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Toxicology

Protective effects of Ganoderma triterpenoids on cadmium-induced oxidative stress and inflammatory injury in chicken livers

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ABSTRACT

Several studies have been conducted on liver damage caused by cadmium, but few on the protective effects of Ganoderma triterpenoids against liver damage due to cadmium. This experiment was designed to evaluate the protective effects of Ganoderma triterpenoids on the liver damage induced by cadmium in chickens. Eighty healthy seven-day-old Hyline male egg-laying chickens were randomly divided into four groups with 20 chickens in each group. All the experiments were carried out in triplicate. The control group (K group) was fed a basal diet, the Cadmium group (Cd group) was fed a basal diet with 140 mg/kg of $CdCl_2$, the Ganoderma triterpenoids treatment group (Cd + GT group) was fed with a full-fodder diet containing 140 mg/kg of $CdCl_2$ and 0.5 mL of Ganoderma triterpenoids solution (20 mg/mL), and the Ganoderma triterpenoids group (GT group) was fed a basal diet and 0.5 mL of Ganoderma triterpenoids solution (20 mg/mL). At the 20th, 40th, and 60th days, fifteen chickens were randomly selected for euthanasia in each group. Livers were quickly removed and stored on ice. Some indicators, such as the cadmium content in the liver, antioxidant enzymes (superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)) activities, malondialdehyde (MDA) content, and inflammatory factors (Tumor necrosis factor alpha (TNF-α), interleukin (IL-1β and IL-6)), heat shock protein (HSP27, 40, 60, 70, and 90) mRNA levels, and protein levels of heat shock proteins (HSP60, 70, and 90) were detected, and chicken liver pathology was taken for each group every 60 days. The results showed that cadmium exposure caused accumulation of cadmium in liver tissue, inhibited antioxidant enzyme activity, and increased MDA content, inflammatory cytokines (TNF-α IL-1β and IL-6), and heat shock protein (HSP27, 40, 60, 70, and 90) mRNA levels, and heat shock protein (HSP60, 70, and 90) levels, with severe tissue damage and inflammatory infiltrates. Ganoderma triterpenoids not only reduced the accumulation of cadmium in the chicken liver, but also significantly increased the activities of antioxidant enzymes which is inhibited by cadmium, reduced the content of MDA, mRNA expressions of inflammatory cytokines (TNF-α IL-1β and IL-6), and heat shock proteins (HSP27, 40, 60, 70, and 90), and protein levels of heat shock proteins (HSP60, 70, and 90). Simultaneously, pathological tissue sections showed that the pathological damage of the liver tissue was significantly reduced. The results showed that Ganoderma triterpenoids can significantly reduce the accumulation of cadmium in the liver of chicken, thereby reducing oxidative stress and inflammation.

1. Introduction

Cadmium is a common toxic metal found in both air and soil. Due to its long half-time, cadmium has the potential to accumulate within the body also under conditions of minor constant exposure. Exposure pathways are through the food chain and tobacco fume [1,2]. Studies have pointed out that cadmium can cause certain damage to the kidney [3], testis [4], spleen [5], liver [6], and other tissues and organs. The liver is an important organ for the storage of cadmium in the body. Currently, the popular research focus has been on the toxicity of cadmium in the cadmium-induced oxidative damage [7,8], inflammation [9], and morphologic damage [10,11].

Ganoderma lucidum, belonging to the genus *Ganoderma lucidum fungus*, has been proposed to promote health and longevity in China and other Asian countries [12]. Previous studies have confirmed the effectiveness of Ganoderma lucidum in the treatment of chronic liver

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disease, hypertension, and hyperglycemia [13]. The body's antioxidant capacity and anti-inflammatory activity could also be enhanced by Ganoderma lucidum [14]. Ganoderma is rich in a variety of biologically active compounds, such as triterpenoids, polysaccharides, organic acids, alkaloids, furans and sterols [15], its triterpenoids were first isolated in 1982 [16]. The basic structure of Ganoderma triterpenoids is lanosterol. Research on the biological active ingredients has shown that Ganoderma triterpenoids have multiple functions, which leads to the extensive studying enclosed its features, including anti-tumor, antioxidant, and sedative properties. Enhancing learning and memory functions and anti-inflammatory activity based on Ganoderma triterpenoids has also been studied by various academic experiments [17–19].

In summary, the liver is a target organ for cadmium poisoning, and no studies have been conducted on the effect of Ganoderma triterpenoids on liver damage in cadmium poisoning animals. In this experiment, chicken were used to establish a chicken cadmium poisoning model with the administration of Ganoderma triterpenoids, through detecting cadmium content of chicken liver, changes of oxidation index, levels of inflammatory cytokines, heat shock proteins, and histopathology checking to evaluate the effect of Ganoderma triterpenoids on cadmium-induced liver toxicity injury.

2. Materials and methods

All procedures used in the current study were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University in China. The methods were carried out in accordance with the approved guidelines [20].

2.1. Extraction and determination of Ganoderma triterpenoids

The dried Ganoderma lucidum was pulverized by a Chinese medicine grinder into a powder. It was then separated and purified to obtain triterpenoid crude extract of Ganoderma lucidum by thin layer chromatography (Qingdao Ocean Chemical Plant, Qingdao, China) and preparative high-performance liquid chromatography (Waters Corporation, Shanghai, China). With oleanolic acid (Aladdin Reagent Company, Shanghai, China) as the standard, the content of Ganoderma triterpenoids in the extract was calculated [21].

2.2. Experimental animals and design

80 seven-day-old Hyline male laying chickens were randomly divided into four groups of 20 each. A total of three parallel experiments were carried out. The control group (K group) was fed a basal diet, the Cadmium group (Cd group) was fed a basal diet with 140 mg/kg of $CdCl_2$, the Ganoderma triterpenoids treatment group (Cd + GT group) was fed a full-fodder diet containing 140 mg/kg of $CdCl_2$ and 0.5 mL of Ganoderma triterpenoids solution (20 mg/mL), and the Ganoderma triterpenoids group (GT group) was fed by a basal diet and 0.5 mL of Ganoderma triterpenoids solution (20 mg/mL). The Cd level within our Ganoderma triterpenoids solution felt below the lower limit of detection (LOD: 0.001 μg/kg). The concentration of cadmium in the feed is 0.8 μg/kg in the both K and GT group, while the other two groups, Cd and Cd + GT group, 140 mg/kg of cadmium is included through the detection. Composition and nutritional level of basic diet shows in Table 1 and Table 2. All Ganoderma triterpenoids solution are orally administered to each chicken to control the ingestion dose to ensure accurate experiments. At the 20th, 40th, and 60th days, fifteen chickens randomly selected in each group were euthanized. Chickens' livers were collected. After removing the gallbladder under aseptic conditions, we separated into five parts of tissue with scissors on each liver. Tissue was divided into two parts, one for detecting cadmium content, antioxidant enzymes, levels of inflammatory cytokines and HSPs, the other part was fixed in paraformaldehyde solution for histopathological observation.

Table 1
Composition of the experimental diets (%).

Ingredient	K Contents	Cd	Cd + GT	GT
$CdCl_2$	0	0.014	0.014	0
Corn	63.69	63.68	63.68	63.69
Soybean meal	14.35	14.35	14.35	14.35
Sunflowers meal	4.09	4.09	4.09	4.09
Corn gluten meal	4.08	4.08	4.08	4.08
Soybean oil	2.73	2.73	2.73	2.73
DL-Methionine (98%)	0.17	0.17	0.17	0.17
L-Lysine HCl (78%)	0.04	0.04	0.04	0.04
Limestone	9.0	9.0	9.0	9.0
$CaHPO_4$	1.0	1.0	1.0	1.0
Premix[a]	0.5	0.5	0.5	0.5
NaCl	0.3	0.3	0.3	0.3
Choline	0.05	0.05	0.05	0.05
Total	100	100	100	100

[a] The premix provided the following per kg of diets: VA 12,500 IU, Cholecalciferol, 4125 IU, VE 15 IU,VK 2 mg, thiamine 1 mg, riboflavin 8.5 mg, calcium pantothenate 50 mg, nicotinic acid 32.5 mg, pyridoxine 8 mg, VB12 5 mg, biotin 2 mg, Fe(as ferrous sulfate) 60 mg, Cu(as copper sulfate) 8 mg, Zn (as zinc sulfate) 66 mg, Mn 65 mg, Se 0.3 mg, I 1 mg.

2.3. The content of Cd in the liver

Try to ensure that each liver is separated in the same way. After pretreatment, ICP-MS (inductively coupled plasma mass spectrometry, Agilent 7800) was conducted on a 0.5 g segment of liver tissue. This test was carried out by HARBIN ENTRY-EXIT INSPECTION AND QUARANTIN (Harbin, China) according to the testing standards. See Table 3 for detection conditions.

2.4. Measurement of antioxidant status

The liver sample was milled in the casing triturated under ice-cooling, homogenized with saline, centrifuged at 3000 rpm after 10 min, and the supernatant. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) activity, and malondialdehyde (MDA) content were measured. The experiment was performed strictly in accordance with the kit instructions, and the OD value was measured. The three kits above were purchased from Nanjing Jiancheng Reagent Company (Nanjing Jiancheng Bioengineering Institute, Nanjing, China)

2.5. Inflammatory cytokines and HSPs mRNA expressions analysis

Total RNA was extracted from 0.1 g of liver tissue according to kit instructions (Bioteke, Beijing, China). The RNA concentration was then detected and reversely transcribed into cDNA (Takara Bio, Beijing, China). The purity and integrity of total RNA were detected by conventional agarose gel electrophoresis and nucleic acid protein ultraviolet analyser. The results showed clear bands of 28 s, 18 s and 5 s and the RNA OD_{260}/OD_{280} value was 1.99, which indicated that the total RNA integrity and quality were good, and the main genetic information was retained. The synthesized cDNA was stored in a refrigerator at −80 °C. The chβ-actin was used as the internal reference, and inflammatory factors (chicken (ch)TNF-α, chIL-1β and chIL-6) were detected by real-time quantitative polymerase chain reaction (PCR) and heat shock protein (HSP27, 40, 60, 70, and 90) mRNA expressions changes. Primers were designed using the design of previous studies in our laboratory. Primers are shown in Table 4 [20]. The synthesis of primers was completed by Sangon Biotech (Sangon Biotech Co., Ltd., Shanghai, China) The reaction system was 20 μL and the amplification experiment was performed using a Roche 480 qPCR instrument. The reaction conditions were: pre-denaturation at 95 °C for 10 min; 95 °C for 15 s, 60 °C for 1 min, 40 cycles; and finally the melt reaction. Three replicate wells were used for each sample, and β-actin was used as an

Table 2
Nutritional levels of the experimental diets.

Nutrient level	K Contents	Cd	Cd + GT	GT
CP	16.52 ± 0.08	16.52 ± 0.12	16.52 ± 0.09	16.52 ± 0.51
ME (MJ/kg)	11.30	11.30	11.30	11.30
Ca	3.08 ± 0.028	3.08 ± 0.009	3.08 ± 0.005	3.09 ± 0.008
TP	0.41	0.41	0.41	0.41
Methionine	0.45 ± 0.016	0.45 ± 0.006	0.45 ± 0.015	0.45 ± 0.006
Lysine	1.03 ± 0.013	1.03 ± 0.006	1.03 ± 0.013	1.03 ± 0.011
Methionine + cysteine	0.78 ± 0.014	0.79 ± 0.008	0.78 ± 0.007	0.78 ± 0.008

Data of nutrients were analyzed value contained except ME and TP. The value of the three batches of feed is expressed as mean ± SD.

Table 3
ICP-MS operating conditions.

Parameter	Cd
Nebulizer gas flow ($L\,min^{-1}$)	0.96
Auxiliary gas flow ($L\,min^{-1}$)	1.4
Plasma gas flow ($L\,min^{-1}$)	18
ICP RF power	1400
Sweeps/reading	30
Readings/replicate	1

Table 4
Gene-special primers used for qRT-PCR.

Gene	Primer (5'-3')	Accession number
TNF-α	Forward: 5′-CAGATGGGAAGGGAATGAAC-3′ Reverse: 5′-AGAGCATCAACGCAAAAGGG-3′	NM_204267.1
IL-6	Forward: 5′-ATGGTGATAAATCCCGATGAAG-3′ Reverse: 5′-CCTCACGGTCTTCTCCATAAAC-3′	NM_204628.1
IL-1β	Forward: 5′-TTCCGCTACACCCGCTCACAGT-3′ Reverse: 5′-CCGCTCATCACACACGACAT-3′	NM_000576.2
β-actin	Forward: 5′-ATTGCTGCGCTCGTTGTT-3′ Reverse: 5′-CTTTTGCTCTGGGCTTCA-3′	NM_205518.1

internal reference to perform relative quantitative analysis of inflammatory cytokine mRNA expression levels. The calculation method uses the $2^{-\Delta\Delta Ct}$ method. The primers and procedures used for testing are previously verified and used [22,23].

2.6. Western blot analysis of HSPs

Western blot was used to detect the expressions of HSP60, HSP70 and HSP90 in the liver tissue. One milliliter of protein lysate was added to a 0.1 g liver sample to extract the total protein of liver tissue, and 100 μL of tissue protein was added to an equal volume of buffer to boil. Then run them in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) [24]. The corresponding antibody prepared by this laboratory is the corresponding polyclonal antibody, which was incubated at a concentration of 1: 1000. The secondary antibody (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Beijing China) was incubated at 1: 5000. To correct the sample volume, β-actin antibody (Beyotime Shanghai China) and a dilution solution were simultaneously incubated at a 1:1000 concentrations, and the secondary antibody (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Beijing China) was incubated at a concentration of 1:5000. Exposure was performed using a biological imaging system (Shanghai Qinxiang Scientific Instrument Co., Ltd. Shanghai China).

2.7. Histopathological analysis

Tissues were fixed in 4% paraformaldehyde and dehydrated by alcohol gradients. Paraffin-embedded sections were counterstained with hematoxylin and eosin and examined by microscopy [25].

2.8. Organ index

The chickens' liver and body weights were determined. The ratio of liver weight to body weight is defined as the organ index.

2.9. Statistical analysis

All experimental data was collected by group and batch. A least squares mehtod based on the SAS JMP7.0 system (SAS Institute Inc., Cary, NC) was used to analyze differences among batches and groups for each parts of the experiment which uses the linear model $Y = \mu + Group + Batch + Group*Batch$. The value $P < 0.05$ was considered as statistically significant. Each value is presented as LSM ± SD of 15 individuals. Drawing was performed using GraphPad Prism 5.01 (GraphPad Inc., La Jolla, CA, USA).

3. Results

The experimental results were analyzed by the least squares method and found no significant difference between the three batches.

3.1. Cadmium content

Fig. 1 shows the cadmium content in the livers at 20 d,40 d, and 60 d. The content of cadmium increased over time. The cadmium content in the Cd group was significantly higher than that in K group and the GT group ($P < 0.05$), while the Cd + GT group was significantly lower than the Cd group ($P < 0.05$). There was no significant difference between the GT group and control group ($P > 0.05$), but at the same time point, the cadmium content of the GT group was lower than K group.

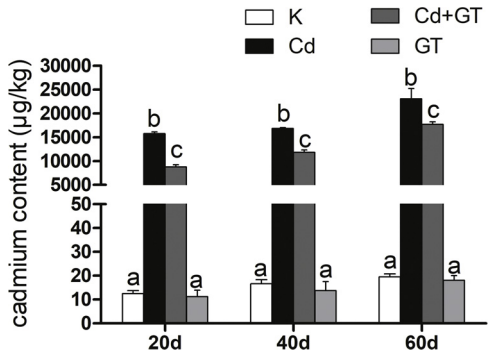

Fig. 1. Demonstrates the response of Ganoderma triterpenoids to the content of cadmium in the liver of chicken. At the same time point, K group is used as a benchmark. Different letters represented significant differences ($P < 0.05$), and each group of data was expressed as LSM ± SD. (n = 15).

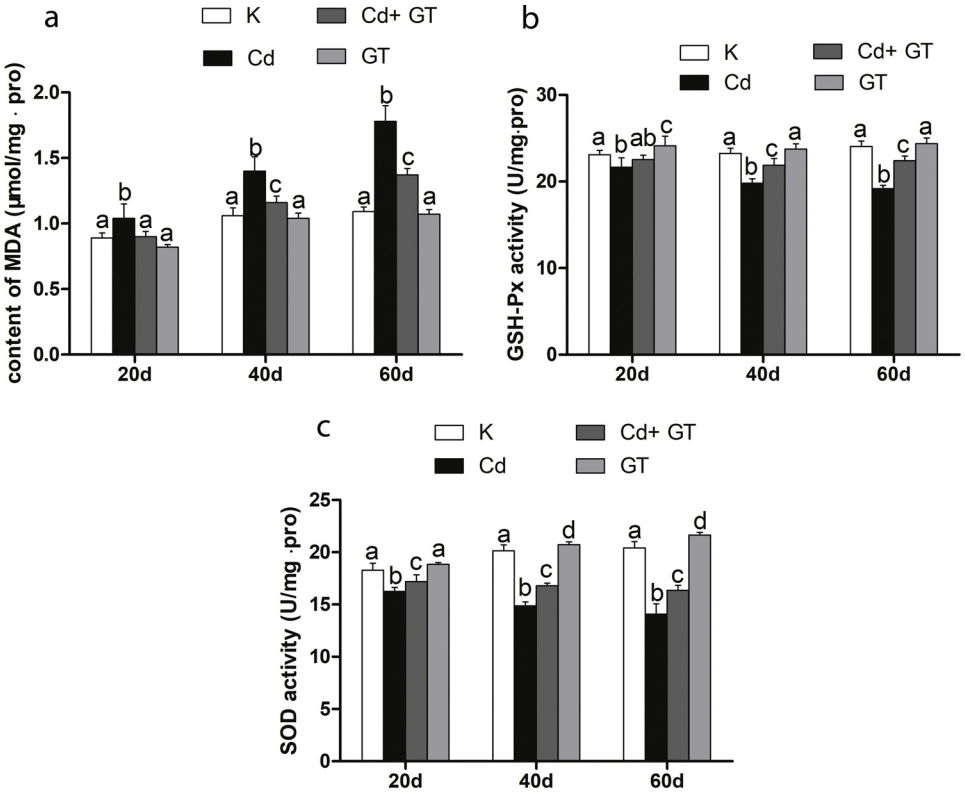

Fig. 2. (a) Is the effect of Ganoderma triterpenoids on cadmium-induced MDA content in chicken liver; (b) is the effect of Ganoderma triterpenoids on the activity of GSH-Px in the liver of chicken induced by cadmium; (c) is the effect of Ganoderma triterpenoids on the activity of SOD in the cadmium-induced liver of chicken. At the same time points, using K group as a benchmark, different letters represent significant differences ($P < 0.05$), and each group of data was expressed as LSM ± SD. (n = 15).

3.2. Results of antioxidant index test

Fig. 2 shows the results of antioxidant index tests at 20 d, 40 d, and 60 d. As shown in Fig. 2a, the content of MDA in the livers of the Cd group was significantly higher than that in K group at 20 d, 40 d, and 60 d ($P < 0.05$). The MDA content in the livers of the GT group was lower than that of K group, but the difference was not significant ($P > 0.05$), and the MDA content in the liver of Cd + GT group was significantly lower than that of the Cd group at the 20th, 40th, and 60th days ($P < 0.05$).

As shown in Fig. 2b, the activity of GSH-Px in the liver of the Cd group was significantly lower than that in K group at 20 d, 40 d, and 60 d ($P < 0.05$). At 20 d, the activity of GSH-Px in the livers of the GT group was significantly higher than that in K group ($P < 0.05$), and there was no significant difference between the levels at 40 d and 60 d ($P > 0.05$).

As shown in Fig. 2c, in 20 d, 40 d, and 60 d, the Cd group chicken liver SOD activity was significantly lower than K group ($P < 0.05$). At 40 d and 60 d, SOD activity of the GT groups was significantly higher ($P < 0.05$), but there was no significant difference at 20 d ($P > 0.05$). At 20 d, 40 d, and 60 d, the SOD activity of Ganoderma triterpenoids-treated groups was significantly higher than that of the Cd group ($P < 0.05$).

3.3. Inflammatory factors mRNA expressions

As shown in Fig. 3a–c, the relative mRNA levels of chTNF-α, chIL-1β, and chIL-6 in the Cd group were significantly higher than those in K group at 20 d, 40 d, and 60 d ($P < 0.05$). At 20 d, 40 d, and 60 d, the levels of chTNF-α, chIL-1β, and chIL-6 mRNA in the liver of the Cd + GT group were significantly lower than those in the Cd group ($P < 0.05$). At 20 d, 40 d, and 60 d, the levels of hepatic chTNF-α, chIL-1β, and chIL-6 mRNA in the livers of the GT group were lower than those in K group ($P > 0.05$).

3.4. Heat shock protein mRNA and protein expressions

As shown in Fig. 4a–e, the levels of HSP27, 40, 60, 70, and 90 mRNA in the Cd group were significantly higher than those in the other three groups ($P < 0.05$). The heat shock protein mRNA level in the treatment group was significantly lower than that in the Cd group ($P < 0.05$), and there was no significant difference in heat shock protein (HSP27, 40, 60, 70, and 90) mRNA levels between the GT group and K group ($P > 0.05$) at three time points.

As shown in Fig. 5, the expressions changes of HSP60, 70, and 90 proteins at the 60th day were consistent with the transcription levels. The levels of HSP60, 70, and 90 proteins in the Cd group were significantly higher than the other three groups ($P < 0.05$). HSP60, 70, and 90 protein levels were significantly lower ($P < 0.05$) compared to the Cd group at three time points. In addition, there was no significant difference in the levels of HSP70 and HSP90 protein in the livers of the GT group and K group($P > 0.05$). The expression of HSP60 protein in the Ganoderma lucidum treatment group was lower than that in K group ($P < 0.05$).

3.5. Histopathological examination

On day 60, samples of this test were embedded into paraffin section and stained with HE. Fig. 6a as control group and normal histomorphological structures are shown; Fig. 6b shows that the hepatic cell cords were arranged in disorder, and the hepatocytes were enlarged. Different sizes and different numbers of vacuoles appear in the cytoplasm. Partial hepatocyte structure is blurred and inflammatory cell infiltration is seen. Fig. 6c shows that there are few changes in the Cd + GT group, and there is no obvious histological change in the liver of the GT group in Fig. 6d.

3.6. Organ index

As shown in Fig. 7, at 20 d, 40 d, and 60 d, the organ index of the Cd group was significantly higher than that of K group ($P < 0.05$). The

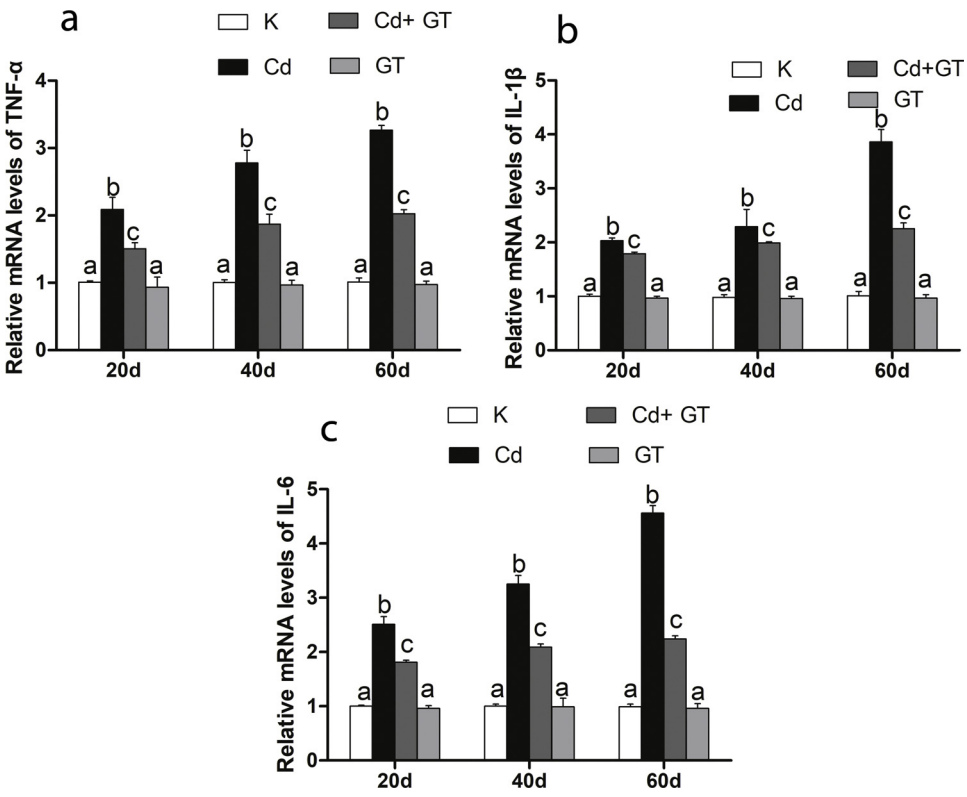

Fig. 3. (a) Shows that the effect of Ganoderma triterpenoids on the expressions of chicken liver chTNF-α mRNA induced by cadmium; (b) shows that the effect of Ganoderma triterpenoids on the expressions of chicken liver chIL-1β mRNA induced by cadmium; (c) shows that the effect of Ganoderma triterpenoids on chicken liver chIL-6 mRNA induced by cadmium. At the same time point control group as a benchmark, different letters represent significant differences ($P < 0.05$) and each group of data was expressed as LSM ± SD. (n = 15).

organ index of the chicken in the Cd + GT group was lower than that of the Cd group ($P < 0.05$). The organ index of K group and the GT group had no significant difference ($P > 0.05$) at 20 d, 40 d, and 60 d.

As shown in Fig. 8, at 20 d, there was no significant difference in body weight among groups of chickens. At 40 d and 60 d, the weight of the Cd group was significantly lower than that of K group ($P < 0.05$). The weight of the chicken in the Cd + GT group was higher than that of the Cd group ($P < 0.05$).

4. Discussion

Cadmium is an accumulative toxic metal that is irreversibly accumulated in various tissues and can be enriched by biological chains. The liver is the animal's main detoxification organ, and it is also one of the first organs to be damaged by toxic substances. The liver plays an important role in cadmium metabolism in the body. A small amount of cadmium that enters the body is rapidly transported to the liver through the blood circulatory system [26]. However, when the absorption of cadmium increases, cadmium begins to accumulate in the liver, damaging liver cells and affecting the liver's metabolic functions. This further increases the accumulation of cadmium in the body. Many studies have shown that cadmium can not only damage the antioxidant system of animals and plants, but also cause inflammation in the body [27]. Previous studies have shown that most of the cadmium content accumulated in the liver and kidneys, where absorption through the cadmium in the blood with the blood combined with a protein transported to the liver [6]. The amount of cadmium in the liver is the most intuitive indicator of the degree of cadmium accumulation in organs. In this experiment, the cadmium content of the Cd group was significantly higher than that of K group and the Cd + GT group, indicating that Ganoderma triterpenoids can effectively reduce the cadmium content in the liver.

Oxidative stress is one of the main mechanisms of cadmium toxicity. Cadmium does not directly produce free radicals. It has only two oxidation states, but several studies have shown that cadmium will indirectly lead to the formation of superoxide radicals, hydroxyl radicals, and other large quantities. Sinha et al. showed that the anti-oxidation ability in mice with chronic cadmium poisoning was reduced [28]. Ramirez et al. found that exposure to chronic cadmium changed the mouse peritoneal macrophage redox balance, resulting in an excess of reactive oxygen species [29]. In this experiment, the liver MDA content of Cd group chickens was significantly higher than K group and increased with time. SOD is considered to play important role to remove free radicals. SOD catalyzes the conversion of superoxide anions (O_2^-) and hydrogen peroxide into water and oxygen. GSH-Px is an important antioxidant enzyme that catalyzes the reduction of hydrogen peroxide to reduce peroxidation. Both antioxidant enzymes are effective in removing excess active oxygen produced in the body. Al Omairi et al. showed that cadmium can cross the blood-brain barrier and cause severe nerve injury. The antioxidant markers of MDA, nitric oxide, and glutathione in the frontal cortex of rats exposed to cadmium for 28 days were determined, and the results showed a reduction of enzyme activity, including SOD, catalase (CAT) and GSH-Px [30]. Studies by Zhang et al. showed that oral administration of cadmium for 35 days resulted in a significant decrease in SOD and GSH-Px activity in the testis, and a significant increase in free radical content [31]. Famurewa et al. found that the activities of antioxidant enzymes SOD, CAT, and GSH-Px in cadmium-induced dyslipidemia were significantly decreased, while the MDA content was significantly increased [32]. The results of this experiment showed that the MDA content in the Cd + GT group was significantly lower than that in the Cd group, and the activities of SOD and GSH-Px were significantly higher than those in the Cd group. Therefore, Ganoderma triterpenoids can protect against lipid oxidative stress induced by cadmium-induced peroxidation damage. It is speculated that the decrease of antioxidant enzymes SOD and GSH-Px is related to the production of a large amount of oxidative enzymes that consume a large amount of reactive oxygen species. It may also be related to the ability of cadmium to replace metal ions in antioxidant enzymes and inhibit the activity of antioxidant enzymes. Further studies suggest that antioxidants can inhibit the toxicity of cadmium, while antioxidant ingredients, such as triterpenoids, significantly increase antioxidant enzyme activity [33].

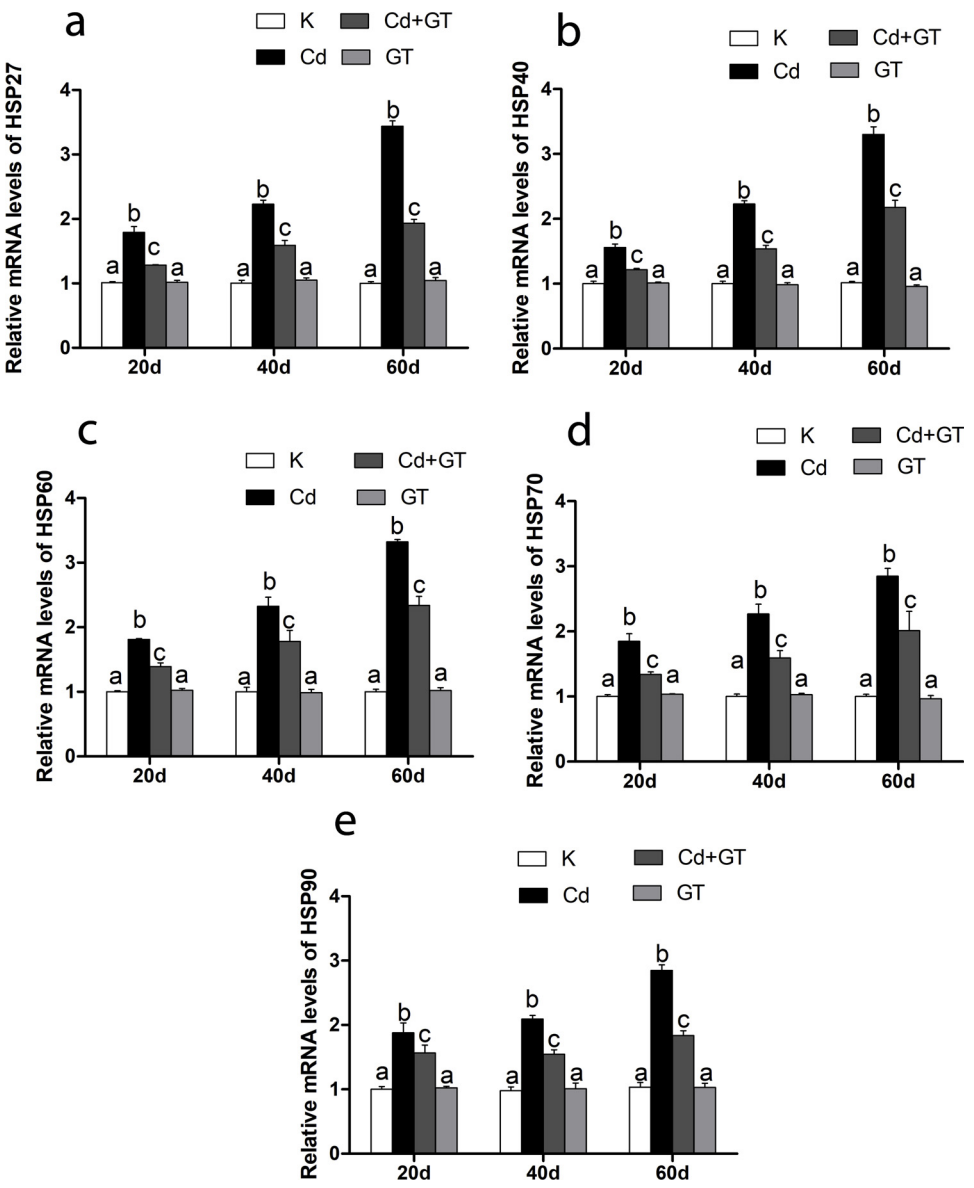

Fig. 4. (a) Shows the effect of Ganoderma triterpenoids on Cd-induced HSP27 mRNA expression in the liver of chicken; (b) shows the effect of Ganoderma triterpenoids on Cd-induced HSP40 mRNA expression in the liver of chicken; (c) shows the effect of Ganoderma triterpenoids on Cd-induced HSP60 mRNA expression in the liver of chicken; (d) Effect of Ganoderma triterpenoids on Cd-induced HSP70 mRNA expression in the liver of chicken; (e) Effect of Ganoderma triterpenoids on Cd-induced HSP90 mRNA expression in the liver of chicken. At the same time point control group as a benchmark, different letters represent significant differences ($P < 0.05$) and each group of data was expressed as LSM ± SD. (n = 15).

Studies have shown that cadmium-induced liver injury is associated with the release of inflammatory factors (TNF-α, IL-1β, and IL-6). TNF-α is one of the most important inflammatory cytokines secreted by macrophages and activates the NF-κB pathway, thereby upregulating the expression of other inflammatory cytokines [34]. An appropriate amount of TNF-α may enhance immune function through the regulation of immune cells to kill a variety of pathogenic factors outside; however, greater than normal expression of TNF-α in the body can cause serious toxic effects that may lead to irreversible damage. IL-1 is a protein polypeptide secreted by monocytes and macrophages and stimulates

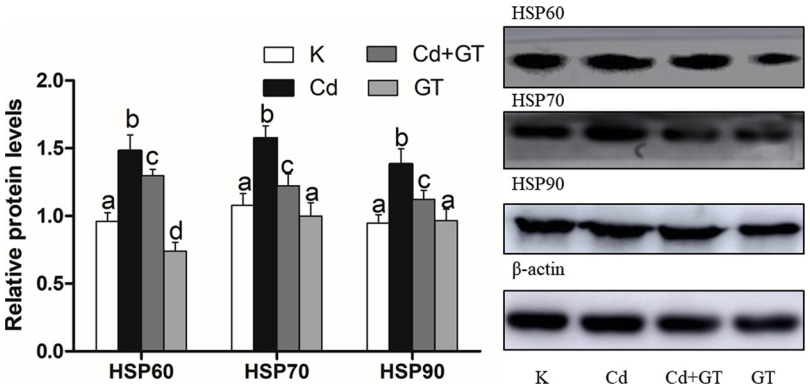

Fig. 5. Shows the effect of Ganoderma triterpenoids on Cd-induced HSP expressions in the liver of chicken. At the same time point control group as a benchmark, different letters represent significant differences ($P < 0.05$), and each group of data was expressed as LSM ± SD. (n = 15).

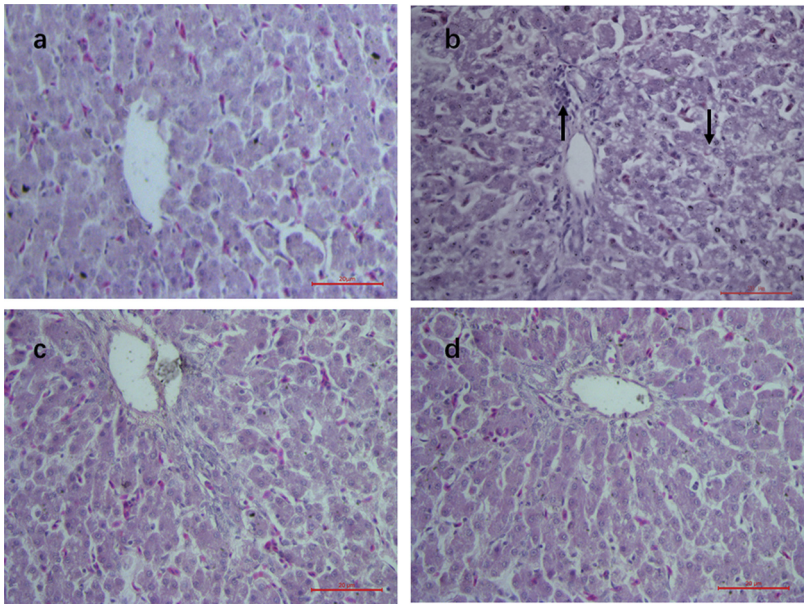

Fig. 6. Shows the chicken liver tissue sections stained with HE. a, K group liver sections. b, liver sections of the Cd group showed leukocyte infiltration (↑) and vacuolar degeneration (↓). c: Liver sections of the Cd + GT group. d: Liver slices of the GT group.

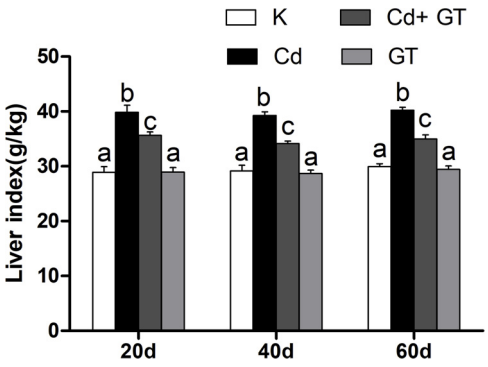

Fig. 7. Effect of Ganoderma triterpenoids on chicken organ index induced by cadmium. At the same time point control group as a benchmark, different letters represent significant differences ($P < 0.05$), and each group of data was expressed as LSM ± SD. (n = 15).

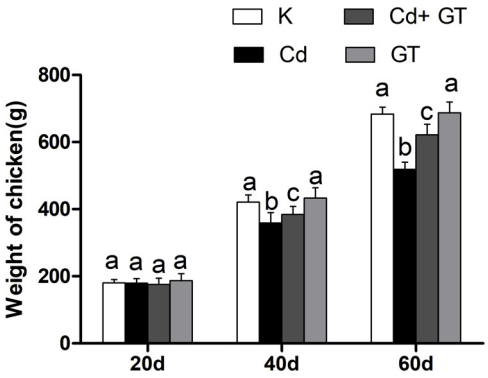

Fig. 8. Effect of Ganoderma triterpenoids on chicken weight induced by cadmium. At the same time point control group as a benchmark, different letters represent significant differences ($P < 0.05$), and each group of data was expressed as LSM ± SD. (n = 15).

the secretion of IL-6 and TNF-α by monocytes and macrophages. IL-6 is a multifunctional cytokine secreted by a variety of inflammatory cells that stimulates the development of inflammatory responses and aggravates the inflammatory response. The results of this experiment show that cadmium exposure can significantly induce the expressions of TNF-α, IL-1β, and IL-6, which is consistent with the results of pathological observation. At the same time, the expressions of inflammatory factors in the Cd + GT group was significantly lower than that in the Cd group, indicating that Ganoderma triterpenoids can reduce the expressions of inflammatory factors in liver tissue induced by cadmium, and alleviate the damaging effects of cadmium on the liver.

When organisms suffer a variety of stresses due to outside influences, the synthesis of most proteins is inhibited, but a series of protective proteins called heat shock proteins possess a fast cytoprotective function [35–37]. In this experiment, cadmium significantly increased mRNA levels of HSP 27, 40, 60, 70, and 90 and protein levels of HSP 60, 70, and 90. The heat shock protein mRNA and protein levels of the Cd + GT group were significantly lower than those of the Cd group. This may be because Ganoderma triterpenoids can promote the discharge of cadmium in the liver, leading to stress on the effects of cadmium reduction in liver tissue, so that the heat shock proteins set lower than that of cadmium. The mechanism requires further study.

Cadmium accumulates in the liver, causes liver cell damage, and reduces the detoxification function of the liver, resulting in a greater amount of accumulated damage and serious consequences. Histopathology can visually indicate the organizational structure and morphological changes, indicating whether the organization is healthy or not. In this experiment, in the Cd group, cadmium induced liver cell degeneration, necrosis, increased red blood cell count, and inflammatory cell infiltration in the chicken. However, these lesions in the Cd + GT group were significantly lighter than those in the Cd group. This shows that Ganoderma triterpenoids can relieve cadmium-induced liver inflammatory lesions.

The organ index is generally the ratio of organ weight to body weight and is often used to predict the patient's potential toxicity. In the medical field, long-term toxicity or drug trials in organ index is one of the indicators that must be tested and observed. Increased or decreased organ index may indicate different pathological changes, increased organ index may indicate congestive organ enlargement, edema, or proliferative hypertrophy. Decreased organ index may indicate atrophy or degenerative changes. With the increase in cadmium dose, liver congestion and liver index will increase. The results of this experiment showed that the liver index was significantly lower in the Cd + GT group than in the Cd group. The increase of liver index caused by

cadmium can be reduced by Ganoderma triterpenoids significantly.

The main absorption pathway of cadmium in the body is intestinal absorption [38]. The change in cadmium content in chicken liver may be due to a decrease in cadmium uptake in the intestine. As a result of the reduction of absorbed cadmium content, the reduction of antioxidant capacity in the body and the improvement of inflammatory response, experimental data indicate that GT leads to an increase in antioxidant capacity and a reduction in inflammatory response. We speculate that in addition to directly participating in the promotion of antioxidant capacity and reducing inflammatory response, it is also possible that GT reduces the absorption of cadmium in the intestine, thereby reducing the cadmium content in the body and indirectly protecting cadmium-induced chicken liver injury.

5. Conclusion

In conclusion, the chicken liver is an important target organ for Cd. Cd can reduce the antioxidant capacity of chicken liver and cause inflammation. Ganoderma triterpenoids may provide protection for chicken liver against Cd toxicity.

Compliance with ethical standards

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University.

Conflict of interest

The authors declare that they have no conflicts of interest.

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