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Antioxidative role of cerium against the toxicity of lead in the liver of silver crucian carp

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Abstract The antioxidative role of cerium was investigated in the liver of silver crucian carp injected with lead. The fish were intraperitoneally injected with 10, 20, or 30 mg/kg wet weight $PbCl_2$. After a 14-day period of incubation, 35 animals were injected with a solution of 1.5 mg/kg wet weight $CeCl_3$. After 42 days, the wet weight and the liver weight of the fish were weighed, and the oxidative stress of the fish liver was estimated by assaying lipid peroxide, superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, glutathione, ascorbic acid, and reactive oxygen species (ROS). The results show that Ce^{3+} could decrease ROS accumulation, relieve the inhibition of the activities of the antioxidant enzyme and the reduction of antioxidants in fish liver caused by Pb^{2+}, and decrease the enhancement of hepatosomatic index of fish under various Pb^{2+} dosages.

Keywords Lead · Cerium · Silver crucian carp · Liver · Oxidative stress

Introduction

Lead (Pb) is a widespread, nonessential heavy metal, which enters the ecosystem from natural (weathering of rocks) as well as anthropogenic sources (industrial effluents, agricultural runoffs) and has a toxic effect on freshwater fish. It has been reported that heavy metals can induce oxidative stress by generating free radicals and toxic oxygen species (Ates et al. 2008; Hartwig 1998; Hong et al. 2006; Kasprzak 1996; Yang et al. 1997). These species react with lipids and inactivate enzymes, impairing cell viability. The deleterious effects resulting from the cellular oxidative state may be alleviated by enzymatic and nonenzymatic antioxidant machinery that vary at various cellular and subcellular levels in fish. Fish use a diverse array of enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APx), glutathione peroxidase (GSHPx), and catalase (CAT), as well as low molecular weight antioxidants like glutathione (GSH) and ascorbic acid (ASA) to scavenge different types of reactive oxygen species (ROS), protecting potential cell injury against tissue dysfunction (Almeida et al. 2002; Campana et al. 2003; Garcia Sampaio et al. 2008; Roberts and Oris 2004; Schlezinger and Stegeman 2001; Zikić et al. 2001). SOD is a key enzyme in protecting cells against oxidative stress and alters superoxide radical ($O_2^{\cdot -}$) to H_2O_2 and oxygen. However, hydrogen peroxide is also toxic to cells and has to be further detoxified by CAT and peroxidases to water and oxygen. GSH and ASA can directly interact with and detoxify oxygen free radicals and, thus, contribute significantly to nonenzymatic ROS scavenging. However, the elimination of Pb toxicity in fish has been rarely reported (Ates et al. 2008).

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Rare earths can promote the growth and development of plants, as well as improving quality and increasing yield (Hong et al. 2000a, b, 2002a, b). However, they also have a metal ion protection effect on enzyme catalytic activity and its catalytic center and definite conformational changes have been frequently reported (Wang et al. 1998; Hong et al. 2003). Previous research demonstrated that cerium ion (Ce^{3+}) enhanced vigor in both the aging seeds of rice and spinach, protected spinach chloroplasts from aging, and could quickly activate SOD in the cell, enhancing its ability to eliminate ROS (Hong 2002; Yang et al. 2005). Research has proven that lower doses of rare earths could increase the activities of NK cells and T cells, causing an improvement in the immunity of animals (Wang et al. 1994; Chen et al. 1995; Deveci et al. 2000; Shen et al. 2004; Tu et al. 2005; Wang et al. 2004). The neutralization of cerium-induced inotropic changes by SOD at concentrations $\leq$5 μM indicated that mechanical changes were mediated by ROS (Manju et al. 2003). Kawagoe et al. (2005) suggested that orally administered cerium increased metallothionein (MT) and GSH content as an antioxidant in the mouse liver. Liu et al. (2008) indicated that, over the range of 2.5–15 μM Ce^{3+}, SOD activity from rat erythrocytes increased rapidly, while over the range of 20–25 μM Ce^{3+}, SOD activity decreased slowly. Wu et al. (2006, 2008) indicated that Ce^{3+} and Ca^{2+} could inhibit the activation of the cadmium ion (Cd^{2+}) on DNase activity, relieve Cd^{2+} inhibition on activities of the antioxidant enzyme, and diminish ROS accumulation in silver crucian carp, while Ce^{3+} could relieve the destruction of mercury ion (Hg^{2+}) on fish DNA structure (Wu et al. 2005).

The aim of this study was to investigate whether Ce^{3+} reduces the oxidative stress caused by Pb^{2+} in the liver of silver crucian carp (*Carassius auratus gibelio*) by analyzing the hepatosomatic index (HSI), ROS accumulation, lipid peroxidation, and enzymatic and nonenzymatic antioxidant machinery.

Materials and methods

Materials

For our experimental subjects, we used allogynogenetic silver crucian carp (*C. auratus gibelio*), which were purchased from the local market. The 70 fish used in the experiment were 2 years old and weighed 200–250 g. Fish were acclimated for 7 days prior to the experiments, kept individually in glass aquaria (one fish per aquarium) with constant aerated freshwater under 12:12 h light–dark cycle and at water temperatures of 22–25°C and pH 6.8. The fish were fed ad libitum once daily in the morning (at 9:00–10:00 h) with commercial dry pellets for silver crucian carp. After acclimatization, the fish were weighed and moved to individual glass aquaria that were randomly allocated to a control group and six experimental groups (10 isolated fish for each group). The control group was injected with a solution of 0.65% NaCl into the abdominal cavity, and the experimental groups were injected with a solution (containing 0.65% NaCl) of 10, 20, and 30 mg/kg wet weight (WW) $PbCl_2$, respectively (two groups for each dose). After a 14-day period of incubation, 35 animals (five isolated fish from the control group, 30 isolated fish from the $PbCl_2$ experimental groups) were injected with a solution (containing 0.65% NaCl) of 1.5 mg/kg WW $CeCl_3$. Twenty-eight days after cerium administration, the fish were weighed, measured, and their liver was excised for subsequent analyses.

HSI

After weighing the body and tissues, the HSI was calculated as the ratio of liver wet weight (mg) to body wet weight (g).

ROS assay of liver

Superoxide ion ($O_2^{\cdot -}$) in liver tissue was measured as described by Oliveira et al. (2001) by monitoring the reduction of XTT in the presence of $O_2^{\cdot -}$, with some modifications. The liver was homogenized with 2 ml of 50 mM Tris–HCl buffer (pH 7.5) and centrifuged at 5,000×*g* for 10 min. The reaction mixture (1 ml) contained 50 mM Tris–HCl buffer (pH 7.5), 20 μg liver proteins, and 0.5 mM sodium, 3′-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzenes sulfonic acid hydrate (XTT). The XTT reaction was measured at 470 nm for 5 min on a UV-3010 spectrophotometer (Hitachi Co., Japan). The production rate of $O_2^{\cdot -}$ was calculated using an extinction coefficient of 2.16×10^4/M cm.

The detection of H_2O_2 production in the liver was carried out by flow cytometry using 2′-7′-dichlorofluoroscein diacetate (DCFH-DA; Sigma). DCFH-DA was added (10 μM final concentration) to the liver and the mixture was incubated for 30 min at 37°C. After incubation, the cells were subjected to flow cytometry analysis (FACScan; Becton Dickinson; Asatiani et al. 2004).

Lipid peroxidation of liver

Lipid peroxidation of liver was determined as the concentration of malondialdehyde (MDA) by the thiobarbituric acid (TBA) reaction as described by Buege and Aust (1978), but with the addition of an isobutanol extraction step for the removal of interfering compounds. For analysis, a subsample of tissue was thawed, homogenized, and cells lysed using a 4% TBA solution in 0.2 M HCl. The reaction mixture was then incubated at 90°C for 45 min. The resulting TBA–MDA adduct was phase-extracted using isobutanol. The isobutanol phase was then read at a wavelength of 535 nm on a UV-3010 spectrophotometer. MDA standard curves were prepared by acid hydrolysis of 1,1,3,3,-tetramethoxypropane (TMP).

Antioxidant enzyme activity of liver

The liver was homogenized in 1 ml of ice-cold 50 mM sodium phosphate (pH 7.0) containing 1% polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 30,000×g for 30 min and the supernatant was used for assays of the activities of superoxide dismutase (SOD), catalase (CAT), ascorbic acid peroxidase (APx), and glutathione peroxidase (GSHPx).

The activity of SOD was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich 1971). Each 3-ml reaction mixture contained 50 mM sodium phosphate (pH 7.8), 13 μM methionine, 75 μM NBT, 2 μM riboflavin, 100 μM EDTA, and 200 μl of the enzyme extract. The reduction of NBT was measured at 560 nm on a UV-3010 spectrophotometer. An assay control in the absence of tissue was used as a control. One unit of SOD was defined as the amount of protein needed to decrease the reference rate to 50% of the maximum inhibition.

The CAT activity was measured by the decrease in the H_2O_2 concentration for 15 s, reading the absorbance at 240 nm on a UV-3010 absorption spectrophotometer according to Claiborne (1985). The reaction volume was 1 ml and contained 500 μl of sample homogenate and 500 μl of sodium phosphate buffer 50 mM, pH 7.0 and 15 mM H_2O_2. The control was assayed without H_2O_2. One unit of enzyme activity was defined as a decrease in absorbance of 0.001/min at 240 nm.

APx activity was assayed using the method described by Reuveni et al. (1992). A reaction mixture consisting of 100 μl supernatant, 17 mM H_2O_2 (450 μl), and 25 mM ascorbate (450 μl) was then assayed for 3 min at 290 nm on a UV-3010 spectrophotometer. Activity was measured as the disappearance of ascorbate. One unit of enzyme activity was defined as a decrease in absorbance of 0.001/min at 290 nm.

GSHPx activity was determined by a modified coupled assay procedure of Paglia and Valentine (1967). This method measures the rate of glutathione oxidation by hydrogen peroxide as catalyzed by GSHPx present in the sample by the addition of endogenous glutathione reductase and NADPH that converts oxidized glutathione (GSSG) to the reduced form. The rate of GSSG formation is measured by following the decrease in absorbance of the reaction mixture at 340 nm as NADPH is converted to NADP. GSHPx was measured using hydrogen peroxide as a substrate. The activity of GSHPx was measured using a UV-3010 spectrophotometer and calculated as the amount of NADPH oxidized per minute using the molar absorption coefficient of 6.22×10^{-6} for NADPH. Enzyme activity was expressed as nmoles of NADPH oxidized per mg protein per minute.

Glutathione and ascorbic acid assay of liver

To perform the reduced glutathione (GSH), oxidized glutathione (GSSG) assay, the liver was homogenized as described above. However, supernatants were not diluted five-fold as described in the antioxidant enzyme assays. GSH and GSSG contents were estimated using the method of Hissin and Hilf (1976). The reaction mixture contained 100 μl of supernatant, 100 μl *o*-phthaldehyde (1 mg/ml), and 1.8 ml phosphate buffer (0.1 M sodium phosphate, 0.005 M EDTA, pH 8.0). Fluorometry was performed

using an F-4500 fluorometer (F-4500, Hitachi Co., Japan) with excitation at 350 nm and emission at 420 nm.

Hepatic-reduced ascorbic acid (ASA) and dehydroascorbic acid (DHASA) determinations were performed as described by Jacques-Silva et al. (2001). Protein was precipitated in 10 volumes of a cold 4% trichloroacetic acid (TCA) solution. An aliquot of homogenized sample (300 ml), in a final volume of 1 ml of the solution, was incubated at 38°C for 3 h, followed by adding 1 ml H_2SO_4 65%(v/v) to the medium. The reaction product was determined using a color reagent containing 4.5 mg/ml dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/ml).

The content of protein was set out following Lowry's method (Lowry et al. 1951).

Statistical analysis

Each biochemical indicator was replicated five times. All data were expressed as mean ± standard error and were analyzed by an analysis of variance (ANOVA). If significance was found in the ANOVA, the group means were compared using Student's *t*-test. Differences were considered to be significant when $P \leq 0.05$.

Results

Effect of Ce^{3+} on the weight and HSI of fish under Pb^{2+} intoxication

It can be seen from Table 1 that the net increase of wet body weight from the Pb^{2+}-treated groups was lower than that of the control group ($P < 0.05$ or 0.01), while the HSI of fish were significantly enhanced by Pb^{2+} treatments ($P < 0.05$ or 0.01). However, after the fish were injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} doses, the net increase of body wet weight was higher than those of fish injected with Pb alone and the HSI were reduced (Table 1).

ROS production of liver

The effects of treatments with various Pb^{2+} doses on the production rate of $O_2^{\cdot -}$ and H_2O_2 in the liver of fish are shown in Figs. 1 and 2. It can be seen that ROS was

Table 1 The net increase of body weight and hepatosomatic index (HSI) of fish after abdominal cavity injections of Pb^{2+} and Ce^{3+} solution

	Pb^{2+} (mg/kg)				1.5 mg/kg WW Ce^{3+} under various Pb^{2+} doses			
	Control	10	20	30	0	10	20	30
Net increase of wet body weight (g)	5.65 ± 0.28	3.81 ± 0.19*	1.89 ± 0.09**	0.25 ± 0.01**	5.89 ± 0.30	4.89 ± 0.24	3.39 ± 0.17*	2.87 ± 0.14**
HSI (mg/g)	16.75 ± 0.84	18.63 ± 0.93*	22.67 ± 1.13**	25.31 ± 1.27**	16.99 ± 0.85.	17.25 ± 0.86	18..39 ± 0.92*	19.51 ± 0.98*

Net increase of wet body weight = wet body weight 42 days after injection − wet body weight after acclimatization (before injection).

Ranks marked with a star or double stars means that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% significance level, respectively

The values represent means ± SE, $n = 5$

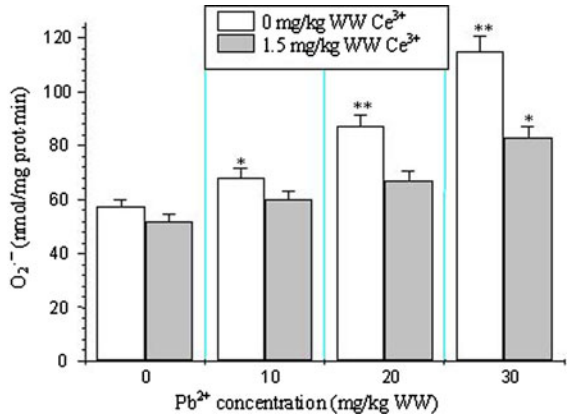

Fig. 1 $O_2^{\cdot-}$-generating rate of the liver after the fish were injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. Bars marked with a star or double stars mean that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% confidence level, respectively. Values represent means ± SE, $n = 5$

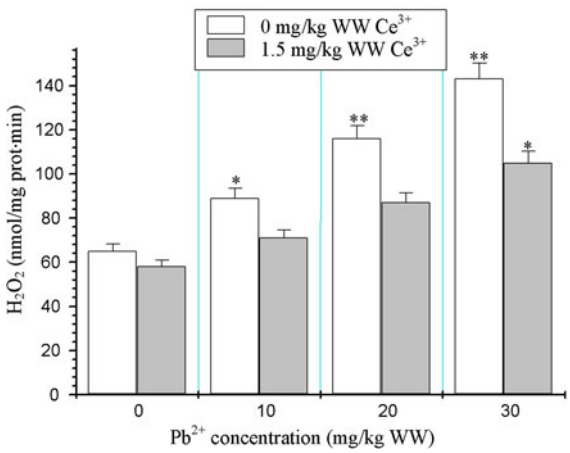

Fig. 2 H_2O_2-generating rate of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. Bars marked with a star or double stars mean that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% confidence level, respectively. Values represent means ± SE, $n = 5$

sharply enhanced by Pb^{2+} treatments from 10 to 30 mg/kg WW; for example, the $O_2^{\cdot-}$-generating rate increased by 19.3%, 52.63%, and 101.75% ($P < 0.05$ or 0.01), respectively, and H_2O_2 was 1.37, 1.78, and 2.2 times as the control ($P < 0.05$ or 0.01), respectively. However, after the fish were injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} stress doses, the ROS accumulation of the liver was notably decreased

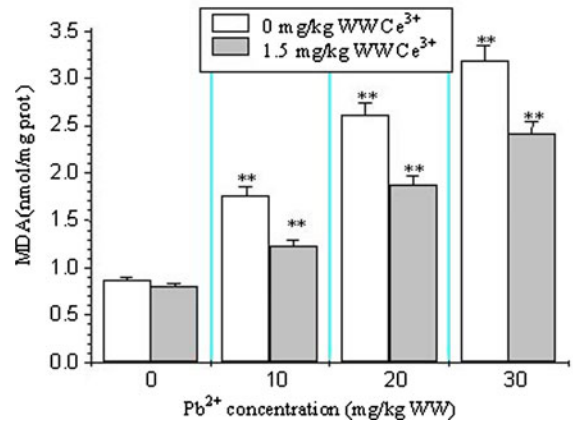

Fig.3 Malondialdehyde (MDA) content of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. Bars marked with a star or double stars mean that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% confidence level, respectively. Values represent means ± SE, $n = 5$

(Figs. 1 and 2); for example, the $O_2^{\cdot-}$-generating rate decreased by 8.77% ($P > 0.05$), 11.76% ($P > 0.05$), 22.99% ($P > 0.05$), and 27.83% ($P < 0.05$), with H_2O_2 reductions of 9.23% ($P > 0.05$), 20.22% ($P > 0.05$), 25% ($P > 0.05$), and 26.57% ($P < 0.05$), respectively, compared to those not injected with Ce^{3+}.

Lipid peroxidation of liver

Analysis of liver lipid peroxide (MDA) content was performed (Fig. 3). MDA content was 104.65%, 203.48%, and 270.93% higher in various Pb^{2+}-treated fish with respect to the control ($P < 0.01$). However, after Ce^{3+} injection under various doses of Pb^{2+}, the increase of MDA content in the liver obviously declined, reduced by 6.98% ($P > 0.05$), 30.11% ($P < 0.01$), 28.35% ($P < 0.01$), and 24.14% ($P < 0.01$), respectively, compared to those not injected with Ce^{3+}.

Antioxidant enzyme activity of liver

Figure 4 compares the activity of SOD in the liver after the fish were injected with 1.5 mg/kg WW Ce^{3+} under various stress doses of Pb^{2+}. From the 10 to 30 mg/kg WW Pb^{2+}-treated groups, the liver showed descending SOD activity that decreased by 21.14%, 42.91%, and 60.2% ($P < 0.05$ or 0.01), respectively. But the SOD activities of the liver after Ce^{3+} injection under various

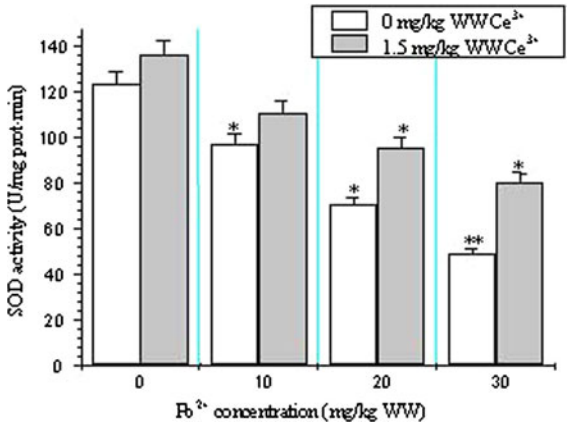

Fig. 4 Superoxide dismutase (SOD) activities of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. Bars marked with a star or double stars mean that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% confidence level, respectively. Values represent means ± SE, $n = 5$

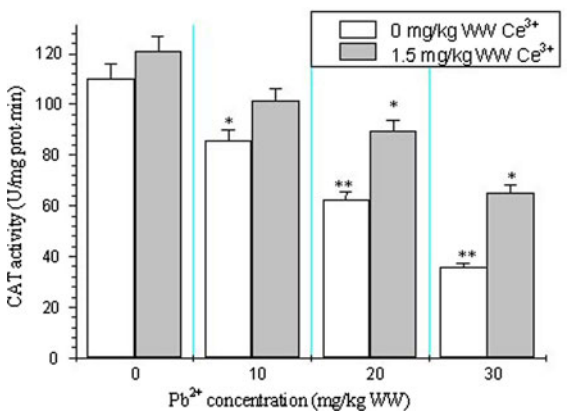

Fig. 5 Catalase (CAT) activities of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. Bars marked with a star or double stars mean that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% confidence level, respectively. Values represent means ± SE, $n = 5$

Pb^{2+} stress doses were significantly elevated, increasing by 10.57% ($P > 0.05$), 14.10% ($P > 0.05$), 35.96% ($P < 0.05$), and 64.17%($P < 0.05$), respectively, compared to those not injected with Ce^{3+}.

Figure 5 presents the CAT activities of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. The liver in Pb^{2+}-treated groups exhibited lower CAT activities, showing 22.25%, 43.44%, and 67.37% reductions ($P < 0.05$ or 0.01),

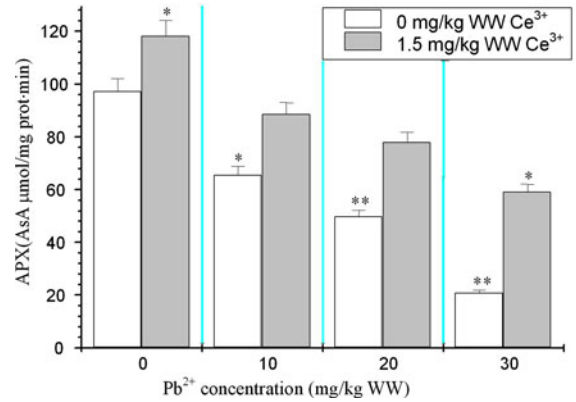

Fig. 6 Ascorbate peroxidase (APx) activities of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. Bars marked with a star or double stars mean that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% confidence level, respectively. Values represent means ± SE, $n = 5$

while the CAT activity of liver after Ce^{3+} injection under various stress doses of Pb^{2+} were increased, suggesting 9.43% ($P > 0.05$), 18.04% ($P > 0.05$), 43.05% ($P < 0.05$), and 82.59% ($P < 0.01$) enhancement, respectively, compared to those not injected with Ce^{3+}.

Figure 6 shows the APx activities of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} doses. In Pb^{2+}-treated groups, the liver of fish exhibited a significant reduction of APx activity, which was 32.45%, 51.11%, and 78.62% compared to the control ($P < 0.05$ or 0.01), respectively. However, after Ce^{3+} injection under Pb^{2+} stresses, the APx activity in the liver were obviously enhanced, elevated by 21.64%, 34.94%, 56.92%, and 184.88%, respectively, compared to those not injected with Ce^{3+} ($P < 0.05$ or 0.01).

The GSHPx activities of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} stresses are shown in Fig. 7. The GSHPx activity in fish liver injected with various Pb^{2+} dosages was significantly lower than the control, having a 19.22%, 38.31%, and 59.67% reduction ($P < 0.05$ or 0.01), respectively, while the GSHPx liver activity after Ce^{3+} injection under various Pb^{2+} stresses improved, showing a 4.21% ($P > 0.05$), 21.11% ($P < 0.05$), 36.54% ($P < 0.05$), and 85.26% ($P < 0.01$) increase, respectively, compared to those not injected with Ce^{3+}.

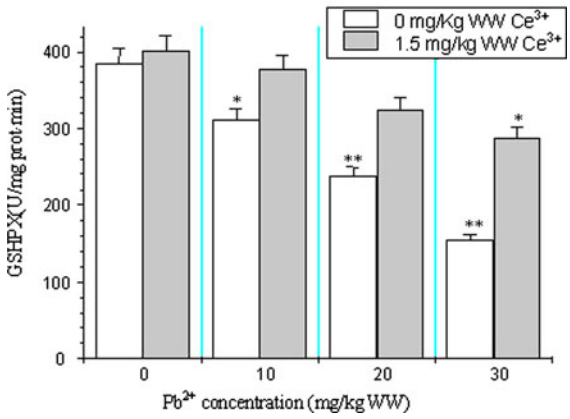

Fig. 7 Glutathione peroxidase (GSHPx) activities of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. Bars marked with a star or double stars mean that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% confidence level, respectively. Values represent means ± SE, $n = 5$

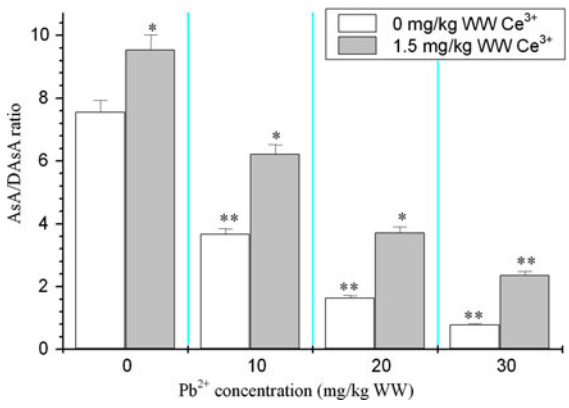

Fig. 8 The ratios of ascorbic acid to dehydroascorbic acid (ASA/DHASA) in fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. Bars marked with a star or double stars mean that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% confidence level, respectively. Values represent means ± SE, $n = 5$

Antioxidant non-enzyme system of liver

Figure 8 compares the ratios of ASA to DHASA of fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various doses of Pb^{2+}. The liver in Pb^{2+}-treated groups exhibited lower ratios of ASA to DHASA, having a 3.89-, 5.92-, and 6.78-fold reduction ($P < 0.01$), while the ratios of ASA to DHASA of liver after Ce^{3+} injection under various Pb^{2+} doses increased, showing a 1.99-, 2.55-, 2.08-, and 1.83-fold

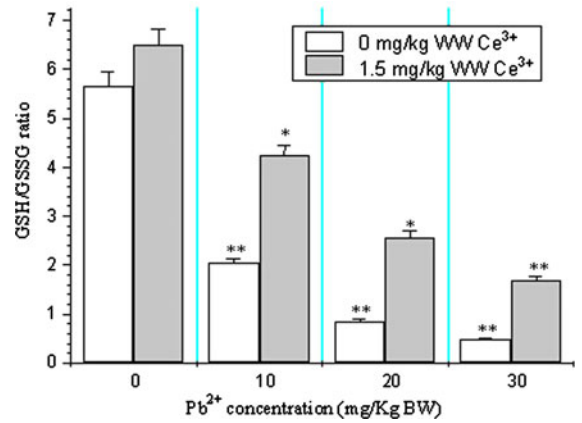

Fig.9 The ratios of glutathione to oxidized glutathione (GSH/GSSG) in fish liver after being injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages. Bars marked with a star or double stars mean that they are significantly different from the control (no Pb^{2+}) at the 5% or 1% confidence level, respectively. Values represent means ± SE, $n = 5$

increase, respectively, compared to those not injected with Ce^{3+} ($P < 0.05$ or 0.01).

The ratios of GSH to GSSG in fish liver after injection with 1.5 mg/kg WW Ce^{3+} under different doses of Pb^{2+} are presented in Fig. 9. The ratios of GSH to GSSG of the liver of fish injected with various Pb^{2+} doses were lower than the control (no Pb^{2+}), which decreased by 3.59, 4.81, and 5.18, respectively ($P < 0.01$). However, after Ce^{3+} injection under various doses of Pb^{2+}, the ratios of GSH to GSSG of liver exhibited a 0.82, 2.21, 1.71, and 1.21 increase, respectively, compared to Pb-treated fish not injected with Ce^{3+} ($P < 0.05$ or 0.01).

Discussion

The mechanism by which Ce^{3+} decreases oxidative stress caused by Pb^{2+} in the liver of silver crucian carp was investigated. The question was raised whether oxidative stress of Pb^{2+} can occur in fish liver by varying Pb^{2+} doses or if Ce^{3+} can decrease the oxidative stress caused by Pb^{2+} during experiments with silver crucian carp.

The observed oxidative stress in fish injected with Pb^{2+} ions do not appear to indicate the presence of being enough to prevent damage and generates swelling of the fish liver, but lower doses of Ce^{3+} ions appear to indicate a decrease of oxidative stress

caused by Pb^{2+} and alleviates the fish's liver enlargement.

One of the mechanisms of heavy metal toxicity on the liver is oxidative stress, probably due to an imbalance between ROS production and their removal, leading to macromolecule and membrane damage, thus, leading to the swelling of fish liver. Our data showed that the production rate of $O_2^{\cdot -}$ and H_2O_2 in the fish liver injected by various Pb^{2+} doses was significantly elevated (Figs. 1 and 2), indicating that $O_2^{\cdot -}$ and H_2O_2 were constantly accumulated in the liver. Interaction between H_2O_2 and $O_2^{\cdot -}$ can create OH and $^1O_2$, which are far more destructive and can peroxidate the unsaturated lipid of the cell membrane (Fridovich 1978). As one kind of peroxide, MDA can intensively react with various cellular components, thus, enzymes and membranes are seriously damaged and membranous electric resistance and fluidity fall (Scandalios 1993). This eventually leads to the destruction of the membrane structure and physiological integrity (Scandalios 1993). In this study, the peroxidation of the liver membrane along with Pb^{2+} dosage was demonstrated by an enhancement in MDA content (Fig. 3). However, after the fish were injected with 1.5 mg/kg WW Ce^{3+} under various Pb^{2+} dosages, the ROS accumulation and the lipid peroxidation level in the liver were significantly decreased (Figs. 1–3), implying that a low Ce^{3+} dosage could alleviate oxidative stress caused by Pb^{2+} in the liver of silver crucian carp. Previous research indicated that a small concentration of Ce^{3+} ions could clear a large amount of $O_2^{\cdot -}$. The mechanism for this was that Ce^{3+} can reduce $O_2^{\cdot -}$ to H_2O_2, and then oxidates itself to Ce^{4+}, and Ce^{4+} can oxidate $O_2^{\cdot -}$ to O_2, while it reduces itself to Ce^{3+} (Wang et al. 1997; Hong 2002; Yang et al. 2005). Therefore, after injecting with Ce^{3+}, Ce^{3+} could directly clear ROS, relieving oxidative destruction in the fish liver. However, the effects of Pb^{2+} and Ce^{3+} on ROS production may be related to the decrease of antioxidant defenses in vivo. Organisms use a diverse array of enzymes like SOD, CAT, APx, and GSHPx, as well as nonenzymatic antioxidants like ascorbate and GSH to remove oxidative stress. SOD can convert $O_2^{\cdot -}$ into H_2O_2 and O_2; moreover, CAT, APx, and GSHPx can reduce H_2O_2 into H_2O and O_2 (Scandalios 1993). Therefore, SOD, CAT, APx, and GSHPx can keep a low level of ROS and prevent ROS from poisoning cells (Scandalios 1993). Ates et al. (2008) showed that, when the activities of SOD, CAT, and GSHPx in the liver of rainbow trout were greatly reduced, the MDA content increased by Pb^{2+} and Cu^{2+}, while Se^{4+} could decrease the inhibition of the activities of the antioxidative enzymes and reduce the lipid peroxidation level caused by Pb^{2+} and Cu^{2+}. In our experiments, we observed that the activities of SOD, CAT, APx, and GSHPx of fish liver injected with various Pb^{2+} doses were significantly inhibited (Figs. 4–7), suggesting that Pb^{2+} caused strong oxidative stress in the liver of fish. Additional evidence pointing to the possibility of oxidative stress was provided by a reduction in ascorbate and GSH contents in the liver injected with Pb^{2+} (Figs. 8 and 9). The depletion of ascorbate and GSH in the fish liver was associated with increases in ROS and MDA, suggesting that the liver was using up antioxidant defenses to prevent oxidative stress. Our data also demonstrated that a low Ce^{3+} dosage could improve the activities of SOD, CAT, APx, and GSHPx and the levels of ascorbate and GSH of (Figs. 4–9) and decrease the ROS accumulation and the lipid peroxidation level in fish liver. Liu et al. [24] proved that Ce^{3+} could significantly increase SOD activity by binding to SOD and improving its structure. It is speculated that Ce^{3+} forces out Pb^{2+} from SOD, CAT, APx, and GSHPx or binds to the enzymes and improves the enzyme's damaged structure caused by Pb^{2+} in the liver of silver crucian carp and that Ce^{3+} can directly interact with and detoxify oxygen-free radicals and, thus, contribute significantly to nonenzymatic ROS scavenging and decreases the depletion of ascorbate and GSH and the oxidative stress in the liver. This, however, needs to be further studied.

Conclusion

These experiments prove that Pb^{2+} induces oxidative damage and Ce^{3+} relieves oxidative damage caused by Pb^{2+} in the liver of silver crucian carp. Pb^{2+} inhibits the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APx), glutathione peroxidase (GSHPx), and other peroxidases, reduces glutathione (GSH) and ascorbicacid (ASA) levels, and leads to reactive oxygen species (ROS) accumulation, which promotes lipid peroxidation and oxidative stress of the liver. This causes enhancement

of the hepatosomatic index (HSI) of silver crucian carp. However, Ce^{3+} can decrease the inhibition of the antioxidant enzymes' activities and the reduction of antioxidants caused by Pb^{2+}, decreasing ROS accumulation, lipid peroxidation, and liver enlargement in silver crucian carp.

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