fed with control diet. The YS group was exposed to tap water (the absence of Pb) and was fed with YS supplemented diet. The Pb group was exposed to $Pb(NO_3)_2$ concentration of 1.5 mg/L and was fed with control diet. The Pb + YS group was exposed to 1.5 mg/L concentration of $Pb(NO_3)_2$ and was fed with YS supplemented diet.

At the end of exposure period, eight fish were removed from each tank and killed by transaction of the spinal cord. The liver and brain tissues of both control and treated fish were dissected. Tissues samples were obtained from an individual fish and prepared for analysis. Tissues were homogenized to 1/5 (w/v) ratio in physiological saline solution (0.8 % NaCl) with homogenizer and then centrifuged at 13,500 rpm for 10 min in a Sigma 2–16 K centrifuge at +4 °C, and supernatant was used for biochemical analyses.

Biochemical assays

The GSH levels of tissues were measured by the method described by Ellman (1959). The homogenated tissues were treated with trichloroacetic acid (TCA, 10 % w/v) and centrifuged. Supernatant was mixed with Tris–HCl (hydrochloric acid) buffer (0.8 M Tris/HCl, 0.02 M ethylenediaminetetraacetic acid (EDTA) (pH 8.9) and 0.01 M 5,5′ dithio-bis (2-nitrobenzoic acid, DTNB). Reaction mixture was incubated at room temperature. The absorbance of GSH–DNTB conjugate was determined at 412 nm using an spectrophotometer. The values were expressed as nmol/mg protein.

The activities of GST in tissues were determined spectrophotometrically by following the formation of GSH conjugate with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm using extinction coefficient of 9.6 mM^{-1} cm^{-1} (Habig et al. 1974). The reaction mixture contained in 1 mL volume: 0.1 M potassium phosphate buffer (pH 6.5), 1 mM GSH, 1 mM CDNB in ethanol and the tissue supernatant. The GST activity was expressed in U/mg protein.

The activities GSH-Px of tissues were determined by Jocelyn method (1970). This method measures the rate of glutathione oxidation by hydrogen peroxide (H_2O_2) as catalyzed by GSH-Px present in the sample by the addition of glutathione reductase (GR) and nicotinamide adenine dinucleotide phosphate (NADPH) that converts oxidized glutathione (GSSG) to the reduced form. The reaction mixture contained in 1 mL volume: 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 0.75 mM H_2O_2, 1 mM GSH, 0.2 mM NADPH, 1.6 IU/mL GR and homogenated tissue. The rate of GSSG formation was measured by following the decrease in absorbance of the reaction mixture at 340 nm by spectrophotometer. The activity of GSH-Px was calculated as the amount of NADPH oxidized per minute using the molar absorption coefficient of 6.22 mM^{-1} cm^{-1}. Enzyme activity was expressed in U/mg protein.

The levels of MDA homogenized tissue, as an index of LPO, were determined by TBA reaction using the method of Yagi (1998). Briefly, to each test tube, 50 µL of homogenized tissue, 100 µL of 8.1 % sodium dodecyl sulfate (SDS), 750 µL of 20 % TCA (pH:3.5) 750 µL of 0.8 % TBA and 350 µL distilled water were added. The test tubes were kept for boiling at 95 °C for 30 min. To each of the tubes, 2.5 mL of n-butanol pyridine (15v/1v) and 500 µL distilled water were added and mixed. The tubes were centrifuged at 3,500 rpm for 15 min. The separated butanol layer was collected and read in a spectrophotometer against reagent blank at 532 nm. The samples were calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Thiobarbituric reactive substances concentration was expressed in terms of nmol of MDA as nmol/mg protein.

The protein contents of tissues were measured only to determine the specific activity of antioxidant enzymes according to the method developed by Lowry et al. (1951) using bovine serum albumin as standard. The tissue sample and the standards (1.0 mg/mL Bovine serum albumin in double distilled water) in different tubes were treated with 5.0 mL of reagent mixture (48 % sodium potassium tartarate, 2 % copper sulfate and 3 % sodium carbonate in 0.1 N sodium hydroxide added in a ratio of 1:1:48 by volume). Then, Folin phenol reagent (1:2) was added to the reaction mixture and allowed to stand for 30 min at room temperature. The absorbances of samples were measured at 750 nm by spectrophotometer.

Statistical analysis

All data were expressed as mean ± standard error (SE) and analyzed using SPSS statistical package

programs. One-way ANOVA was used to compare variables among control and treatments. Duncan's multiple range tests were used to analyze differences between groups. The differences were defined as statistically significant when $p < 0.05$.

Results

In this experiment, no mortality was observed. The GSH levels in tissues were given in Fig. 1. There was no significant change in tissues GSH content in YS group when compared with control group. GSH levels of liver and brain were significantly decreased ($p < 0.05$) in lead-exposed group when compared with the control. The decrease was 37 % in liver and 44 % in brain. The administration of Se in Pb + YS group significantly increased GSH contents in tissues as compared to Pb group and normalized without reaching control values.

The GST activities in tissues of fish were shown in Fig. 2. Sublethal concentration of Pb (1.5 mg/L) caused a significant increase ($p < 0.05$) by 89 %, in liver, but a significant decrease ($p < 0.05$) by 45 % in brain of Pb-exposed fish. In addition, administration of Se improved this enzyme activity in Pb + YS group as compared to Pb group without reaching in liver and with reaching in brain control values.

Exposure to concentration of Pb significantly increased GSH-Px activity ($p < 0.05$) by 100 % in liver and decreased ($p < 0.05$) by 40 % in brain of fish at the end of the experiment period (Fig. 3). Administration of Se improved this enzyme activity in Pb + YS group as compared to Pb group without reaching in liver and with reaching in brain control values (Fig. 3).

MDA levels of tissues were decreased in YS group compared with control group (Fig. 4). MDA levels of liver and brain tissues were significantly increased by 45 and 41 %, respectively, in Pb-exposed group ($p < 0.05$) when compared with control group (Fig. 4). The administration of supplemented Se in Pb + YS group significantly decreased MDA contents in tissues as compared to Pb-exposed group with reaching control levels.

Discussion

Glutathione is a main molecule in cellular antioxidant systems, acting as a detoxifying agent for endogenous radical species. It can also be involved in enzymatic detoxification reactions as a cofactor or a coenzyme for enzymes such as GST and GSH-Px (Shelly and Lu 2009). In the present study, GSH levels in the liver and brain were decreased in lead-treated group compared with control fish. In many studies were definited that GSH levels of tissues were decreased by Pb exposure. Baysoy et al. (2012) reported that level of GSH was decreased in liver of *O. niloticus* by exposure to Pb. The

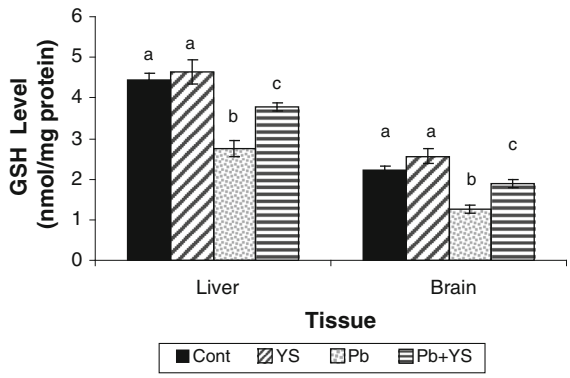

Fig. 1 GSH level in liver and brain tissues of *C. carpio* exposed to sublethal concentration of $Pb(NO_3)_2$ (1.5 mg/L), with or without a dietary supplementation of Se. Each value is the mean ± SE ($n = 8$). Multiple comparisons were made separately for each tissue, and means with different *superscript* in tissues are significantly different ($p < 0.05$)

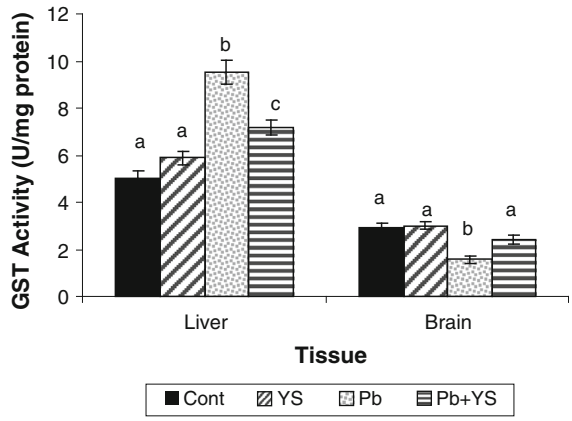

Fig. 2 GST activity in liver and brain tissues of *C. carpio* exposed to sublethal concentrations of $Pb(NO_3)_2$ (1.5 mg/L), with or without a dietary supplementation of Se. Each value is the mean ± SE ($n = 8$). Multiple comparisons were made separately for each tissue, and means with different *superscript* in tissues are significantly different ($p < 0.05$)

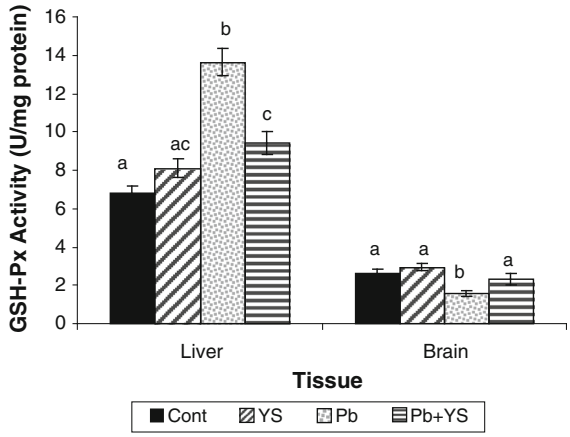

Fig. 3 GSH-Px activity in liver and brain tissues of *C. carpio* exposed to sublethal concentrations of $Pb(NO_3)_2$ (1.5 mg/L), with or without a dietary supplementation of Se. Each value is the mean ± SE ($n = 8$). Multiple comparisons were made separately for each tissue, and means with different *superscript* in tissues are significantly different ($p < 0.05$)

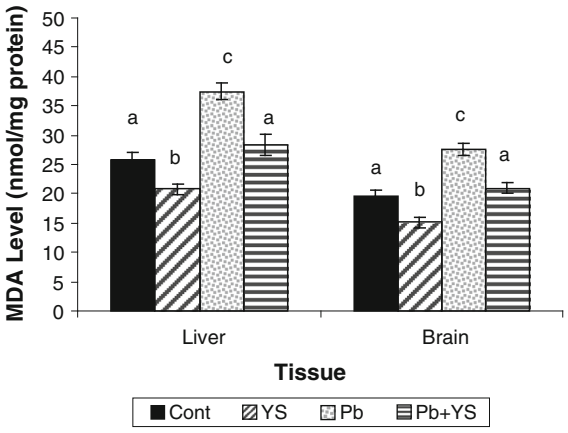

Fig. 4 MDA level in liver and brain tissues of *C. carpio* exposed to sublethal concentration of $Pb(NO_3)_2$ (1.5 mg/L), with or without a dietary supplementation of Se. Each value is the mean ± SE ($n = 8$). Multiple comparisons were made separately for each tissue, and means with different *superscript* in tissues are significantly different ($p < 0.05$)

authors supposed that the decrease in GSH levels may be an indication of its exhaustion on phase II biotransformation as confirmed by increased GST activity. Also, they explained that GSH decrease can occur due to GSH binding to metals to prevent the membrane integrity, and GSH depletion seems to reflect an aggravation status due to reduced cell protection ability (Baysoy et al. 2012). Dai et al. (2012) showed that GSH level was decreased in tissue of *O. niloticus* by exposure to Pb. They explained that Pb has a high affinity for sulfhydryl (SH) groups and altered antioxidant activities by inhibiting functional SH groups in enzymes (Dai et al. 2012). GSH is a tripeptide containing cysteine that has a reactive SH group with reductive potency. Metal-induced decreases in GSH levels could be the result of direct binding of the metal to GSH through its SH group (formation of metal-SG complexes) or of enhanced oxidation of this thiol (Elia et al. 2003; Sevcikova et al. 2011).

Glutathione S-transferases (GSTs) are a group of intracellular enzymes with the main function in detoxification processes by catalyzing the conjugation of tripeptide glutathione (GSH) with some endogenous toxic metabolites and many environmental contaminants (Nimmo 1987). This study demonstrated an increase in GST activity in liver, but a decrease in brain of Pb-exposed fish. Several studies have demonstrated the increase in GST activity of tissues after the lead exposure (Daggett et al. 1998; Wright et al. 1998). This induction may be due to the glutathione-dependent enzymes system that provides major protection against the toxic agents. It could explained that the metal-induced decrease in glutathione content could be mainly related to a stimulation of GST activity because GSH is necessary for proper functioning of GST (Elia et al. 2003). Oxidative damage is considered a probable cause of lead-induced brain damage, because the brain is believed to be particularly susceptible to oxidative stress due to high rate of oxygen-free radical generation without corresponding levels of antioxidant defenses (Savolainen 1987). Previous studies on brain have revealed the susceptibility for lead to catalyze oxidative reactions generating the reactive oxygen species (ROS) that inhibits the production of sulphydryl antioxidants (Flora et al. 2003; Maiti et al. 2010). Decreased GST activity in brain tissue in this study might be correlated with these reasons.

In the present study, Pb exposure caused significant increase in liver GSH-Px activity but decrease in brain. GSH-Px plays the preliminary role in cellular defense against peroxides, superoxide anions and hydro peroxides (Doyen et al. 2008). Baysoy et al. (2012) showed that GSH-Px activity in liver of *O. niloticus* was significantly increased by Pb-exposed groups. Ling and Hong (2010) observed that the activities of GSH-Px in liver of *C. auratus gibelio* exposed with Pb doses were significantly increased. The authors

suggested that Pb caused strong oxidative stress in the liver of fish. Our current data suggest that Pb treatment may result in increased formation of oxygen-free radicals, which could stimulate GSH-Px activity to cope with this increased oxidative stress and protecting membranes from damage due to LPO (Adonaylo and Oteiza 1999; Van der Oost et al. 2003). We found that GSH-Px activity was decreased in brain. Ateş et al. (2008) showed brain tissue of rainbow trout after exposure to Pb caused decrease in GSH-Px activity. Antioxidant enzymes also show tissue-specific differences in activities that reflect the functions of the tissues and the oxidative stress load that they experience (Lushchak et al. 2009). In the present work, this enzyme responded in a different level in liver and brain tissues. The response of antioxidant system to oxidative stress in various tissues shows differences from one species to another due to the differences in free radical generation and different antioxidant potential of these tissues (Adonaylo and Oteiza 1999). In the previous studies, it was found that brain was a more sensitive target organ to oxidative damage than liver (Song et al. 2006; Özkan et al. 2012).

In this study, lipid peroxidation end product MDA were significantly elevated in liver and brain tissues of *C. carpio* exposed to lead compared with control group. These results are parallel to the results of many authors. Ates et al. (2008) reported that MDA levels in the liver and brain of *Oncorhynchus mykiss* were increased by exposure to Pb. Maiti et al. (2010) described elevated MDA levels in the brain of *Clarias batrachus* following a longtime exposure to lead. Lipid peroxidation is the reaction of oxidative deterioration of membrane polyunsaturated fatty acids (PUFA). Fish tissues are characterized by high concentration of PUFA and may therefore be susceptible to lipid peroxidation (Stephan et al. 1995). Lead exposure could produce free radicals, which resulted in the increase in lipid peroxidation. The increase in lipid peroxidation may be attributed to alterations in the antioxidant defense system (Ademuyiwa et al. 2009). The liver and brain are sensitive to peroxidative damage because they are rich in oxidizable substrates. An increase in the MDA levels of tissues might be induced by the possible involvement of ROS in Pb-induced toxicity (Patra et al. 2011).

The results of the present study indicate that enzymes activities in liver and brain of fish were altered by Pb and were normalized by organic Se supplementation. In groups treated with Pb plus YS, enzyme activities, glutathione and MDA levels converge to control group values. Also, MDA levels of tissues significantly lower in YS supplemented group than in the control. Earlier studies have shown that they inhibit free radical production and decrease in MDA levels by Se in experimental animal (Othman and El Missiry 1998; Ozluer-Hunt et al. 2011). The decreased MDA levels can be explained by the recovery in the physiological activity of GSH-Px or by the important role of Se in preventing LPO and in protection of functioning of tissues and cells (Klotz et al. 2003). The protective action of Se against lead-induced changes could be due to increased antioxidant capacity in cells. In the mechanism, Se enhances the availability of glutathione, which is one of the most intrinsic antioxidants that prevent cell damage, and Se has the capability to neutralize ROS and protect the formation and function of proteins, lipids against oxidative damages (Othman and El Missiry 1998). In addition, lead can bind to Se and form highly bonded Se-lead complexes, which have been proposed as a mechanism for Se's protective effect in lead toxicity (Flora et al. 1982).

In our study, GSH-Px and GST activities and GSH levels of tissues were not changed in Se supplemented group when compared with the control. In fact, we observed slightly increase in enzymes activities of liver, but these values were not significant statistically. In our study, experiment fish were fed with supplemented Se diet for 14 days. Elia et al. (2011) demonstrated that GSH-Px activity in liver of juvenile carp was increased with Se supplemented diets for 30 and 60 days. Lin and Shiau (2005) reported that GSH-Px activity in liver of *E. malabaricus* was increased with dietary Se (2.02 and 1.23 mg/kg Se for 8 weeks). On the other hand, Cotter et al. (2008) reported that hepatic GSH-Px activity of hybrid striped bass was decreased with organic Se supplemented diets (0.4–3.2 mg/kg Se for 6 weeks). Lorentzen et al. (1994) showed that supplemented Se diets did not affect the hepatic GSH-Px activity of Atlantic salmon (1 and 2 mg Se/kg as selenite or selenomethionine, respectively, for 8 weeks). Several studies have reported increased or decreased effects of Se on antioxidant capacity of fish species (Jovanovic et al. 1997; Han et al. 2011). The different reasons in studies could be related to the form of the nutrient interaction, which may be either synergistic of antigenic,

physiological and pathological condition of the fish (Watanabe et al. 1997; Elia et al. 2011). In addition, feeding period might be caused by another reason.

In conclusion, the present results indicated that Pb can stimulate oxidative stress by inducing lipid peroxidation, altering antioxidants system. In addition, organic Se treatment could protect the liver and brain tissues against the toxicity of lead since it reduced MDA level and normalized the activities of antioxidant enzymes in these tissues. The treatment with YS could minimize environmental hazards.

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