Alleviative effect of threonine on cadmium-induced acute liver injury in mice

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Abstract

As a toxic pollutant, cadmium (Cd) can cause severe liver injury. In the previous study, we found that threonine (Thr) significantly alleviated the toxic effect of Cd in yeast. To investigate the effect of Thr on Cd-induced liver damage in mice, twenty-four mice were evenly divided into control, Cd-exposed, and low/high dose of Thr-treatment groups (0.04, 0.08 mmol/kg/day) for 7 days of continuous treatment. Thr significantly reduced the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, exhibiting alleviative effect on hepatic injury. Further investigation showed that Thr significantly reduced the Cd-induced inflammation (tumor necrosis factor-α and interleukin-6), and inhibited the increase of apoptosis factors (Bax and Caspase-3). Thr also reduced liver malondialdehyde (MDA) and O$_2^-$ level, and restored superoxide dismutase (SOD) activity. In conclusion, Thr alleviated Cd-induced hepatic tissue and dysfunction through reducing the formation of free radicals O$_2^-$, lipid peroxidation, inflammation, and inhibiting the expression of Bax and Caspase-3.

Introduction

In recent years, excessive cadmium (Cd) in foods has been frequently reported, which seriously threatens human health [1]. Cd can be easily accumulated in the human body through the food chain due to its strong water solubility [2]. Consumption of Cd-contaminated foods will lead to metabolic disorders, oxidative damage, and apoptosis of cells, causing irreversible impairment to multiple organs and tissues of the human body [3, 4]. Among those organs, the liver is considered the major target organ that undergoes acute damage induced by Cd [5]. Therefore, the way to effectively prevent and potently reduce liver damage caused by Cd exposure has always been an urgent problem to be solved.

Threonine (Thr), an essential amino acid of the human body, has promising effects in alleviating fatty liver tissue damage and reducing liver fat accumulation [6]. Recent studies have shown that Thr dehydratase can effectively enhance the Cd tolerance of microorganisms [7]. The content of Thr was significantly reduced after Cd exposure [8]. Our previous study also showed that Thr could significantly alleviate the toxic effect of Cd on yeast, and reduce the membrane lipid damage and oxidative damage caused by Cd [9]. However, it is unknown whether Thr is effectual in alleviating Cd-induced liver injury. Hence, this study explored the alleviating effects of Thr on liver injury in Cd-exposed mice, which may provide new ideas for the prevention and treatment of Cd-induced liver tissue damage.

Materials And Methods

2.1 Reagents

Cadmium chloride (CdCl$_2$, CAS: 7790-78-5) was purchased from Xilong Scientific Co., Ltd. L-threonine (CAS#:72-19-5, A610919), Trizol reagent and Tissue or Cell Total Protein Extraction Kit (C510003-0050) were purchased from Sangon Biotech (Shanghai, China). SOD erythrocytes lyophilized powder (S36430-15 ku) was purchased from Acmec Biochemical (Shanghai, China). Superoxide Dismutase (SOD) Activity
Kit (BC0175) and Lipid peroxidation (malondialdehyde, MDA) Assay Kit (BC0025) were purchased from Solarbio Science Technology (Beijing, China). Aspartate aminotransferase (AST) Assay Kit and Alanine aminotransferase (ALT) Assay Kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). BCA Protein Assay Kit was purchased from Beyotime Biotechnology (Shanghai, China). Mouse interleukin 6 (IL-6) ELISA kit (MM-0163M2) and Mouse tumor necrosis factor α (TNF-α) ELISA kit (MM-0132M2) were purchased from Jiangsu Meimian industrial (Nanjing, China). PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, RR047A) was purchased from Takara Bio Inc (Japan). Strong RIPA Lysis Buffer for Cell Tissues Processing (G2002-100mL) and Cocktail Protease Inhibitor for Proteinic Integrity (G2006-250 μL) were purchased from Wuhan Servicebio Technology (Wuhan, China). Other reagents were purchased from Sangon Biotech (Shanghai, China).

2.2 Methods

2.2.1 Animals experiments

24 SPF male mice (25-35 g) were purchased from Tianqin Biotech (Changsha, China). These SPF mice were kept in plastic cages containing sawdust at 22-25 °C under a standard 12:12 h light/dark cycle with ad libitum access to standard feed and water. As previously described [10-12], after adapting for one week, mice were randomly divided into 4 groups: (1) control group, intraperitoneal injection of 0.9% NaCl once a day; (2) Cd-exposed group, intraperitoneal injection of 5 mg/kg/day CdCl₂; (3) Cd+L-Thr group, of intraperitoneal injection of 5 mg/kg/day CdCl₂ and oral administration of 0.04 mmol/g/day of Thr; (4) Cd+H-Thr group, intraperitoneal injection of 5 mg/kg/day CdCl₂ and oral administration of 0.08 mmol/kg/day. Mice were continuously treated for 7 days, and then sacrificed. Mice livers were collected and stored at -80 °C. All protocols were subject to the operating standard approved by the Laboratory Animal Ethics Committee of Guangdong Ocean University (approval number: GDOU-LAE-2020-009).

2.2.2 Blood sampling

Blood was sampled for the subsequent tests as previously reported [13]. Mice were given free access to water and blood samples were collected from their eyeballs. Blood samples were then centrifuged at 3000 rpm for 15 min. Serum was collected and stored at 4 °C.

2.2.3 Determination of liver oxidative stress

Determination of MDA, O²⁻ and SOD were performed as previously described [14]. 0.2 g of liver tissue was rinsed with 4 °C salines. 0.5 mol/L phosphate buffer (pH=7.4) at a ratio of 1:9 was added into the prepared liver tissues, and then the tissues were homogenized on the iced box to generate 10% tissue homogenate. Homogenate was centrifuged at 10000 rpm for 10 min at 4 °C to collect its supematant for determining the levels of MDA, O²⁻ and SOD according to the instructions.

2.2.4 Determination of serum AST and ALT
Determination of serum AST and ALT were performed as previously described [15]. AST and ALT levels of the serum samples were determined according to the instructions of kits with Varioskan Flash Multiplate Reader (Thermo Fisher, MA, USA).

2.2.5 Histopathological observation

The liver tissues were fixed with 4% paraformaldehyde universality fixative and dehydrated with absolute alcohol. Then the tissues were embedded with paraffin and sectioned. After dewaxing with xylene for 15 min, and hydrating with graded alcohol, the tissues were routinely stained with hematoxylin and eosin staining (H&E) according to the method described [13]. Liver tissues were observed with a DMI4000B inverted fluorescence microscope at 100× magnification.

2.2.6 Evaluation of liver inflammation

IL-6 and TNF-α of the liver tissues were evaluated via enzyme-linked immunosorbent assay (ELISA). Liver inflammatory levels of mice injected with Cd and orally administrated with Thr were determined. Liver tissues were homogenized with lysis buffer. The homogenate was centrifuged at 10000 rpm for 10 min at 4°C. The total protein concentration was determined using a BCA protein detection kit. The contents of IL-1β and IL-6 were quantified according to the kit instructions.

2.2.7 Quantitative real-time PCR (qRT-PCR)

Total RNA from liver tissues was extracted with Trizol. First-strand cDNA was synthesized using the reverse transcription kit StarScript II according to the kit instructions. qRT-PCR was performed on the cDNA samples using CFX96 Touch Real-Time PCR Detection System. The following primer sequences were designed according to references [16, 17]. The PCR procedures were performed with the primers listed in Table 1. The levels of gene expression were analyzed with $2^{-\Delta\Delta Ct}$ method. All experiments were performed in triplicate.

2.2.8 Western Blot analysis

The liver tissues were lysed with RIPA lysis buffer supplemented with protease inhibitors, and then subjected to ultrasonic homogenization [18]. Total protein of the liver tissues was extracted with an animal total protein extraction kit (Servicebio technology, Wuhan, China). BCA kit was applied for the measurement of protein concentration. The extracted proteins were transferred to polyvinylidene fluoride (PVDF) membranes and blocked with 5% skim milk (0.5% TBST) for 1 h. Then, PDVF membranes were incubated with primary mouse antibodies against Bax (1:1000,60267-1-Ig, Proteintech) and Caspase-3 (1:1000,66470-2-Ig, Proteintech) at 4°C overnight, and subsequently washed three times with TBST on a destaining shaker at room temperature. After washing, HRP-conjugated GAPDH Monoclonal antibody (1:3000, HRP-60004, Proteintech) was incubated at room temperature for 2 h. Finally, the developing and fixing reagent is used to develop and fix. The optical density value of the target band was analyzed via the Alpha software processing system.
2.2.9 Determination of the secondary structure of SOD with Circular dichroism spectroscopy

100 mmol/L of Thr and SOD were prepared for Circular dichroism spectroscopy (CD). SOD enzyme was thawed on ice and then diluted to 100 mmol/L. The diluted SOD enzymes were treated with Cd or Cd+Thr, respectively. Then the secondary structure of the protein was determined by CD. Scanning parameters were set as follows: slit width 1 nm, reaction time 5 s, wavelength scanning range 190-260 nm, and scanning speed 5 nm/s. Phosphate buffer acted as the reference to correct the absorption background. Thus, the change in the CD value was observed in the reaction system, and the content of α-helix, β-sheet, and random coil in the samples were quantitatively calculated.

Statistical methods

Statistically significant differences between groups were calculated with JMP Pro 13. Graphs were plotted with GraphPad Prism (version 8.0, USA). The data were expressed as mean ± standard deviation, and the comparison between groups was performed by analysis of variance. *p*<0.05 was considered statistically significant.

Results

3.1 Thr alleviated Cd-induced hepatic dysfunction and damage

Serum AST and ALT activities are sensitive biomarkers of liver injury [19]. Compared to control, the serum AST and ALT activities in the Cd group increased 66.12% and 81.59%, respectively, while AST and ALT activities of mice in the Thr-treated group decreased to varying degrees (Fig. 1 A, B). AST level of Cd+L-Thr treated mice failed to exhibit a significant decrease but the ALT significantly decreased by 16.31% (Fig. 1 A, B). AST and ALT levels of mice in the Cd+H-Thr group declined by 19.16% and 20.65%, respectively, indicating that Thr attenuated the hepatic dysfunction (AST and ALT) in Cd-exposed mice (Fig. 1 A, B). The results of the histological analysis showed that Cd exposure caused obvious pathological changes in liver tissue, including the irregular arrangement of hepatic cords, cytoplasmic vacuolization, increasing hepatic steatosis, band-shaped necrosis, and infiltration of inflammatory cells (Fig. 1 C). In the Thr-treated group, the pathological changes in the liver tissue of the mice were observed with intact liver cord structure, the declining hepatic steatosis, and zonal necrosis, as well as the relieved inflammatory cell infiltration (Fig. 1 C). These results further showed the alleviating effect of Thr on liver injury in Cd-exposed mice.

3.2 The effects of Thr on TNF-α and IL-6 in Cd-exposed mice

TNF-α is an important biological marker in the substantial hepatic tissue damage [20]. IL-6 is a stimulator of the hepatic synthesis of acute-phase proteins in response to trauma, and is also an important marker of hepatic inflammation [21]. Compared to control, the TNF-α and IL-6 levels in the Cd group increased by 35.10% and 49.16% respectively (*p*< 0.05) (Fig. 2 A, B). Compared to the Cd group, the TNF-α and IL-6 levels of the Cd+L-Thr group decreased by 22.25% and 27.96% respectively, while the TNF-α and IL-6 levels...
levels of the Cd+H-Thr group decreased by 24.36% and 29.93% respectively (Fig. 2 A, B). Results from qRT-PCR exhibited that the expression of TNF-α and IL-6 in the Cd group increased by 30.46% and 37.57% respectively when compared to the control group (Fig. 2 C). Compared with the Cd group, the expression levels of IL-6 and TNF-α in the L-Thr group decreased by 18.09% and 22.81% respectively, while the expression levels of IL-6 and TNF-α in the Cd+H-Thr group decreased by 18.28% and 24.57%, respectively (Fig. 2 C). These results revealed the alleviative effect of Thr on hepatic inflammation in Cd-exposed mice.

3.3 Thr dampened the increase of Caspase-3 and Bax induced by Cd

Hepatic apoptosis is closely related to hepatic injury and inflammation. The apoptosis pathway can be activated when cells are stimulated by Cd or other stress [22]. The apoptosis-related mitochondrial Bcl2-associated protein X (Bax) and Caspase-3 are important markers in the process of apoptosis. Under the stimulation of oxidative stress caused by heavy metals, the level of Bax in liver cells increased, then activated and upregulated downstream Caspase-3, and eventually, induced apoptosis [23]. The western blot assay showed that Caspase-3 and Bax protein levels in the Cd group increased by 33.11% and 135.74%, respectively (Fig. 3 B, C). Compared to the Cd group, the Caspase-3 and Bax protein levels in the Cd+L-Thr group decreased by 28.06% and 42.20% respectively, and the Cd+H-Thr group decreased by 28.86% and 46.51%, respectively (Fig. 3 B, C). Compared with the control group, the mRNA expression levels of Bax and Caspase-3 in the Cd group were significantly increased (p < 0.01) (Fig. 3 D).

Furthermore, the treatment of Thr inhibited Cd-induced expression of Bax and Caspase-3 compared with Cd group (Fig. 3). The results suggested an obvious alleviating effect of Thr on Cd-induced apoptosis.

3.4 Thr alleviated hepatic oxidative damage induced by Cd

Oxidative damage is a pivotal part of toxicity induced by Cd, and also a key stimulator for the Caspase-3 and Bax [24, 25]. Oxidative damage assays of hepatic cells showed that, compared to the control group, hepatic MDA and O$_2^-$ levels in the Cd group rose by 66.22% and 71.42%, respectively (Fig. 4 A, B). Decreasing rates of MDA and O$_2^-$ levels in the Cd+L-Thr group were 28.76% and 19.08% respectively, while the decreasing rates of the Cd+H-Thr group were 35.32% and 22.59%, respectively (Fig. 4 A, B). The results above indicated an obvious alleviative effect on Cd-induced oxidative damage.

The antioxidase SOD activity of the Cd group decreased by 35.22% compared to the control group (Fig. 4 C). Compared to the Cd group, the Cd+L-Thr group exhibited an increase in SOD activities by 35.94% while the SOD activities of the Cd+H-Thr group increased by 20.09% (Fig. 4 C). All in all, Thr enhanced the hepatic SOD activity in Cd-exposed mice.

3.5 Thr protected SOD secondary structure
Circular dichroism (CD) has unique advantages in studying protein conformation, accompanied by obtaining protein conformational change information and quantitatively calculating α-helix, β-turn, and random coil content [26]. To investigate the protective mechanism of Thr on SOD against Cd in vitro, the secondary structure of bovine SOD protein (98% similar to mouse SOD) before and after treated with Cd and Thr was analyzed using CD spectroscopy. Cd exposure alters the CD spectrum of SOD protein. Significant differences could be easily observed between the treatment of SOD with Cd and the cotreatment with Cd and Thr. H-Thr significantly increased the intensity of positive ellipticity bands (Fig. 5). The distributions of α-helix, β-antiparallel, β-paralled, β-turn, and random coil ratio of the protein secondary structure were shown in Table 2. Under the treatment of Cd, the proportion of α-helix in animal proteins decreased by 14.5%, and the proportion of β-antiparallel and random coil increased by 6.4% and 6.2%, respectively, when compared to the control (untreated SOD) (Table 2). Compared to the Cd treatment, Thr restored the proportions of α-helix and β-antiparallel of SOD, which exhibited great resemblance to that of the control. Therefore, Thr conceivably protected SOD from Cd intercalation, and maintained the SOD secondary structure.

Discussion

Hepatic injury is one of the symptoms of acute Cd poisoning [27]. Oxidative damage and cell apoptosis are two important origins of Cd-induced hepatic injury and dysfunction. In this study, we found that Thr was able to reduce Cd-induced hepatic injury through effectively decreasing the generation of oxygen free radical cell apoptosis and inflammation.

Thr exhibited significantly inhibitory effects on the liver function index, serum AST and ALT. Compared to the Cd group, both L-Thr and H-Thr groups showed a significant decline in serum AST and ALT (p < 0.05), which exhibited a dose-dependent effect. The results of serum AST and ALT assays indicated that Thr had significantly improved effects on Cd-induced liver dysfunction. ALT and AST are released into the blood circulation during hepatocyte damage [28, 29]. Hwang and Wang (30) found that taurine could also alleviate the Cd-induced increased serum AST and ALT level. Dong, Jiang (31) reported that ALT and AST notably decreased in the serum of juvenile grass carp when they were fed with a Thr diet. Hepatic histopathological changes manifested that acute Cd exposure resulted in massive contraction band necrosis-like necrosis and inflammatory cell infiltration in liver tissue. With the addition of Thr, liver tissue necrosis and inflammatory infiltration were significantly reduced. Gonzalez, van Liempd (32) found that serum Thr increased in acute drug-induced liver injury. Nonetheless, excessive intake of Thr did not cause damage to chicken liver tissue but significantly reduced tetrachloromethane-induced rat hepatic injury and cirrhosis [33, 34]. These findings suggested that Thr was able to alleviate the hepatic injury and improve the hepatic dysfunction in Cd-exposed mice.

As one of the target organs attacked by Cd, the liver plays an important role in the metabolism of Cd [35]. Tissue inflammation and apoptosis are two important biomarkers in the process of liver injury in Cd-exposed mice [27]. Reducing Cd-induced apoptosis is also considered to be one of the important ways to prevent and cure Cd-induced liver and kidney injury [36]. TNF-α and IL-6 are two key inflammatory
mediators of tissue injury-induced inflammatory response [37]. This study showed that the level of TNF-α in Cd-exposed mice was significantly increased, which might be related to the expression and release of TNF-α induced by liver tissue injury [20]. The results showed that the level of TNF-α was mainly increased under Cd exposure and inflammation was detected by inducing the expression of TNF-α. Dong, Simeonova (38) found that the Cd-induced expression of TNF-α was associated with the oxidative stress induced by Cd. IL-6, one of the secondary inflammatory mediators, was also significantly increased in Cd-exposed mice. The results suggested that Cd-induced liver injury was accompanied by an inflammatory response, and its mechanism was also related to oxidative stress and apoptosis factors [39]. In this study, Thr could effectively reduce the levels of inflammatory factors TNF-α and IL-6 in Cd-exposed mice (24.36% and 29.93%, respectively, \( p < 0.05 \)). Chen, Zhang (40) found that daily supplementation of 25 mM/g of Thr could effectively reduce the expression of TNF-α (15.87%, \( p < 0.05 \)) in the treatment of lipopolysaccharide-induced intestinal inflammation in chickens, which might be attributed to the reduction of intestinal injury by Thr. The results of apoptosis factors showed that the levels of apoptosis factors Caspase-3 and Bax in livers of Cd-exposed mice were significantly increased (33.11% and 135.74%, \( p < 0.05 \)). Yuan, Dai (41) detected the elevation of Caspase-3 and Bax in the liver and kidney tissues in the sub-chronic lead and Cd-exposed mice, and the reason for the elevation might be related to mitochondrial damage. In this study, Thr showed a prominent inhibitory effect on the elevation of Caspase-3 in Cd-exposed mice. More importantly, Thr also exhibited a significant inhibitory effect on Bax factor, which was located in the upstream of Caspase-3 in apoptosis pathway. Baird, Niederlechner (42) found that Thr decreased apoptosis in heat-stressed intestinal epithelial cells, and significantly inhibited the elevation of Caspase-3. It was found that Thr deficiency would aggravate cell apoptosis in gill of grass carp infected with \textit{Flavobacterium columnarum}, while additional Thr supplementation could significantly hamper the elevation of Bax expression [43]. These evidences suggest that the alleviative effects of Thr on Cd-induced liver injury may be related to the prevention of apoptosis.

Reactive oxygen species (ROS) are the key mediator of Cd-induced oxidative damage and activation of Bax and Caspase-3 pathways in apoptosis and inflammation [24, 25]. Liver injury in Cd-exposed mice usually involved lipid peroxidation of cell membranes [44]. In this study, the levels of \( \ce{O2^-} \) and MDA in liver tissue significantly increased in Cd-exposed mice (respectively 66.22% and 71.42%, \( p < 0.05 \)). Thr exhibited significantly declining effects on \( \ce{O2^-} \) and MDA generation in Cd-exposed mice. Thr also showed alleviative effects on \( \ce{O2^-} \) and MDA of Cd-exposed yeast, and the mechanism may be related to the protection of the SOD enzyme from Cd damage [9]. Ross-Inta, Zhang (45) found that Thr deficiency led to the imbalance of mitochondrial respiration and capacity metabolism in hepatocytes. Moreover, the imbalance of mitochondrial energy metabolism led to the formation of oxygen free radicals [46]. Azzam, Dong (47) found that although Thr did not affect serum MDA in laying hens under high temperature and humidity stress, it significantly enhanced plasma SOD activity. However, Chen, Cheng (48) found that 25 mM/g of Thr reduced the serum MDA content in chicken. Cd mainly inhibits the activity of antioxidant enzymes such as SOD by replacing the coenzyme factor on the antioxidant enzyme to generate \( \ce{O2^-} \) and finally causes oxidative damage. The results of SOD assays showed that the hepatic SOD activity significantly decreased in Cd-exposed mice (\( p < 0.05 \)). Sun, Cui (49) found that CdTe quantum dots also
significantly inhibited SOD activity in mice. Thr can effectively restore the activity of antioxidant enzymes such as SOD in Cd-exposed HEK293T cells [50]. Huang, Fang (9) reported that Thr may protect SOD by directly blocking Cd binding to SOD. In this study, Thr protected the α-helix and β-antiparallel structures of SOD protein against Cd, and thereby effectively reduced the adverse effects of Cd on the secondary structure of SOD and maintained the activity of SOD. Polykretis, Cencetti (50) reported that Cd caused the formation of intra-unit disulfide bond between CYS57 and CYS146 in SOD protein. Thr may hinder the formation of the disulfide bond. However, the mechanism of Cd-induced liver injury and oxidative damage is very complex, which makes it difficult to fully and deeply reveal the mechanism of Thr for alleviating Cd-induced liver injury. Although additional supplementation of Thr can alleviate Cd-induced liver injury and oxidative damage to a certain extent, it is still not fully understood whether excessive Thr will have adverse effects. For instance, 2–4% of excessive Thr in mice feed ended up in a decline of weight gains or food intake [51]. Therefore, the molecular mechanism of alleviative effects of Thr on Cd toxicity needs to be elucidated via a comprehensive evaluation of the interaction among Thr, Cd, and key factors in Cd-induced liver injury-related pathways in follow-up studies.

Conclusion

This study investigated the alleviative effect of Thr on Cd-induced acute liver injury in mice. We found that Thr may alleviate Cd-induced hepatic tissue damage and dysfunction through reducing the formation of free radicals O$_2^-$, lipid peroxidation, inflammation (TNF-α and IL-6), and inhibiting the elevation of apoptosis factors (Bax and Caspase-3) in Cd-exposed mice. Moreover, Thr exhibited significant protective effects on the secondary structure of the SOD enzyme against Cd in vitro, which effectively reduced the inhibitory effect of Cd on the activities of SOD.

Declarations

Funding

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CRediT author statement


Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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https://doi.org/https://doi.org/10.1016/j.redox.2019.101102


Tables

**Table 1** Primer sequences of genes analyzed in qRT-PCR.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward Primer (5´-3´)</th>
<th>Reverse Primer (5´-3´)</th>
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<tr>
<td>IL-6</td>
<td>TCCAGTTGCCCTCTTGAGGAC</td>
<td>AGTCTCTCTTCCGGACTTGT</td>
</tr>
<tr>
<td>TNF-α</td>
<td>ACTGAACTCTGCGGTCTTGGT</td>
<td>GCTTTGGTGTTTGTACGAC</td>
</tr>
<tr>
<td>Bax</td>
<td>CTGAGCTCTTTGGAGC</td>
<td>GACTCCAGCCACAAGAGATG</td>
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<tr>
<td>Caspase-3</td>
<td>GAGCTTGGAAACGGTACAGCTA</td>
<td>CCGTACAGACGGAGATGAC</td>
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<tr>
<td>GAPDH</td>
<td>TCACACCATGGAGAAGGC</td>
<td>GCTAAGCAGTTGGTGGTGA</td>
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**Table 2** The proportion distribution of α-helix, β-parallel and random coils in the secondary structure of SOD protein.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>α-helix (%)</th>
<th>β-antiparallel (%)</th>
<th>β-parallel (%)</th>
<th>β-turn (%)</th>
<th>Random coil (%)</th>
<th>Total (%)</th>
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<td>control</td>
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<td>5.5</td>
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<td>24.4</td>
<td>104.9</td>
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<tr>
<td>Cd</td>
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<td>5.0</td>
<td>20.0</td>
<td>30.6</td>
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<tr>
<td>Cd+L-Thr</td>
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<td>6.6</td>
<td>19.4</td>
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<tr>
<td>Cd+H-Thr</td>
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<td>16.4</td>
<td>7.6</td>
<td>18.3</td>
<td>31.0</td>
<td>102.1</td>
</tr>
</tbody>
</table>

Figures
Figure 1

Effects of Thr on liver function and hepatic histopathological changes in Cd-exposed mice (A) Serum AST activity. (B) Serum ALT activity. (C) Micrograph of liver tissue section. (Scale bar: 100 μm.)
Quantitative data are the mean ± SEM. *p < 0.05 and **p < 0.01 versus control group. # p < 0.05 and ## p < 0.01 versus Cd group.
Figure 2

Effects of Thr on the expression of apoptosis factors TNF-α and IL-6 in Cd-exposed mice. (A) IL-6 level. (B) TNF-α level. (C) the relative mRNA expression levels of IL-6 and TNF-α by qRT-PCR. *$p < 0.05$ and **$p < 0.01$ versus control group. #$p < 0.05$ and ##$p < 0.01$ versus Cd group.
Figure 3

Effects of Thr on the expression of apoptosis factors Bax and Caspase-3 in Cd-exposed mice. (A) Analysis of the effects of Thr on the expression of apoptosis factors Bax and Caspase-3 in Cd-exposed mice using Western blot. (B) The quantitative densitometric analysis of Bax. (C) The quantitative densitometric analysis of Caspase-3. (D) Relative mRNA expression of Bax and Caspase-3. Quantitative
data are the mean ± SEM and *p < 0.05 and **p < 0.01 versus control group. # p < 0.05 and ##p < 0.01 versus Cd group.

Figure 4

Effects of Thr on the Cd-induced hepatic oxidative damage levels in mice. (A) MDA level. (B) O$_2^-$ level. (C) SOD activity. *p < 0.05 and **p < 0.01 versus control group. # p < 0.05 and ##p < 0.01 versus Cd group.
Figure 5

Protective effect of Thr on the CD spectra of SOD protein *in vitro.*