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Dietary silymarin, *Silybum marianum* extract ameliorates cadmium chloride toxicity in common carp, *Cyprinus carpio*

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Abstract

The present study evaluated the protective effects of silymarin extract (SIE) on cadmium chloride toxicity in common carp, *Cyprinus carpio*. Four experimental group were considered for the experiment including: SIE_0 (control): non-SIE-supplemented fish, SIE_1: fish supplemented with 400 mg SIE/kg diet, SIE_2: fish supplemented with1400mg SIE/kg diet, SIE_3: fish supplemented with 2400 mg SIE/kg diet). Fish were fed experimental diet for 60

days and then exposed to cadmium chloride (1.5 mg/l or 25% of LC50-96 h) and antioxidant defense components and the survival rate assayed. After 60 days feeding trial, total antioxidant capacity (TAC) levels significantly increased (P<0.01) in 1400-2400 mg SIE/kg diet treatments compared to those in control and 400 mg SIE/kg diet treatment. Malondialdehyde (MDA) (P>0.01) and acetylcholinesterase (AChE) levels (P>0.01) remained unchanged during the feeding period in all treatments. Hepatic catalase (CAT) in all SIE supplemented groups and superoxide dismutase (SOD) and glutathione peroxidase (GPx) in 1400-2400 mg SIE/kg diet treatments significantly elevated (P<0.01) in response to SIE. Plasma levels of hepatic metabolic enzymes [alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK) , lactate dehydrogenase (LDH)] remained unchanged (P>0.01) in all experimental groups over feeding period. After exposure to cadmium chloride, TAC levels were significantly elevated (P<0.01) in all experimental groups. In control and 400 mg SIE/kg diet treatment, TAC showed lower levels (P<0.01) compared to other groups. MDA levels were significantly increased (P<0.01) in control and fish supplemented with 400 and 1400 mg SIE/kg diet. TAC in the fish of 2400 mg SIE/kg diet treatment remained unchanged (P>0.01), following the exposure. CAT (P<0.01), SOD (P<0.01) and GPx (P<0.01) were significantly elevated in response to cadmium chloride in all groups. However, the treatments, 1400 and 2400 mg SIE/kg diet, showed lower increases (P<0.01) of enzymes. AChE activity (P<0.01) in the liver were significantly decreased in control and fish fed 400 and 1400 mg SIE/kg diet. Exposure to cadmium significantly increased (P<0.01) the plasma levels of ALT, AST, ALP and LDH in control and those fed 400 and 1400 mg SIE/kg diet. The findings of the current study indicated that SIE at a rate of 1400-2400mg/kg diet might enhance antioxidant defense and protect hepatocytes against toxic effects of cadmium.

Key words: Heavy metal, liver, blood, herbal extract, fish

Contamination of aquatic ecosystems with heavy metals and their effects on aquatic organisms has been the subject of many studies (Ansari et al., 2004; Bhuyan and Islam, 2017; Sodango et al., 2018; Joksimović et al., 2020; Hajirezaee et al., 2021).Heavy metals usually enter aquatic environments through effluents from industrial and agricultural activities (Tahar and Keltoum, 2011; Miskowiec et al., 2015; Yang et al., 2018).

In fish, it recognized that heavy metal toxicity could alter all aspects of life such as growth, reproduction, osmoregulation, and immunity (Ebrahimi and Taherianfard, 2011; Saglam et al., 2013; Guardiola et al., 2015; Jinhui et al., 2019). Therefore, finding efficient

ways to reduce the harmful effects of heavy metals on aquatic organisms would be of great importance. As a natural way, few studies indicate that herbal supplements and their derivatives can mitigate the negative physiological effects of contaminants in fish (Hajirezaee et al., 2019; Rafieepour et al., 2019 a,b; Ahmadifar et al., 2020; Elumalai et al., 2020; Mohammed et al., 2020; Dawood et al., 2020; Khafaga et al., 2020; Zhu, 2020; Ahmadifar et al., 2021). As a medicinal plant, the therapeutic properties of the Milk thistle, *Silybum mariamum* have been well recognized for many years (Křen and Walterová, 2005). Silymarin is a polyphenolic compound extracted from *Silybum mariamum* with strong antioxidant activity (Kvasnička et al., 2003). Banaee et al. (2015) used silymarin extracts to moderate the toxicity of malathion in zebrafish and *Cichlasoma nigrofasciatum*. They reported that the protective effects of silymarin are probably exerted through enhancing the hepatic antioxidant capacity and hepatic enzymes. Nazdar et al. (2018) demonstrated the protecting effects of silymarin on pancreatic tissue and digestive capacity of rainbow trout exposed to nickel oxide nanoparticles. As a medicinal plant, dietary *S. marianum* enhanced some non-specific immune responses in *C. carpio* (Alishahi et al., 2011). The oxidative stress induced by heavy metals causes a wide spectrum of physiological and histological lesions in fish (Chowdhury and Saikia, 2020).In toxicological studies, biochemical changes in the liver and blood are usually used to evaluate the mode of action of toxicants (Chupani et al., 2017; Chupani et al., 2018). Plasmatic and hepatic enzymes were a good reflection of environmental stressors and provide a comprehensive view on the status of immunity (Yousafzai et al., 2011). In this research, we have studied the effects of silymarin extract (SIE) on the antioxidant defense system in cadmium chloride -exposed common carp, a species with a high value of culture in the world. Heavy metals, particularly, cadmium have been found as the main source of aquatic pollution and have been detected in alarming quantities in many water environments, especially at or near industrial localities where effluents are usually discharged (Chandra and Khuda-Bukhsh, 2004). The findings of the present study suggest a possible natural may to ameliorate the deleterious effects caused by cadmium in fish.

Material and methods

Preparation of herbal extract

The silymarin in dry form was provided from a local medicinal plants shop. The extraction was done based on the method suggested by Hajirezaee et al. (2019) by a rotary

(Buchi, Switzerland) at 80 °C. The concentrated extract was finally dried at 40 °C to make powder. The powders were eventually kept at 0°C till the beginning of the experiment.

Fish and experimental design

Common carp (n= 1650; TW: 22.1 ± 4.1 g; TL: 11.8 ± 2.2 cm) were distributed into 15 tanks (500 l) (110 fish per tank) containing aerated and disinfected water. After a 10-dayacclimation period, fish were divided into four groups in three replicates within *thirty* tanks containing 100L continually aerated water and fed for 60 days with various dietary levels of silymarin extract (SIE). The dietary treatments were: SIE_0 (control): non-supplemented fish, SIE_1: 400 mg SIE/kg diet, SIE_2: 1400 mg SIE/kg diet, SIE_3: 2400 mg SIE/kg diet. A commercially basal diet (pellet, crude protein: 36 %, crude lipid: 6 %, carbohydrates: 12 %, crude fiber: 4 %, ash: 9 %, Faradaneh, Company, Iran) was used to make experimental diets. In this regard, the basal diet was powdered by a mill, and then the dried extracts (SIE) were added to the diet at a rate of 0 mg, 400 mg, 1400 mg, and 2400 mg/kg diet. After complete mixing, the mixture became paste by adding 100 ml of distilled water. Then, the appropriate size of food (as granule; diameter: 3 ± 0.1 mm, length: 3.5 ± 0.2 mm) was made using a sieve. Then, the foods were respectively kept at room temperature (away from light) and an oven (at 30 °C) to dry. Finally, the foods were stored in a refrigerator (4 °C) (Nya and Austin 2009). Feeding operation was performed daily and at a rate of 3% total fish weight. The amount of needed food was regulated at intervals of 10 days by estimating total fish weight. After 60 days feeding trial, fish were exposed to 1.5 mg/l cadmium chloride (Merck CO., Germany, CAS number: 35658-65-2) for 96 h. The exposure concentration was calculated based on 25 % (1.5 mg/l) of LC50-96 h (6 mg/l). The amount of LC_{50} was determined in a previously separate experiment. In this regard, fish with a density of 10 per 10-liter aquarium were exposed to different doses of cadmium chloride in three replications, including: 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8 mg/l. Finally, the concentration, which yielded 50% mortality over 96 h, was determined as the amount of LC_{50}.

Throughout the experiment, the water quality properties were pH: 6.8 ± 0.1 (APX15/C-WTW-330i), temperature: 22.5 ± 1.2°C, ammonia: 0.02 ± 0.002 mg/L (colorimetrically at 670 nm) and dissolved oxygen:7.2 ± 0.15 mg/L (OxyGouard). Also, the numbers of dead fish were recorded during the experiment to estimate mortality percent. In addition, the growth parameters, including final weight, final weight gain, specific growth rate and feed conversion ratio (FCR), were determined after the feeding trial according to following formula:

$$\text{Feed conversion ratio (FCR)} = \text{Dry weight of feed given (g)/weight gain (g)}$$

$$\text{Specific growth rate} = (\ln w_f - \ln w_0/T) \times 100$$

where: w_f: final weight, w_0: initial weight

Blood and liver sampling

The blood and liver samples were collected after 60 days feeding trial. The blood sampling was done 6 h after last feeding. For this purpose, fish were anesthetized with clove oil (100 mg/l) (Malekpouri et al., 2011) and the blood samples were taken from the caudal fin by 2 ml syringe (10 fish/tank). The plasma samples were obtained through centrifuging at 13,700 g for 10 min. Plasma samples were then frozen in liquid nitrogen ($-196°C$) till further assays. Besides, liver tissue samples were taken after dissecting out the fish and then kept in liquid nitrogen ($-196 °C$) for further assays.

Biochemical assays of plasma

All assays were conducted according to manufacturer's instructions.

LDH levels were measured at 450 nm based on the oxidation rate of NADH (Sigma-Aldrich CO, USA, catalogue number: MAK066).

CK activity was assayed at 340 nm by measuring the amount of phosphate transferred from phosphocreatine to ADP during phosphorylation of glucose (Sigma-Aldrich CO, USA, catalogue number: MAK116).

AST levels were measured at 450 nm by calculating the deamination rate of aspartate to α-ketoglutarate and the following generation of glutamate (at 37 C, pH: 8.0) (Sigma-Aldrich CO, USA, catalogue number: MAK055).

ALT levels were assayed at 570 nm based on the production rate of pyruvate (Sigma-Aldrich CO, USA, catalogue number: MAK052).

ALP activity was measured at 405 nm by estimating the conversion rate of p-nitrophenol phosphate to nitrophenol (Sigma-Aldrich CO, USA, catalogue number: GTX85593).

Biochemical assays of liver

The total antioxidant capacity (TAC) was assayed using the ferric reducing ability of plasma (FRAP) method, according to Benzie and Strain (1996). Briefly, the liver tissues were homogenized in cooled phosphate buffer (pH 7.4) in the ratio of 1:10 (w/v) and then centrifuged (15 min; 13700 g, at 4 °C) to separate the supernatant. After that, a 0.01 µl of the supernatant sample was poured into 0.3 ml FRAP reagent, and the absorbance was read at 593 nm.

Catalase (CAT) activity was assayed at 240 nm based on the production of water and oxygen from catalysis of hydrogen peroxide (H_2O_2) (Sigma-Aldrich CO, USA, catalogue number: CAT100) (Claiborne 2018).

Superoxide dismutase (SOD) activity was determined colourimetrically at 440 nm through oxidation of xanthine to superoxide radicals (SOR) and further reaction of SOR with WST-1 reagent to create a red formazan dye (Marklund and Marklund 1974) (Sigma-Aldrich CO, USA, catalogue number: 19160 SOD).

Glutathione peroxidase (GPX) levels were determined at 340 nm based on the generation of oxidized glutathione from glutathione (GSH) upon GPx action (Sigma-Aldrich CO, USA, catalogue number: CGP1).

Liver acetylcholinesterase (AChE) activity was also measured at 412 nm according to an optimized method of Ellman et al. (1961). In this assay, thiocholine is generated from acetylthiocholine iodide (0.015 M) as a result of AChE action and then the produced thiocholine reacts with Ellman's reagent (0.01 M) to generate a dye product (Sigma-Aldrich CO, USA, catalogue number: MAK119).

The lipid peroxidation was spectrophotometrically (at 532 nm) determined upon reaction of malondialdehyde (MDA) with thiobarbituric acid and following production of a dye product (Utley et al. (1967) (Sigma-Aldrich CO, USA, catalogue number: MAK085).

Statistical analysis

All statistical analysis was performed using SPSS software. After the normality of data (mean ± standard deviation) was verified and confirmed, one-way analysis of variance was used to detect statistical differences. Finally, the difference between the means was determined using the Tukey test. The percent data were converted by arcsin transformation before ANOVA.

Results

Biochemicals after feeding trial

The supplementation of fish with 1400–2400 mg/kg diet SIE significantly increased TAC levels compared to control and those supplemented with 400 mg/kg diet SIE (Figure 1, $P<0.01$). The MDA (Figure 2, $P>0.01$) and AChE (Figure 3, $P>0.01$) levels remained unchanged during the feeding period in all experimental groups ($P>0.01$). The levels of antioxidants, SOD (Figure 4 a, $P<0.01$) and GPx (Figure 4 b, $P<0.01$), significantly increased in the fish supplemented with 1400-2400 mg/kg diet SIE. There were no significant differences in SOD (Figure 4 a, $P>0.01$) and GPx (Figure 4 b, $P>0.01$) levels between control and fish feed 400 mg/kg diet SIE. The levels of CAT elevated in all SIE supplemented fish compared to control (Figure 4 c, $P<0.01$).The metabolic enzymes of liver, ALT (Figure 5 a), AST (Figure 5 b), ALP (Figure 5 c), CK (Figure 5 d), LDH (Figure 5 e) remained unchanged in all experimental groups after 60 days feeding period ($P>0.01$).

Biochemicals after exposure to cadmium chloride

After exposure to cadmium chloride, TAC levels significantly increased in fish supplemented with 1400 and 2400 mg SIE/kg diet (Figure 1, $P<0.01$). Fish of control and those supplemented with 400 mg SIE/kg diet decreased after exposure (Figure 1, $P<0.01$). The MDA levels significantly elevated in control and fish fed 400 and 1400 mg SIE/kg diet (Figure 2, $P<0.01$). The fish fed 2400 mg SIE/kg diet showed no significant changes after exposure (Figure 2, $P>0.01$). The antioxidant enzymes, CAT (Figure 4 c, $P<0.01$), SOD (Figure 4 a, $P<0.01$) and GPx (Figure 4 b, $P<0.01$) significantly increased in response to cadmium chloride in all groups. The treatments 1400 and 2400 mg SIE/kg diet showed lower increases in antioxidant enzymes after exposure compared to other groups (Figure 4 a, b, c, $P<0.01$). The liver AChE activity (Figure 3, $P<0.01$) significantly declined in control and fish fed 400 and 1400 mg SIE/kg diet ($P<0.01$). The exposure to cadmium significantly increased the plasma levels of ALT (Figure 5 a), AST (Figure 5 b), ALP (Figure 5 c), LDH (Figure 5 e) in control and those supplemented with 400 and 1400 mg SIE/kg diet ($P<0.01$).

Growth performance and survival rate

The growth parameters including final weight, final weight gain, FCR and survival rate showed no significant difference between all groups after 60 days feeding trial (Table 1, $P>0.01$). After exposure to cadmium chloride, the survival rate significantly decreased in control and 400 mg SIE/kg diet treatment compared to 400–2400 mg SIE/kg diet treatments (Figure 6, $P<0.01$).

Discussion

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The use of immunostimulants has considerably increased in aquaculture to improve fish growth, immunity, and survival (Bricknell and Dalmo, 2005; Ringø et al., 2012; Ahmadifar et al., 2020). Among immunostimulants, special attentions have been paid to natural components as an alternative to chemicals (Van Hai, 2015; Mohammadi et al., 2020). In this respect, many studies have focused on medicinal plants (MEPs) and their effects on fish and shellfish (Dügenci et al., 2003; Jian and Wu, 2004; Citarasu et al., 2006; Ardó et al., 2008; Aly and Mohamed, 2010; Abdel-Tawwab, 2012; Choi et al., 2014; Güroy et al., 2014).Although the immunostimulant properties of MEPs have been largely reported, little data is available related to the protective role of them against toxicity caused by heavy metals in fish (Banaee et al. 2015; Rabie et al., 2016).

In the present study, GPX, SOD and CAT showed higher activitiesin SIE supplemented fish, particularly in 1400 and 2400 mg SIE/kg diet treatments compared to control. These results demonstrated that silymarin at optimized dietary levels could improve the hepatic antioxidative abilities of common carp, as TAC also increased in SIE supplemented fish. The enhancing effects of silymarin on hepatic antioxidant enzymes have been previously documented in fish and other vertebrates (Jia et al., 2013; Wang et al., 2019; Veisi et al., 2021).

SOD is responsible for removal of superoxide radicals (Kohen and Nyska, 2002). CAT exerts its antioxidant function by removing hydrogen peroxide radicals (Van der Oost et al., 2003). Furthermore, GSH-Px catalyzes hydrogen peroxide radicals to water and also eliminates lipid peroxide radicals by converting them to lipid alcohols. During this process, glutathione is oxidized to glutathione disulfide as a consequence of GSH-Px action.

After exposure to cadmium chloride, the antioxidant enzymes significantly elevated in all groups, which may be an antioxidant response to reduce the oxidative stress induced by the heavy metal, as previously reported in other exposed fish (Almeida et al., 2002; Basha and Rani, 2003). In this study, MDA levels, significantly elevated in control and fish fed 400 and 1400 mg SIE/kg diet after exposure, indicating cadmium chloride-inducing oxidative stress in the fish. In biological systems, MDA is well known as the main indicator of oxidative stress (Janero, 1990; Dragun et al., 2017). In contrast to control and 400-1400 mg SIE/kg treatments, fish supplemented with 1400 and 2400 mg SIE/kg diet showed lower increases of the antioxidant enzymes, indicating the ameliorating effects of SIE supplementation on hepatic antioxidant defense system. The protective effects of silymarin may be related to antioxidative properties of this plant and thus its potentials in scavenging the free radicals and sparing the

levels of antioxidant enzymes in 1400 and 2400 mg SIE/kg diet treatments. The composition of silymarin includes some components with free radical-scavenging and antioxidant activity such as taxifolin, silychristin, silydianinsilybin B, iso-silybin A and iso-silybin B (Anthony and Saleh, 2013). Taxifolin is known as the most effective component for scavenging free radicals (Anthony and Saleh, 2013).

The plasma levels of liver metabolic enzymes are usually used as biomarkers of hepatic damages (Ghelichpour et al., 2017). An elevation in plasma levels of these enzymes is usually attributed to liver tissue damages and following release of them into the circulatory system. However, these enzymes are non-specific, and their levels may fluctuate in response to other factors such as diseases. In this study, the exposure to cadmium chloride significantly increased the plasma levels of ALT, ALP, AST and LDH in control and those fed diet containing 400 and 1400 mg SIE. This result may be a consequence of the toxic effects of cadmium chloride on hepatocytes and following release of these enzymes to bloodstream (Vaglio and Landriscina, 1999; Heydarnejad et al., 2013; Al-Asgah et al., 2015; Atli et al., 2015).

In 2400 mg SIE/kg treatment, although the plasma levels of AST, ALP, ALT and LDH elevated after exposure, these increases were lower compared to other groups. These results clearly show that SIE at a concentration of 2400 mg/kg diet protects the hepatocytes against cadmium chloride-induced tissue damages.

AChE is responsible for inactivating the neurotransmitter acetylcholine and identified as a specific indicator of PC toxicity (Khan and Law, 2005; Deb and Das, 2017). Any disruptions in AChE activity results in the accumulation of acetylcholine and following blockage of signal transmission (Fulton and Key, 2001). Disruptions in swimming pattern, feeding and reproductive behaviour have been reported as consequences of signal transmission disrupted by acetylcholine (Pavlov et al., 1992; dos Santos Miron et al., 2005; Kavitha and Rao, 2008). After exposure to cadmium chloride, the liver the AChE activity significantly reduced in control and fish supplemented with 400 and 1400 mg SIE/kg, indicating the depressing effects of cadmium on AChE activity, as reported in other studies (Gill et al., 1991; Sen et al., 1995; Pretto et al., 2010; Banaee et al., 2015; Zhang et al., 2017). In 2400 mg SIE/kg diet treatment, AChE activity remained unchanged after exposure, which apparently indicates the ameliorating effects of SIE at optimized concentration on cadmium chloride-related depressions of AChE activity.

Conclusion

The findings of the present study suggested a protective role for silymarin extract at optimized dietary levels against toxic effects caused by cadmium chloride. Silymarin at optimized dietary levels (1400-2400 mg/kg diet) improved the antioxidant system and survival in cadmium chloride-exposed common carp. Identification of compounds with detoxifying effects in the chemical composition of silymarin and study of their mechanism of action can be suggested for future studies.

Conflict of interest statement

The authors state that no conflicts of interest exist.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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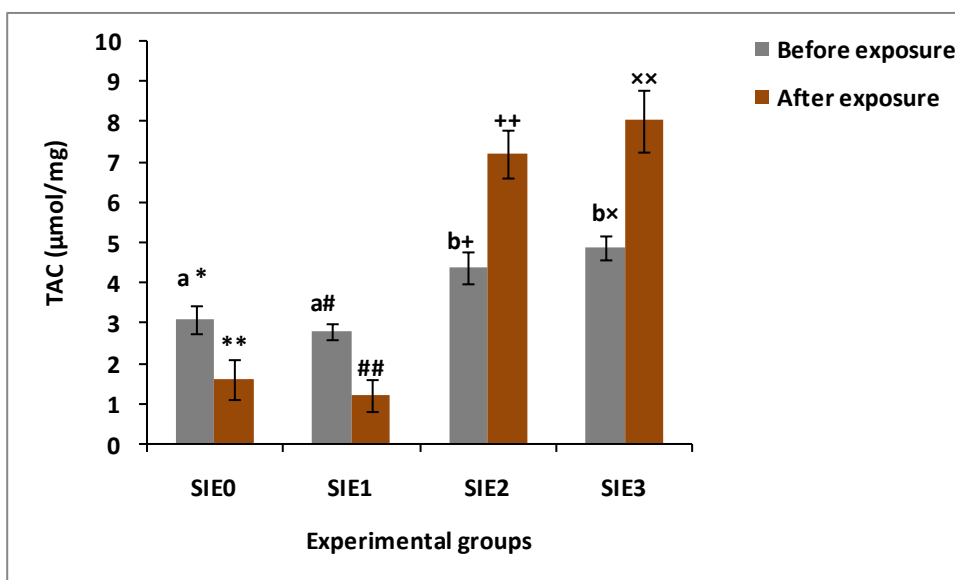
Figure 1. Effects of silymarin extract (SIE) supplementation on the liver TAC (total antioxidant capacity) activity before and after exposure to cadmium chloride in common carp. SIE_0 (control): non-supplemented fish, SIE_1: 400 mg SIE/kg diet, SIE_2: 1400 mg SIE/kg diet, SIE_3: 2400 mg SIE/kg diet. Significant differences are shown with different letters (P<0.01). For each group, significant differences between "before" and "after" exposure is shown as different symbols (P<0.01)
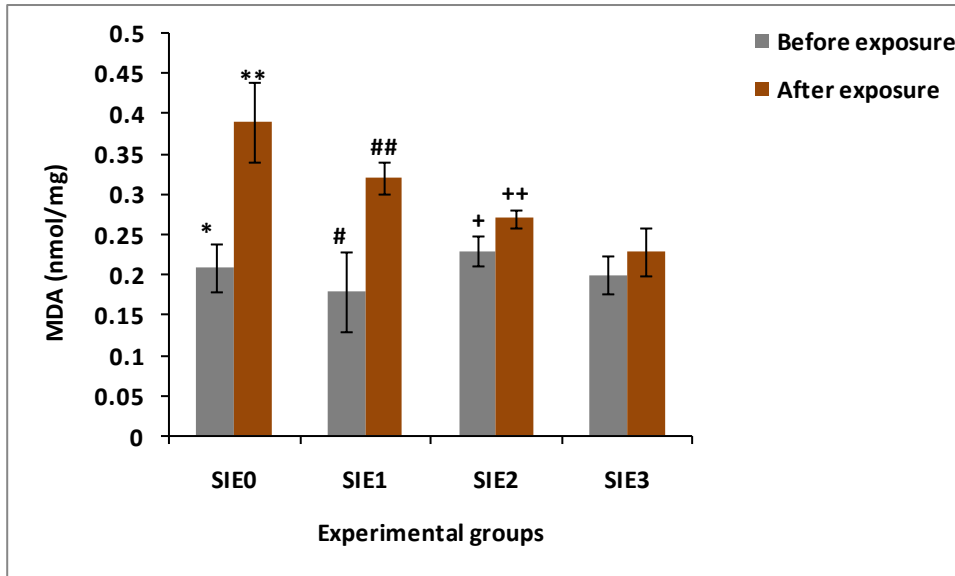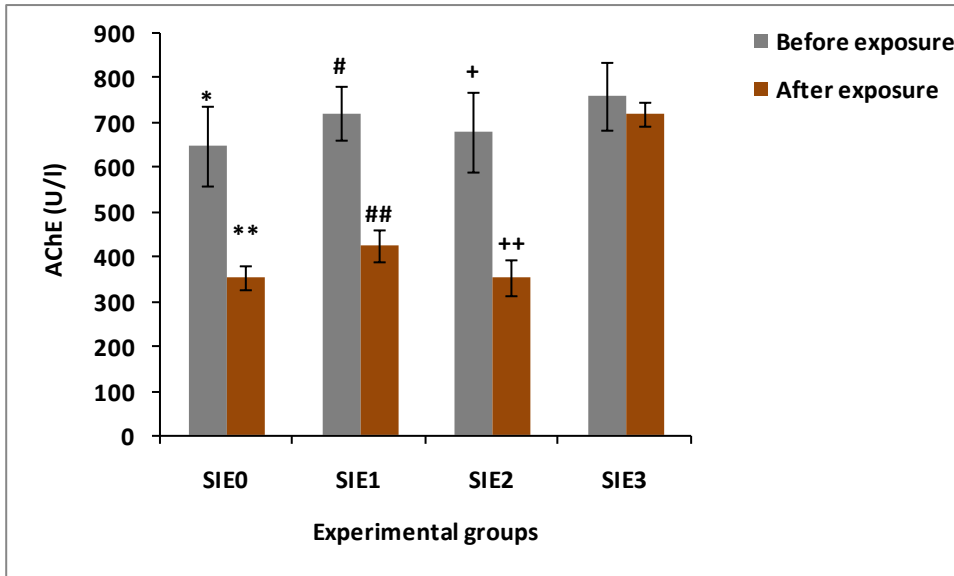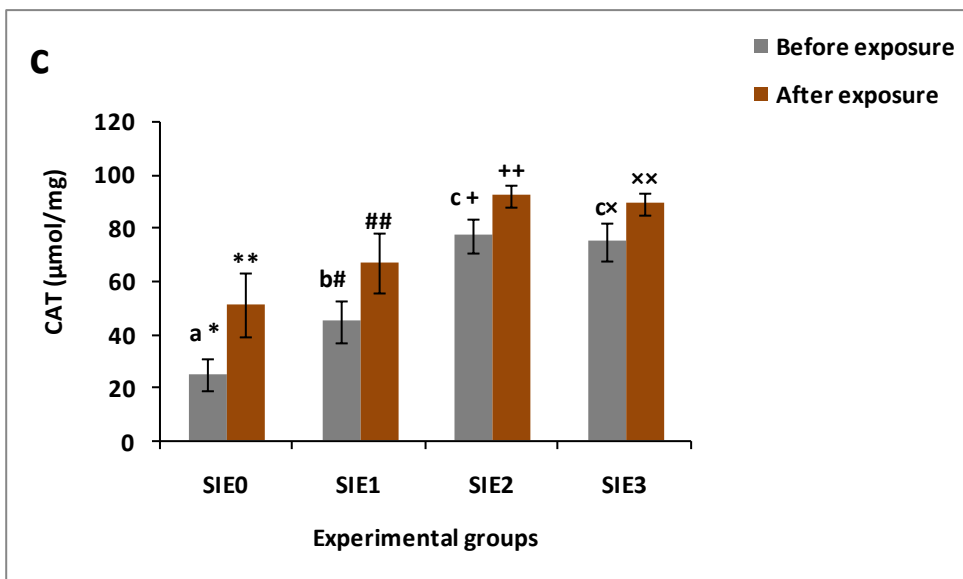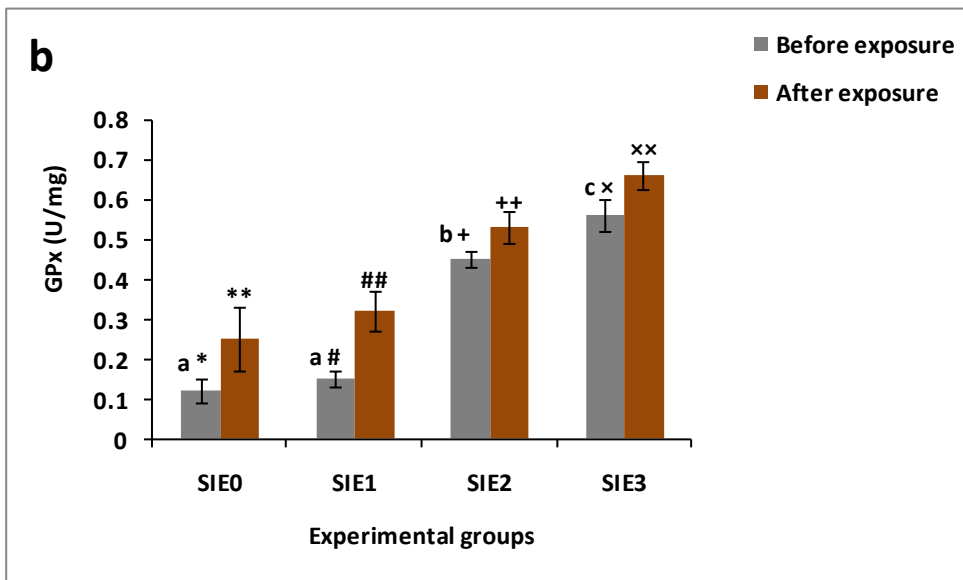
Figure 2. Effects of silymarin extract (SIE) supplementation on the liver MDA levels (malondialdehyde) before and after exposure to cadmium chloride in common carp. SIE_0 (control): non-supplemented fish, SIE_1: 400 mg SIE/kg diet, SIE_2: 1400 mg SIE/kg diet, SIE_3: 2400 mg SIE/kg diet. Significant differences are shown with different letters (P<0.01). For each group, significant differences between "before" and "after" exposure is shown as different symbols (P<0.01)
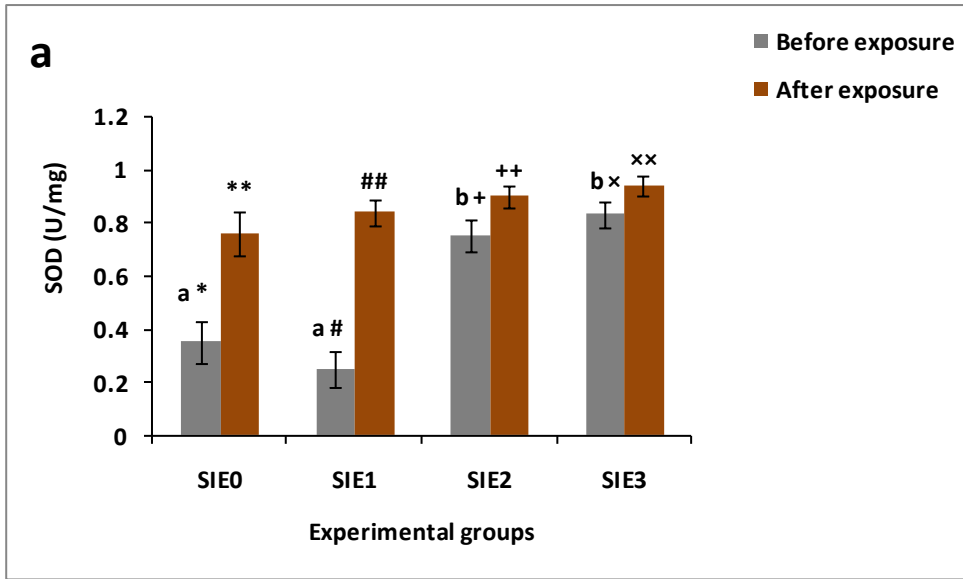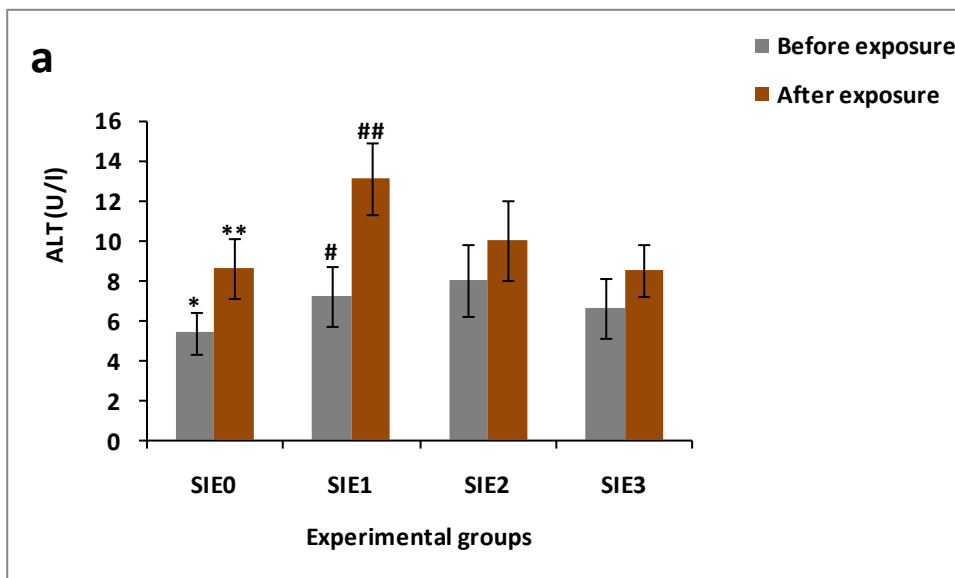
Figure 3. Effects of silymarin extract (SIE) supplementation on the liver AChE levels (acetylcholinesterase) before and after exposure to cadmium chloride in common carp. SIE_0 (control): non-supplemented fish, SIE_1: 400 mg SIE/kg diet, SIE_2: 1400 mg SIE/kg diet, SIE_3: 2400 mg SIE/kg diet. Significant differences are shown with different letters (P<0.01). For each group, significant differences between "before" and "after" exposure is shown as different symbols (P<0.01)
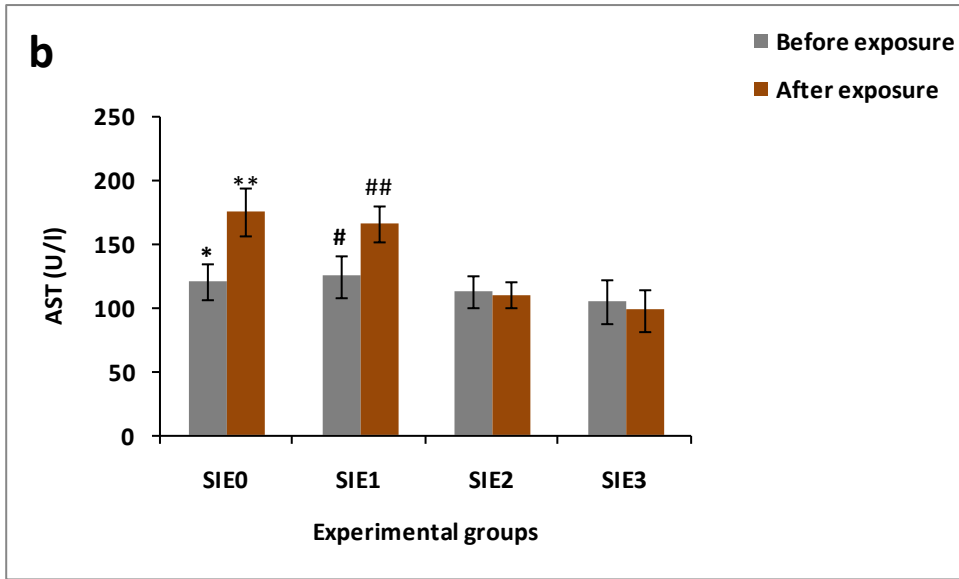
Figure 4. Effects of silymarin extract (SIE) supplementation on the liver antioxidant enzymes [SOD (superoxide dismutase) (plot a); GPx (glutathione peroxidase) (plot b); CAT (catalase) (plot c)] before and after exposure to cadmium chloride in common carp. SIE_0 (control): non-supplemented fish, SIE_1: 400 mg SIE/kg diet, SIE_2: 1400 mg SIE/kg diet, SIE_3: 2400 mg SIE/kg diet. Significant differences are shown with different letters (P<0.01). For each group, significant differences between "before" and "after" exposure is shown as different symbols (P<0.01)
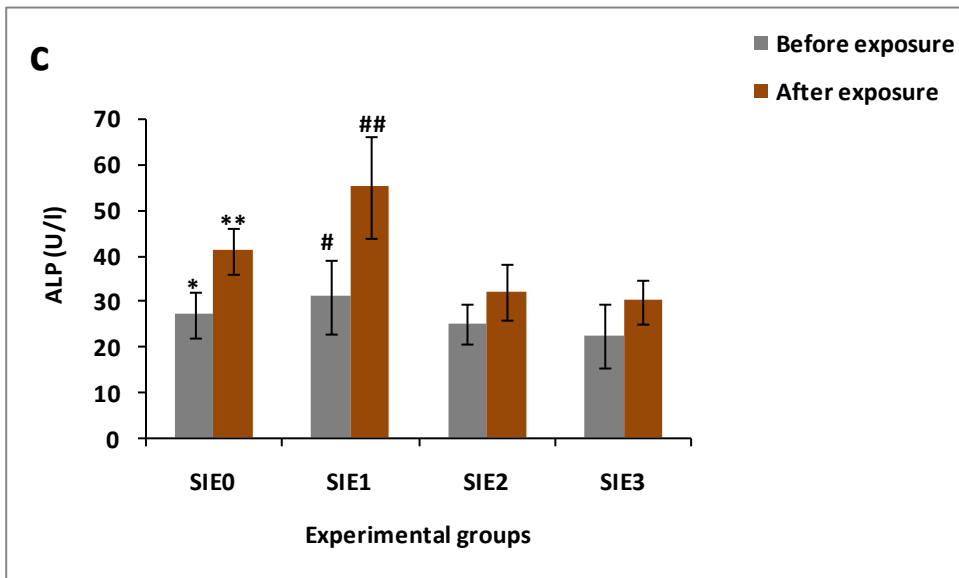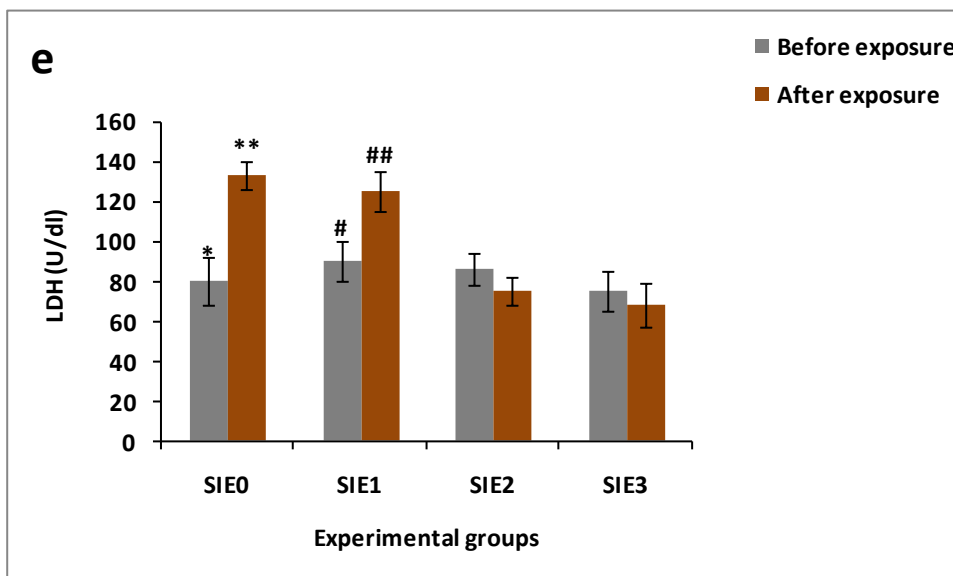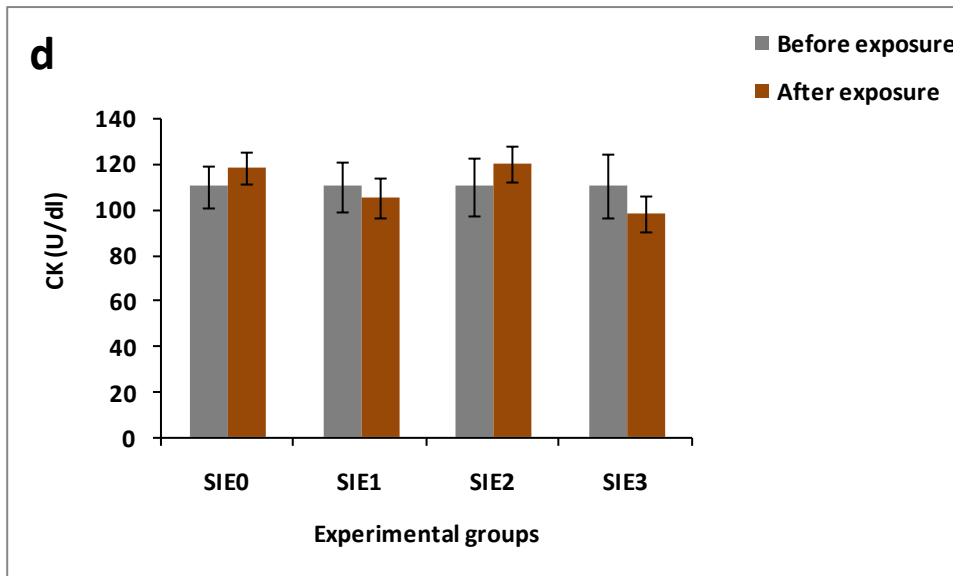
Figure 5. Effects of silymarin extract (SIE) supplementation on the plasma levels of hepatic metabolic enzymes [ALT (alanine transaminase) (plot a), AST (aspartate aminotransferase) (plot b), ALP (alkaline phosphatase) (plot c), CK (creatine kinase) (plot d), LDH (lactate dehydrogenase) (plot e)] before and after exposure to cadmium chloride in common carp. SIE_0 (control): non-supplemented fish, SIE_1: 400 mg SIE/kg diet, SIE_2: 1400 mg SIE/kg diet, SIE_3: 2400 mg SIE/kg diet. Significant differences are shown with different letters (P<0.01). For each group, significant differences between "before" and "after" exposure is shown as different symbols (P<0.01)
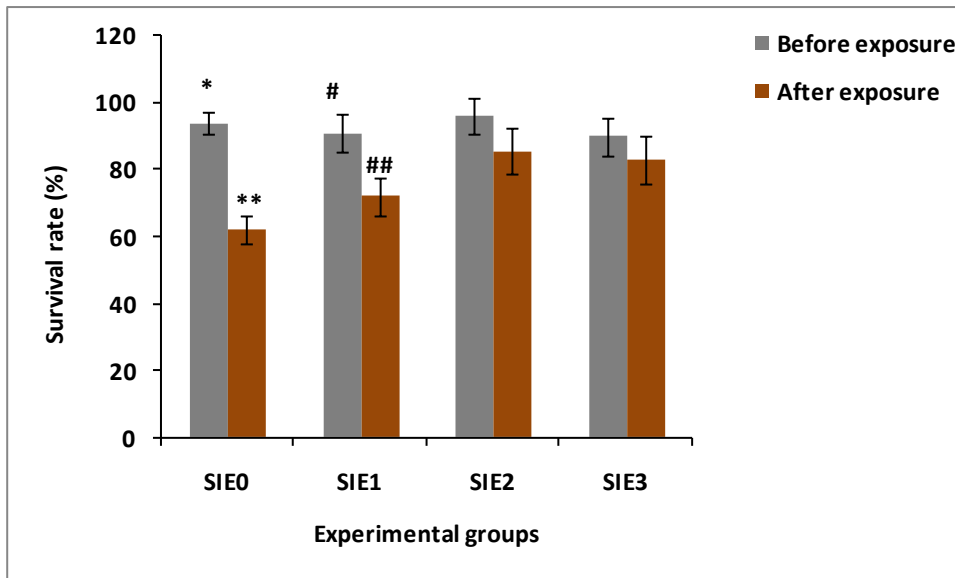
Figure 6. Effects of silymarin extract (SIE) supplementation on the survival rate before and after exposure to cadmium chloride in common carp. SIE_0 (control): non-supplemented fish, SIE_1: 400 mg SIE/kg diet, SIE_2: 1400 mg SIE/kg diet, SIE_3: 2400 mg SIE/kg diet. Significant differences are shown with different letters ($P<0.01$). For each group, significant differences between "before" and "after" exposure is shown as different symbols ($P<0.01$)

Table 1. The growth parameter changes of common carp during 60 days feeding with diet containing various levels of silymarin extract (SIE). SIE_0 (control): non-SIE-supplemented fish, SIE_1: 400 mg SIE/kg diet, SIE_2: 1400 mg SIE/kg diet, SIE_3: 2400 mg SIE/kg diet. Significant differences are shown with different letters ($P<0.01$)

Growth parameters	Treatments			
	SIE$_0$ (control)	SIE$_1$	SIE$_2$	SIE$_3$
Initial weight (g)	23.2 ± 4.5	21.8 ± 3.3	22.6 ± 4.8	24.3 ± 5.5
Final weight (g)	125.6 ± 10.2	130.9 ± 12.5	128.1 ± 15.5	130.5 ± 13.3
Final weight gain (%)	441.3 ± 126.6	500.4 ± 278.7	466.8 ± 222.9	437.1 ± 141.8
Specific growth rate	4.89 ± 1.02	5.21 ± 1.2	5.05 ± 1.06	4.81 ± 1.1
FCR	1.25 ± 0.06	1.21 ± 0.05	1.24 ± 0.07	1.22 ± 0.08