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RESEARCH ARTICLE

Protective effects of *Ganoderma lucidum* triterpenoids on oxidative stress and apoptosis in the spleen of chickens induced by cadmium

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Abstract

Cadmium (Cd) is a heavy metal that poses a huge potential threat to human and animal health. Therefore, it is necessary to study its damage mechanism. In the present study, we have examined the protective effects of *Ganoderma lucidum* triterpenoids on oxidative stress and apoptosis in the spleen of chickens induced by Cd. One hundred and twenty healthy Hailan white chickens (7-day-old) were randomly divided into the following four groups: control group, Cd group, triterpenoid group, and Cd–triterpenoid group. The chickens were euthanized on the 20th, 40th, and 60th days, and the spleens were removed. Cd and malondialdehyde (MDA) content, antioxidant enzyme (superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)) activities, and inflammatory factor (tumor necrosis factor alpha (TNF-α) and interleukin (IL-1β and IL-6)) and apoptotic factor (caspase-3, BAX, and Bcl-2) expressions were detected. The results showed that *Ganoderma lucidum* triterpenoids could reduce the content of Cd and MDA; increase the antioxidant enzyme activities (SOD and GSH-Px); decrease the expression of inflammatory factors (TNF-α) and interleukin (IL-1β and IL-6); increase the expression of apoptotic factor (Bcl-2); and decrease the expression of apoptotic factors (caspase-3 and Bax). It showed that the triterpenoids of *Ganoderma lucidum* had significant protective effects on oxidative stress and apoptosis of chicken spleen, which provided a theoretical basis for further prevention and treatment of cadmium poisoning.

Keywords *Ganoderma lucidum* triterpenoids · Cadmium · Spleen · Antioxidant · Apoptosis

Introduction

The increasing number of heavy metals entering the environment may lead to the accumulation of these pollutants in humans and animals, posing a serious threat to ecosystems and human health (Wang et al. 2018d, f). Cd is a typical environmental pollutant. The Cd pollution in the environment mainly comes from industry and agriculture. Some studies have shown that Cd has a certain toxic effect on animals and humans (Järup and Åkesson 2009; Abdelrazek et al. 2016; Elkhadragy et al. 2017). Cd toxicity can be amplified through the food chain. Cd accumulates in humans and animals by ingesting plants and vegetables grown in soils with high levels of Cd (Nordberg et al. 2007; Bergstrom et al. 2015). Excessive Cd in the body can cause damage to the liver, kidneys, testicles, and immune organs, and the spleen is one of the main target organs of Cd (Modi et al. 2008; Angenard et al. 2010; Chen et al. 2017). Studies have shown that cadmium can inhibit the function of spleen lymphocytes, cause splenomegaly, and damage the body's immune system, leading to immune dysfunction (Liu et al. 2014; Xu et al. 2015). Cd toxicity can affect the number of spleen B lymphocytes and T lymphocytes, resulting in an increase or decrease in B lymphocytes and T lymphocytes. This different result may be related to the dose and duration of cadmium in the body (Swiergosz-

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Kowalewska 2001; Pathak and Khandelwal 2007). Cd affects not only the number of T lymphocytes but also the differentiation of T lymphocyte subsets. Studies have also found that Cd can induce toxic effects, such as oxidative stress, morphological changes, and apoptosis in poultry (Xiaolong et al. 2015; Xie et al. 2017). Exposure to Cd inhibits the activity of antioxidant enzymes, such as SOD, malondialdehyde (MDA), and GSH-Px. At the same time, the action of Cd on the spleen also leads to a significant increase in inflammatory factors, including IL-2, IL-4, and TNF-α. The large amount of TNF-α synthesis activates the NF-κB pathway, and the activation of NF-κB can feedback the synthesis of inflammatory factors, eventually leading to inflammation (Dan et al. 2000; Mayara Lutchemeyer et al. 2012; Li et al. 2016; Wang et al. 2018c).

Ganoderma lucidum is a functional food and medicine that has been widely used in traditional Chinese medicine because of its ability to prolong life and maintain human health (Li et al. 2018). *Ganoderma lucidum* has good immune regulation and antioxidation effects, and it has been proven to prevent and treat various diseases, such as chronic hepatitis, nephritis, hypertension, tumor, and neurasthenia (Qing et al. 2014; Wang et al. 2018a). The most important pharmacologically active compound in *Ganoderma lucidum* is triterpenoid (Boh et al. 2007). *Ganoderma lucidum* triterpenoid was first separated from the *Ganoderma lucidum* fruiting body in the 1980s (Kubota et al. 1982). It is a compound composed of isoprene units (Qing et al. 2014) and is a highly oxidized lanostane derivative (Hu et al. 2013). *Ganoderma lucidum* has a wide range of biological activities, such as liver protection, blood pressure lowering, blood fat reduction, antitumor, antioxidation, antibacterial, anti-inflammatory, and improving hematopoietic function (Luo and Zhao 2002; Jie et al. 2004; Wang et al. 2004; Chu et al. 2007; Tung et al. 2013).

Although there have been many studies on *Ganoderma lucidum* triterpenoids so far, there is a lack of research on its effects on cadmium poisoning, and its protective mechanism against cadmium-induced spleen damage is still unclear. For further study, this experiment created a model of chicken cadmium poisoning. The damage of Cd to spleen and the protective effect of *Ganoderma lucidum* on spleen were evaluated by detecting the Cd content, oxidation index, inflammatory factor, and expression level of apoptotic protein in chicken spleen and observing the pathological histomorphology.

Materials and methods

Preparation of *Ganoderma lucidum*

The *Ganoderma lucidum* fruit body was pulverized, and anhydrous ethanol was used as the extracting agent. The ratio of material to liquid was 1:20, the leaching temperature was 80 °C for 3 h, and the filtered solution was concentrated and dried to obtain a crude extract, which was extracted with chloroform for 3 times. The mixture was firstly extracted three times with saturated sodium hydrogen carbonate, then the pH was adjusted to 2–3 with hydrochloric acid, and finally the mixture was extracted three times with chloroform and concentrated to obtain purified *Ganoderma lucidum* triterpenoid compound. A total of 100 μg/mL oleanolic acid reference solution 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL was weighed in a test tube, and a blank was taken as a control. Next, all the tubes were heated in a water bath until the solvent was dried, then 0.4 mL of 5% vanillin-glacial acetic acid and 1 mL of perchloric acid were quickly mixed in, and the tubes were heated again in a water bath at 65 °C for 15 min; after, the solution was naturally cooled, and the volume was adjusted to 5 mL. After setting the wavelength, the average absorbance was measured three times. The absorbance and the concentration values of the standard were set as the ordinate and abscissa of the standard curve, respectively; then, the regression equation was established according to the relationship between the two; and the oleanolic acid standard curve was obtained. A total of 250 mL of *Ganoderma lucidum* triterpenoid extract was obtained. The extract in the tube to be tested was firstly heated in a water bath, then rapidly mixed with 0.4 mL of 5% vanillin-glacial acetic acid and 1 mL of perchloric acid, and finally subjected to 65 °C for 15 min. After heating in a water bath, it was naturally cooled; at a constant volume of 5 mL, the absorbance was averaged at the same wavelength against the blank reagent, and the content of *Ganoderma lucidum* triterpene in the extract was calculated to be 20 mg/mL by combining the regression equation.

Experimental animals and experimental design

One hundred and twenty healthy Hailan white chickens (7-day-old) were randomly divided into the following four groups: control group, Cd group, triterpenoid group, and Cd–triterpenoid group. The normal control group was fed the basal diet, and each chicken was fed 0.5 mL of distilled water; the Cd group was fed a basal diet mixed with 140 mg/kg $CdCl_2$, and 0.5 mL of distilled water was fed daily; the triterpenoid group was fed the foundation, in which the diet given was 0.5 mL of *Ganoderma lucidum* triterpenoid solution (20 mg/mL) per day; the Cd–triterpenoid group was fed a basic diet containing 140 mg/kg $CdCl_2$, and 0.5 mL of *Ganoderma lucidum* was administered per day solution (20 mg/mL). The experiment lasted for 60 days. During the experiment, all the experimental animals were free to eat and drink. Ten experimental animals were randomly selected for euthanasia on the 20th, 40th, and 60th days. Before the euthanasia, the experimental animals were fasted for 12 h to remove the spleen. The collected spleen tissue was divided into two

Table 1 Composition of the experimental diets (%)

Ingredient	The normal control group Contents	The Cd group	The triterpenoid group	The Cd–triterpenoid group
$CdCl_2$	0	0.014	0	0.014
Corn	63.69	63.68	63.68	63.69
Soybean meal	14.35	14.35	14.35	14.35
Sunflower 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

parts; one part was fixed in 4% paraformaldehyde solution and stored at 4 °C for histopathological examination, and the other part was stored at − 80 °C for detection of antioxidant index, inflammatory factor, apoptosis, and the level of protein expression.

The dietary composition and nutritional level of the experimental animals are shown in Tables 1 and 2, respectively.

Determination of cadmium concentration

A total of 0.5 g of spleen was taken as a test sample, and the graphite digestion system (Polytech ST60) was pretreated. The cadmium content was determined by the Harbin Entry-Exit Inspection and Quarantine Bureau using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method. The test conditions are shown in Table 3:

Antioxidant index detection

A suitable amount of spleen sample was taken, placed in a grinder and added with physiological saline, ground to a 1% homogenate in an ice water bath, and homogenized at 4000 r/min for 10 min, and the supernatant was taken. Using TBA, colorimetric, and hydroxylamine methods, strictly follow the steps of the kit's instructions, detect MDA, SOD, GSH-Px, and finally determine the corresponding OD value and obtain the antioxidant index by calculation. All kits were purchased from Nanjing Jiancheng Reagent Company.

Table 2 Nutritional levels of the experimental diets

Ingredient	The normal control group Contents	The Cd group	The triterpenoid group	The Cd–triterpenoid group
CP	16.52 ± 0.08	16.52 ± 0.12	16.52 ± 0.51	16.52 ± 0.09
ME (MJ/kg)	11.30	11.30	11.30	11.30
Ca	3.08 ± 0.028	3.08 ± 0.09	3.09 ± 0.008	3.08 ± 0.005
TP	0.41	0.41	0.41	0.41
Methionine	0.45 ± 0.016	0.45 ± 0.006	0.45 ± 0.006	0.45 ± 0.015
Lysine	1.03 ± 0.013	1.03 ± 0.006	1.03 ± 0.011	1.03 ± 0.013
Methionine + cysteine	0.78 ± 0.014	0.79 ± 0.008	0.78 ± 0.008	0.78 ± 0.007

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
Tuning	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
Timing	Sweeps/reading	30
	Readings/replicate	1
	Number of replicates	3

Quantitative real-time polymerase chain reaction

The extraction of RNA and the preparation of cDNA were done according to the method described by Guo et al. (2019). Taking 0.1 g frozen spleen sample, strictly extract total RNA according to the instructions, detect RNA concentration, reverse-transcribe cDNA, synthesize cDNA, and store in − 80 °C refrigerator; taking samples of 20, 40, and 60 days, detect TNF-α, IL-1β, IL-6, caspase-3, Bax, and Bcl-2 mRNA levels by qPCR method; each sample is 20 μL system and β-actin was used as an internal reference. Reaction mixtures were incubated in the LightCycler 480 real-time PCR system. The reaction conditions are pre-denaturation in 1 cycle at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and extension at 72 °C for 15 s in 40 cycles. Three replicate wells were used for each sample to perform a relative quantitative analysis of inflammatory cytokine mRNA expression levels. The calculation method was $2^{-\Delta\Delta Ct}$, in which ΔΔCt = (Ct target gene − Ct reference gene) treatment group − (Ct target gene − Ct reference gene) blank group. Primer design is shown in Table 4:

Cytokine content

The contents of TNF-α, IL-1β, and IL-6 in spleens were evaluated by ELISA. The spleen supernatants were collected by the method described previously. The concentrations of TNF-α, IL-1β, and IL-6 were measured using ELISA kits following the manufacturer's instructions (Elabscience Biotechnology Co., Ltd.).

Apoptosis protein expression

A total of 0.1 g of frozen spleen tissue was weighed into a homogenizer, and 1 mL of protein lysate was added to prepare total protein of spleen tissue. A total of 100 μL of total tissue protein was taken, and an equal volume of 2× loading buffer was added to boil; then, SDS-PAGE was performed. After transferring, the NC membrane was blocked in 5% skim milk at 4 °C. The primary antibody is a polyclonal antibody prepared in this laboratory. Incubation was carried out at a concentration of 1:1000, and the secondary antibody was an HRP-labeled goat anti-rabbit IgG antibody purchased from Nakasugi Jinqiao Co., Ltd., which was incubated at a concentration of 1:5000; for correcting the amount of the sample, the same was purchased from Biotech. The β-actin antibody was used as internal controls. Exposure was performed using a bio-imaging system form Shanghai Qinxiang Scientific Instrument Co., Ltd. The ImageJ software was used to detect the integral density (IntDen) of each band, and the protein expression level was expressed as the ratio of IntDen of the target protein to IntDen of β-actin.

Histopathological examination

The spleen tissue samples fixed in 4% paraformaldehyde were prepared, and paraffin sections were prepared for HE staining to observe pathological changes.

Statistical analysis

All data were statistically analyzed and analyzed using SPSS 17.0 software. The results were analyzed by one-way analysis of variance using SPSS17.0 software, and the histograms were drawn by GraphPad Prism 5 software. The blanks were used as the benchmark at the same time point, and the different letters represented significant differences ($P < 0.05$). The results were averaged and standard deviation (mean ± SD) was indicated.

Table 4 Gene-special primers used for qPCR

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

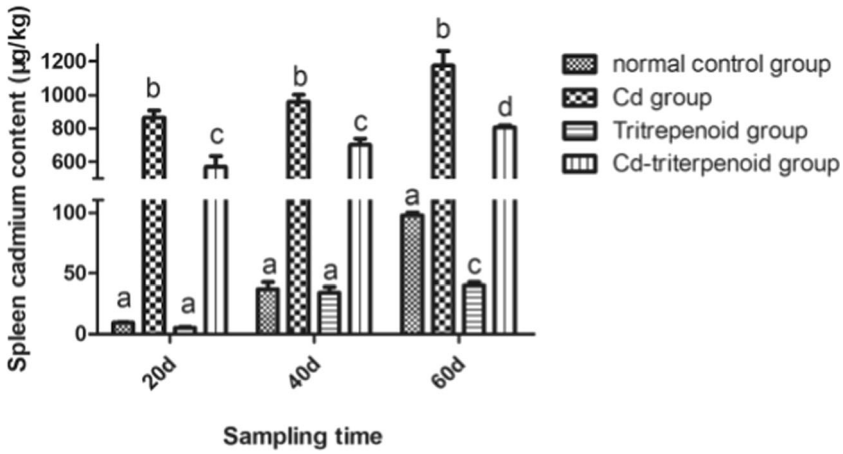

Fig. 1 Spleen cadmium content. Effect of *Ganoderma lucidum* triterpenoids on cadmium content in chicken spleen. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant difference ($P < 0.05$). The data of each group were expressed as mean ± standard deviation. $n = 10$

Results

Cadmium content

The Cd contents in the spleen at different time points were shown in Fig. 1. The Cd content in the spleen of the Cd group is significantly higher than that of the normal control group and the triterpenoid group. The Cd content in the Cd–triterpenoid group is significantly lower than that in the Cd group. On the 20th and 40th days, the cadmium content in the spleen of the triterpenoid group was lower than that in the normal control group, but the difference was not significant; however, on the 60th day, it was shown that the Cd content in the spleen of the triterpenoid group was significantly lower than that in the normal control group.

Antioxidant index test results

As shown in Fig. 2, the content of MDA in the spleen of the Cd group was significantly higher than that of the other three groups in the three sampling periods; the MDA content in the Cd–triterpenoid group was significantly lower than that in the Cd group, slightly below the normal control group but not significantly different.

As shown in Figs. 3 and 4, the activity of SOD and GSH-Px in the spleen of the Cd group was significantly lower than that of the other three groups; the activity of SOD and GSH-Px of the Cd–triterpenoid group was significantly higher than that of the Cd group. In the three sampling periods, the GSH-Px activity in the triterpenoid group was significantly higher than that in the normal control group.

Inflammatory factor expression test results

As shown in Figs. 5, 6, and 7, it can be seen that the cytotoxic levels of TNF-α, IL-1β, and IL-6 in the Cd group are significantly higher than those in the other three groups. The transcript levels of TNF-α, IL-1β, and IL-6 in the Cd–triterpenoid group were significantly lower than those in the Cd group, and the inflammatory factors TNF-α, IL-1β, and IL-6 in the triterpenoid group were compared with those of the normal control group, which showed no significant difference.

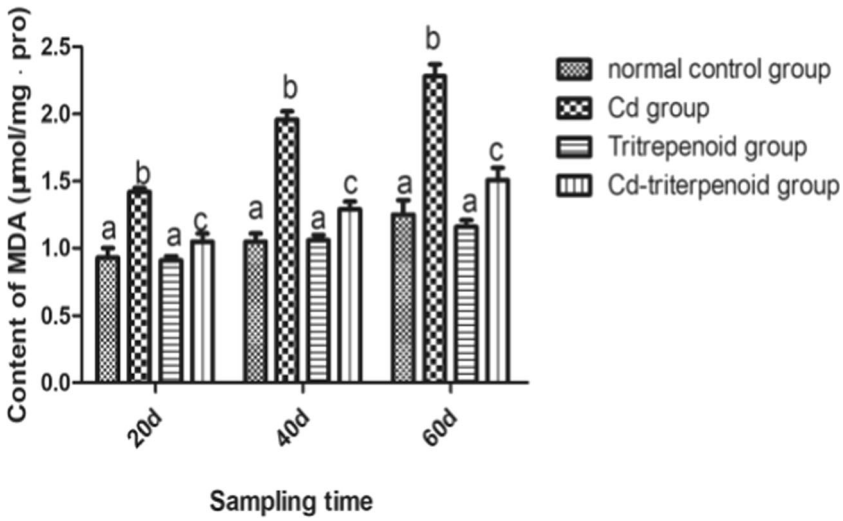

Fig. 2 Content of MDA in chicken spleen. Effect of *Ganoderma lucidum* triterpenoids on cadmium-induced activity of antioxidant enzymes (SOD and GSH-Px) in spleen and MDA content. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Group data are expressed as mean ± standard deviation. $n = 10$

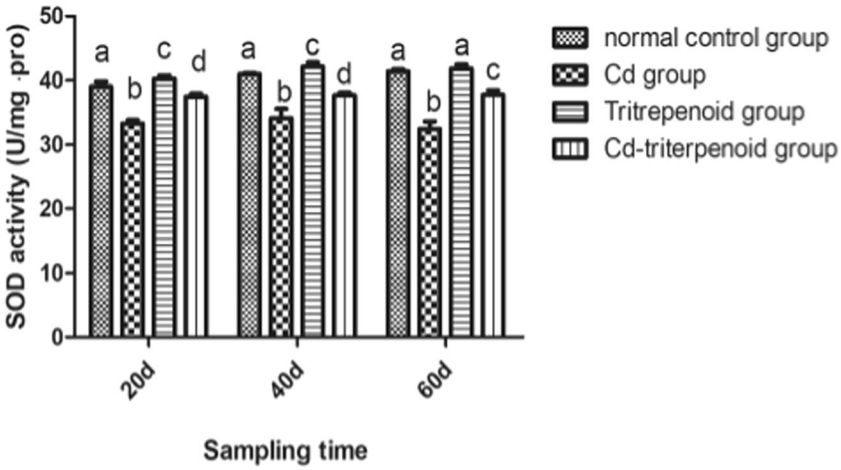

Fig. 3 Activity of SOD in chicken spleen. Effect of *Ganoderma lucidum* triterpenoids on cadmium-induced activity of antioxidant enzymes (SOD and GSH-Px) in spleen and MDA content. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Group data are expressed as mean ± standard deviation. $n = 10$

As shown in Figs. 8, 9, and 10, it can be seen that the levels of inflammatory factors, such as TNF-α, IL-1β, and IL-6, in the Cd group are significantly higher than those in the other three groups. The expression levels of TNF-α, IL-1β, and IL-6 in the Cd–triterpenoid group were significantly lower than those in the Cd group, and the expression levels of inflammatory factors TNF-α, IL-1β, and IL-6 in the triterpenoid group were compared with those in the normal control group, which showed no significant difference.

Apoptosis protein transcription level and protein expression detection results

It can be observed from Figs. 11 and 12 that the transcript levels of caspase-3 and Bax in the Cd group were significantly higher than those in the other three groups during the three sampling periods.

The transcript levels of caspase-3 and Bax were significantly lower in the Cd–triterpenoid group than in the Cd group, and there was no significant difference between the triterpenoid group and the normal control group.

As shown in Fig. 13, the transcript level of Bcl-2 in the Cd group was significantly lower than that in the other three groups. The Cd–triterpenoid group was significantly higher than the Cd group. There was no significant difference between the triterpenoid group and the normal control group.

As shown in Fig. 14, the expression levels of caspase-3 and Bax protein in the spleen of the Cd group were significantly higher than those of the normal control group at 60 days, and the Cd–triterpenoid group was significantly lower than the Cd group. Furthermore, the triterpenoid group was lower than the normal control group, but there was no significant difference. The expression of Bcl-2 protein in the spleen of the Cd group was significantly lower than that of the normal control group at 60 days, and the Cd–triterpenoid group was significantly higher than the Cd group.

Histopathological changes

As shown in Fig. 15, the pathological tissue sections of the spleen tissue were selected on the 60th day. Figure 15a shows the spleen tissue section of the normal control group, in which

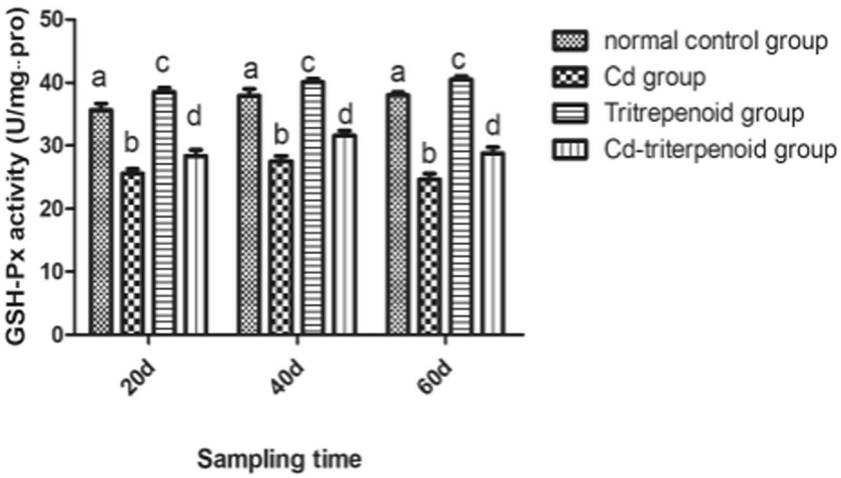

Fig. 4 Activity of GSH-Px in chicken spleen. Effect of *Ganoderma lucidum* triterpenoids on cadmium-induced activity of antioxidant enzymes (SOD and GSH-Px) in spleen and MDA content. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Group data are expressed as mean ± standard deviation. $n = 10$

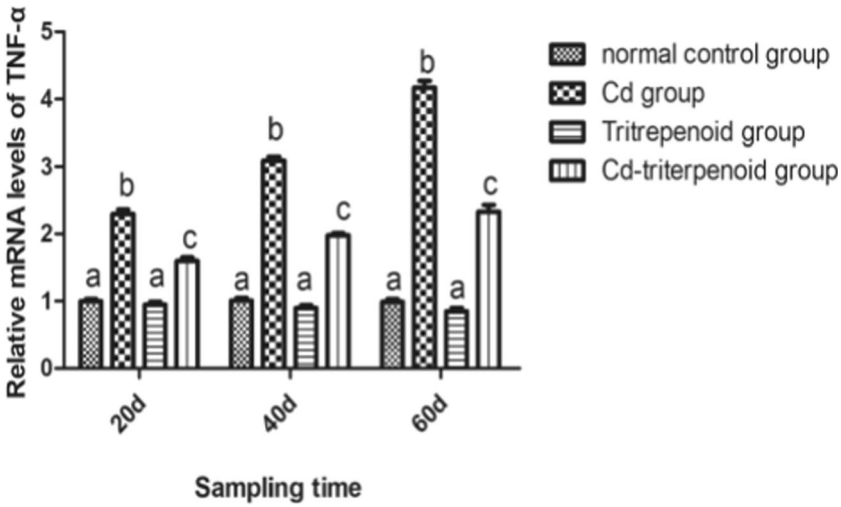

Fig. 5 The mRNA levels of TNF-α in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on cadmium-induced inflammatory factor mRNA expression in the spleen. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Group data are expressed as mean ± standard deviation. $n = 10$

the cells are arranged neatly, the red pulp and white pulp junction is obvious, and there are a large number of lymphocytes; Fig. 15b shows the spleen tissue section of the Cd group, in which the white pulp and red pulp are unclear, the number of lymphocytes is reduced, the cells are arranged in disorder, and there are a lot of red blood cells in the red pulp; Fig. 15c shows the spleen tissue section of the triterpenoid group, in which there is no significant difference between the triterpenoid group and the normal control group; Fig. 15d shows the spleen tissue section of the Cd–triterpenoid group, in which the lymphocytes of the spleen and cadmium poisoning groups increased significantly, the cells were arranged neatly, and the number of red blood cells in the red pulp was significantly reduced.

Discussion

Cd is a toxic heavy metal widely found in the environment. It has a long half-life and can exist in the body for 15–30 years. It can be enriched through the food chain and eventually accumulated in organs in the body (Stohs et al. 2001). Cd has many target organs in humans and animals. The spleen is an important immune organ of the body and is one of the target organs of cadmium toxicity (Liu et al. 2015; Zhang et al. 2017). Cd reaches the spleen through the blood circulation system and accumulates, causing tissue damage. The accumulation of Cd in the body depends on its exposure route, dose, and duration (El-Sharaky et al. 2007). As an active ingredient extracted from *Ganoderma lucidum*, *Ganoderma lucidum* triterpenoid has attracted attention in recent years due to its extensive biological effects, such as antioxidation and anti-inflammation. Studies have shown that *Ganoderma lucidum* can protect the spleen from a variety of harmful ingredients, including chemical agents, physical damage, and microbial infections (Wang et al. 2014). The results of this study showed that cadmium accumulation in the spleen of the Cd group was significantly higher than that of the normal control group, which was consistent with the results of Ninkov et al. (2016), while the Cd content in the spleen of the Cd–

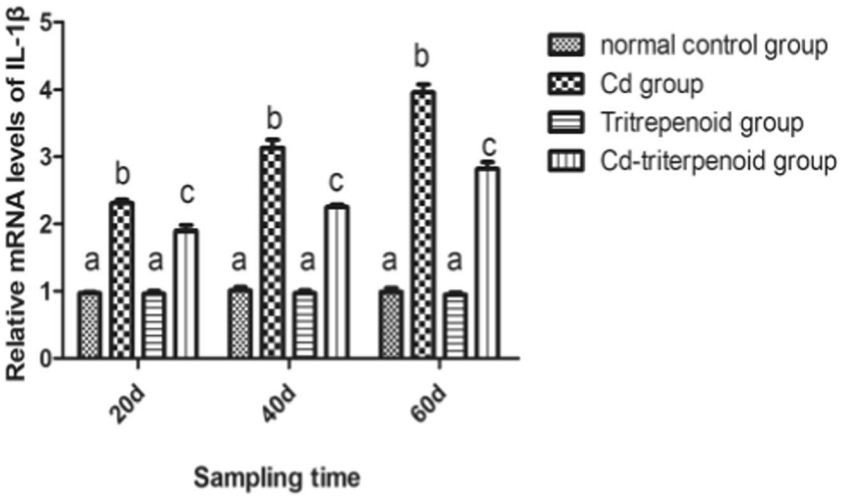

Fig. 6 The mRNA levels of IL-1β in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on cadmium-induced inflammatory factor mRNA expression in the spleen. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Group data are expressed as mean ± standard deviation. $n = 10$

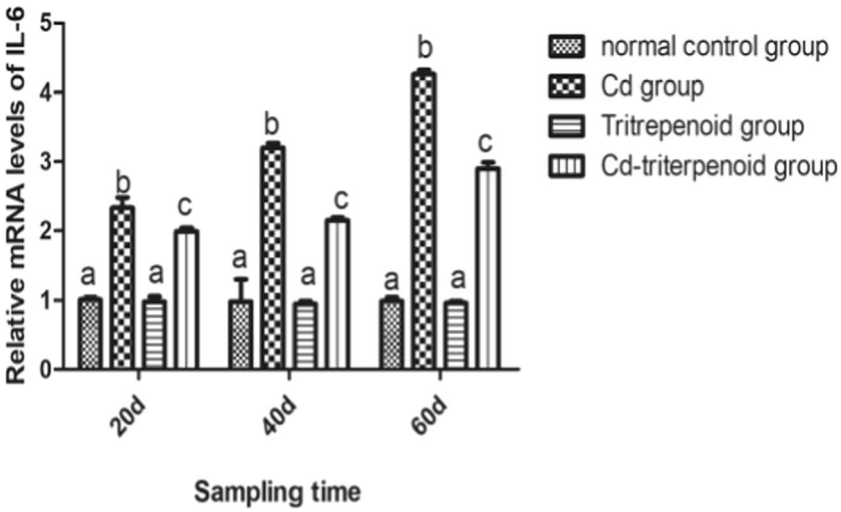

Fig. 7 The mRNA levels of IL-6 in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on cadmium-induced inflammatory factor mRNA expression in the spleen. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Group data are expressed as mean ± standard deviation. $n = 10$

triterpenoid group was significantly lower than that of the Cd group. Cd accumulation can cause pathological damage in various tissues and organs. El-Ebiary et al. (2016) observed that cadmium causes pulmonary interstitial hyperplasia, vascular congestion, inflammatory cell infiltration, and fibrous tissue hyperplasia. In this experiment, the spleen tissue section of cadmium-poisoned chicken showed that the spleen's white pulp and red pulp were unclear, the number of lymphocytes was reduced, the cells were arranged in disorder, and there were a lot of red blood cells in the red pulp, which was consistent with the observations of Cao et al. (2016). The pathological damage of chicken spleen in the Cd–triterpenoid group was significantly reduced. It can be seen that *Ganoderma lucidum* triterpenoid has a good protective effect on the spleen of cadmium-poisoned chicken.

Cd is an inducer of oxidative stress and does not produce free radicals by itself. However, a large number of studies have shown that cadmium indirectly leads to the formation of superoxide radicals and hydroxyl radicals. Many experiments have shown that the large amount of free radicals is due to the Fenton reaction and the Haberves reaction (Ambily Ravindran et al. 2013). Studies have found that chronic exposure to cadmium in mice alters the redox balance of mouse peritoneal macrophages, resulting in excess reactive oxygen species (Ramirez and Gimenez 2003). SOD is considered to be an important enzyme for scavenging free radicals in the body, and the metal auxiliary groups contained zinc, copper, manganese, and iron. Three of the complexes of SOD are important, including manganese superoxide dismutase (located in the mitochondria), copper–zinc superoxide dismutase (located in the cytoplasm), and extracellular superoxide dismutase (located in the blood vessels). SOD catalyzes the conversion of superoxide anion (O-2) and hydrogen peroxide to water and oxygen (Chan et al. 1996; Lu et al. 2015). GSH-Px is an important antioxidant enzyme whose active center is selenocysteine. GSH-Px catalyzes the reduction of hydrogen peroxide to reduce peroxidation (Guéraud et al. 2010; Lu et al. 2015). Both antioxidant enzymes are effective in removing excess reactive oxygen species produced in the body. The level of MDA can directly reflect the level of oxygen free radicals in the body, as well as the intensity and rate of peroxidation. It is an important indicator reflecting the degree of damage to body tissues and cells caused by peroxidation (Zhao et al. 2017). Therefore, MDA has been widely studied

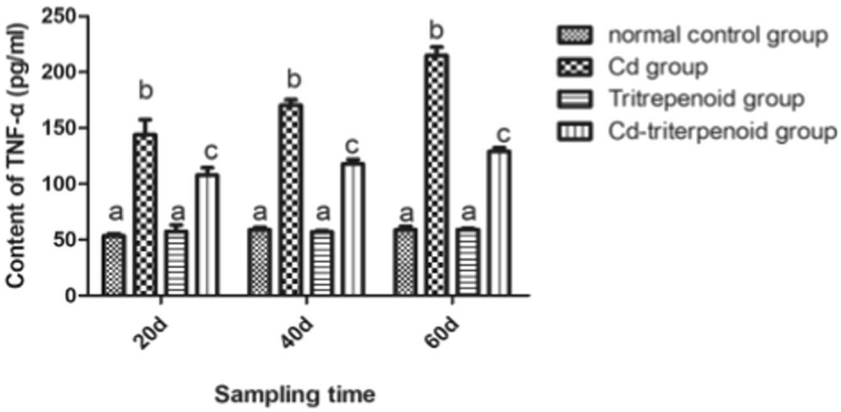

Fig. 8 Expressivity dynamics changes of the TNF-α concentration in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on cadmium-induced inflammatory factors in the spleen. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Expressed as mean ± standard deviation. $n = 10$

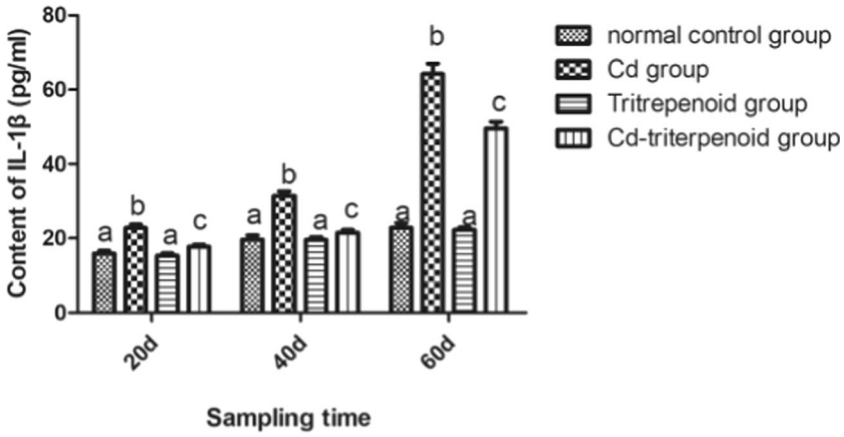

Fig. 9 Expressivity dynamics changes of the IL-1β concentration in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on cadmium-induced inflammatory factors in the spleen. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Expressed as mean ± standard deviation. $n = 10$

as an important indicator of oxidative stress system (Zheng et al. 2019). *Ganoderma lucidum* triterpenoid has good antioxidant capacity and ability to scavenge free radicals. Studies have reported that cadmium accumulation leads to decreased activity of antioxidant enzymes in tissues and an increase in MDA content (Li et al. 2019). In addition, triterpenoids extracted from purslane have shown strong resistance in diabetic rat models. Oxidative capacity significantly increases antioxidant enzyme activity (Tirtha et al. 2011). In this test, the intensity of oxidative stress was detected by detecting MDA, SOD, and GSH-Px. The results showed that the amount of MDA was significantly higher than that in the normal control group, and the activities of SOD and GSH-Px in the Cd–triterpenoid group were lower than those in the normal control group, and it was time-dependent. *Ganoderma lucidum* triterpenoids exhibit anti-chemical activity and normalize MDA expression. It can be considered that *Ganoderma lucidum* triterpenoids can reduce free radical damage caused by cadmium, which is consistent with previous studies.

In addition to causing oxidative stress, Cd can also cause inflammation in the body (Kayama et al. 1995). TNF-α is mainly produced by macrophages and has various biological effects, such as promoting the expression of adhesion factors (Beutler and Cerami 1989) and exerting a strong pro-inflammatory function by activating immune cells and vascular endothelial cells. TNF-α plays a key role in the initiation and regulation of the cytokine cascade during initiation of immunization (Beutler and Cerami 1989; Conrad et al. 2018). IL-1β is mainly produced by bone marrow mononuclear cells and is rapidly synthesized into a factor released by inactive tissue of proactive cytoplasmic precursor (pro-IL-1β) by triggering a Toll-like receptor (TLR) or other pattern-recognition receptor (PRR) by a pathogen product (Dinarello 2018). IL-6 is a pleiotropic cytokine produced primarily by lymphocytes, monocytes/macrophages, epithelial cells, and tumor cells (Ekshyyan et al. 2016). Some researchers have found that cadmium can induce a large increase in inflammation-related factors, such as TNF-α and IL-1β in the lung tissue, as well as overexpression of related chemokines CXCL2 and CXCL3 (Låg et al. 2010). Studies by Demenesku et al. (2016) and Phuagkhaopong et al. (2017) have shown that cadmium induces an increase in the levels of TNF-α, IL-1, IL-6, and IL-8 in the spleen and brain tissue. *Ganoderma lucidum* triterpenoids have significant anti-inflammatory effects, and studies have shown that *Ganoderma lucidum* triterpenoids can down-regulate the

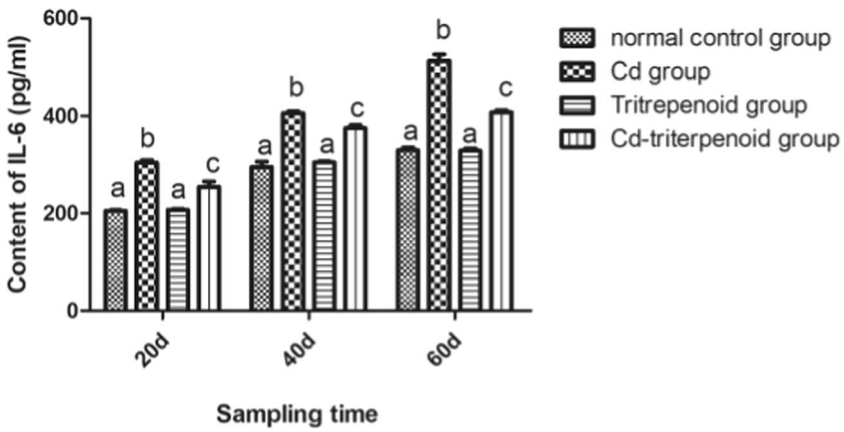

Fig. 10 Expressivity dynamics changes of the IL-6 concentration in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on cadmium-induced inflammatory factors in the spleen. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Expressed as mean ± standard deviation. $n = 10$

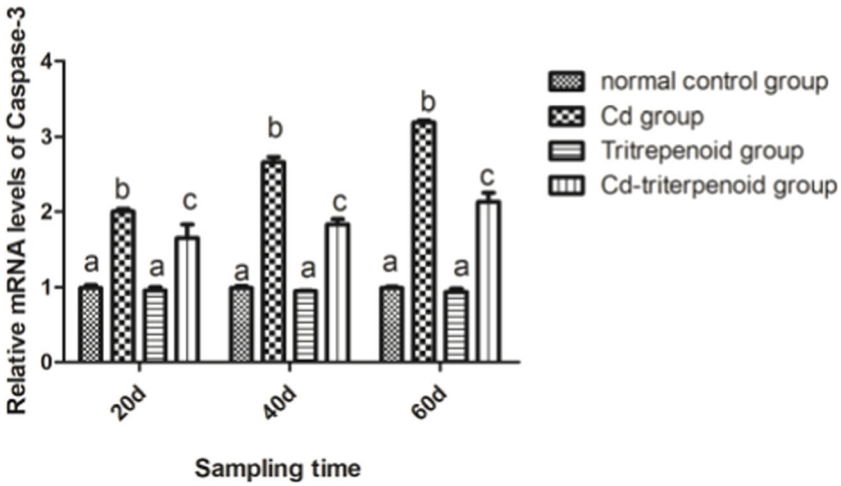

Fig. 11 The mRNA levels of caspase-3 in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on the expression of Bax, Caspase-3 and Bcl-2 mRNA in spleen induced by cadmium. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Expressed as mean ± standard deviation. $n = 10$

expression of TNF-α, IL-1β, and IL-6 by inhibiting NF-κB signaling pathway in mice (Chi et al. 2018). Studies by Solip et al. (2014) showed that *Ganoderma lucidum* triterpenoids inhibited the expression of TNF-α and IL-6 in RAW 264.7 cells. The results of this study showed that the expression levels of TNF-α, IL-1β, and IL-6 in the spleen of chickens in the Cd–triterpenoid group were significantly lower than those in the Cd group, indicating *Ganoderma lucidum* triterpenes can effectively inhibit the inflammatory response caused by cadmium and reduce the inflammatory injury to tissues. Reducing the inflammatory response induced by cadmium by *Ganoderma* triterpenes may be related to the inhibition of the expression of related inflammatory cytokines and alleviating the oxidative stress level induced by cadmium.

Apoptosis is a normal physiological phenomenon of cell death regulated by genes, but Cd can induce apoptosis in many aspects and cause damage to tissues and organs, which has a serious impact in organ function. Apoptosis involves a family of proteases, and an excess of pro-inflammatory mediators can trigger apoptosis and ultimately activate the family of cysteine proteases, of which caspase-3 is the most important member (Wang et al. 2018b; Zhao et al. 2018). Research have shown that TNF-α induces apoptosis or necrosis by activating caspase-3, which cleaves various cellular substrates and triggers apoptosis (Nagase et al. 2002; Fan et al. 2014). It is the meeting point of the apoptotic signaling pathway. Once caspase-3 is activated, the apoptotic pathway is also activated (He et al. 2018). It is well-known that the Bcl-2 family of proteins is a key factor in regulating apoptosis by controlling mitochondrial outer membrane permeabilization (Wang et al. 2019). Bax is a protein that is homologous but opposite in function to Bcl-2 and is also localized in cells. Its self-forming homodimer can promote apoptosis, while the heterodimer formed by binding to Bcl-2 can inhibit apoptosis (Wang et al. 2018e). Studies have shown that both caspase-dependent and independent pathways are involved in cadmium-induced apoptosis and caspase-3 is activated in the apoptotic pathway (Liu et al. 2016). Mahdavi et al. (2017) reported that cadmium induced neuronal apoptosis in rats, resulting in increased expression of caspase-3 mRNA in a dose-dependent manner and decreased Bcl-2/Bax mRNA values, confirming that cadmium-induced apoptosis was through caspase-3-activated mitochondrial pathway. Interestingly, we found that the levels of caspase-3 and Bax mRNA in the spleen of the Cd group

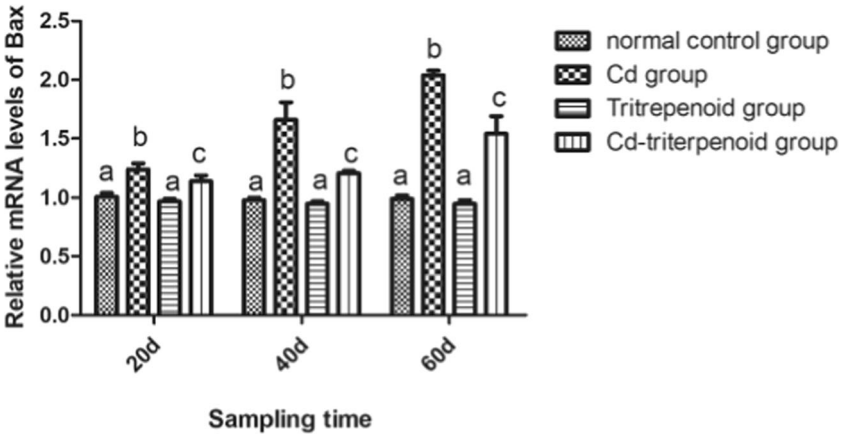

Fig. 12 The mRNA levels of Bax in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on the expression of Bax, Caspase-3 and Bcl-2 mRNA in spleen induced by cadmium. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Expressed as mean ± standard deviation. $n = 10$

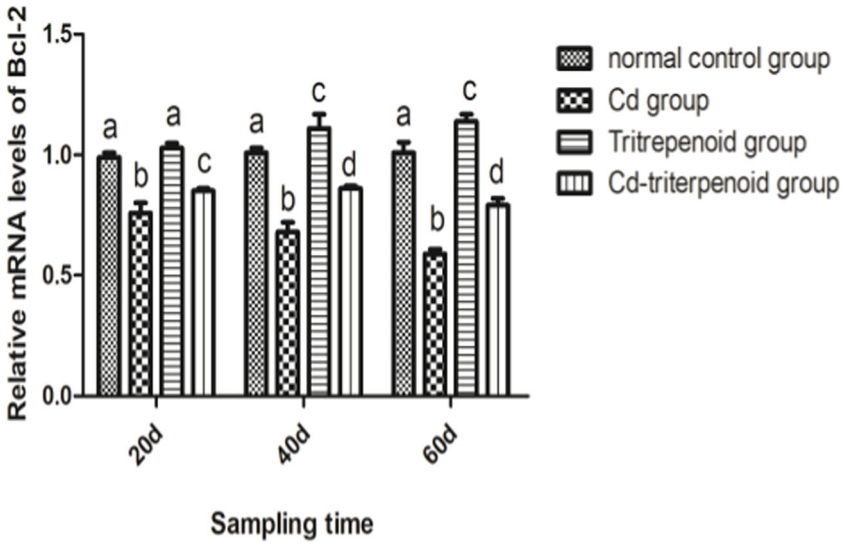

Fig. 13 The mRNA levels of Bcl-2 in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on the expression of Bax, Caspase-3 and Bcl-2 mRNA in spleen induced by cadmium. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Expressed as mean ± standard deviation. $n = 10$

were significantly higher than those in the normal control group, while that of Bcl-2 was significantly decreased throughout the experiments, which is consistent with those in the previous studies. Cadmium induces the permeability of mitochondrial membrane, changes the intracellular calcium homeostasis, and releases cytochrome C in mitochondria, which activates caspase, while Bcl-2 completely inhibits these effects of cadmium. Studies have shown that caspase-3 is induced by cadmium. The role of apoptosis is important, and the inhibition of Bcl-2 may be related to the regulation of cytochrome C release (Bao et al. 2017). Studies by Smina et al. (2011) have shown that *Ganoderma lucidum* triterpenoids can effectively protect DNA-induced DNA damage and apoptosis. The results of this experiment showed that the levels of caspase-3 and Bax mRNA and protein expression in the spleen of the Cd–triterpenoid group were significantly lower than those in the Cd group, while the mRNA and protein expression levels of Bcl-2 were significantly increased. Evidences suggested that Chinese herbal extracts have a strong inhibitory effect on cadmium-induced apoptosis, and its mechanism may be related to the regulation of the expression of apoptotic genes. For instance, green tea extract had protective effects on cadmium-induced testicular apoptosis in male Wistar rats, and the caspase-3 level in the Cd–triterpenoid group was significantly lower than that in the Cd group (Abdelrazek et al. 2016). Sinapic acid can effectively inhibit cadmium-induced up-regulation of caspase-3 and Bax expression and down-regulation of Bcl-2 expression (Ansari et al. 2017). Strawberry extract has protective effects against acute nephrotoxicity induced by cadmium in rats by reducing the expression of pro-apoptotic factor mRNA and up-regulating the expression of anti-apoptotic factor mRNA (Elkhadragy et al. 2017). These studies support the results obtained from our study. *Ganoderma lucidum* triterpenoids can inhibit the expression of cadmium-induced proapoptotic factors, caspase-3 and Bax, promote the expression of Bcl-2,

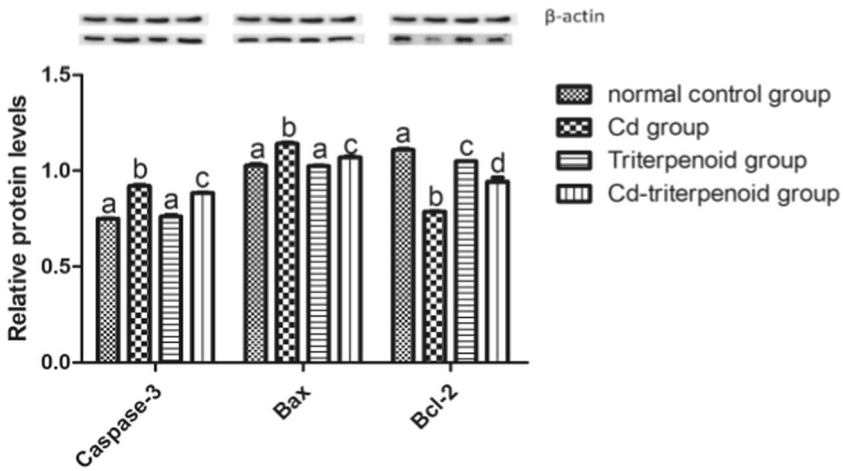

Fig. 14 The levels of apoptotic protein in chicken spleen. The effect of *Ganoderma lucidum* triterpenoids on the expression of Bax, caspase-3, and Bcl-2 in spleen induced by cadmium. At the same time point, the normal control group was used as the benchmark, and the different letters represented significant differences ($P < 0.05$). Expressed as mean ± standard deviation. $n = 10$

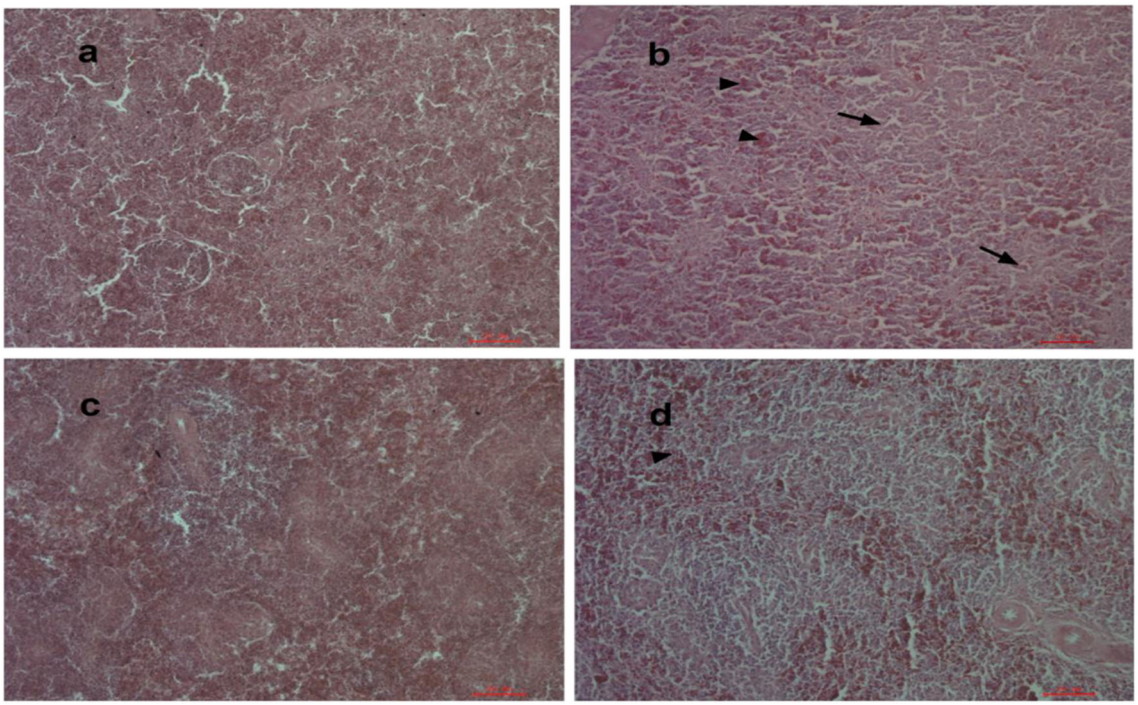

Fig. 15 Sections of the spleen stained with H&E. (**a**) Spleen of the normal control group. In the spleen of the Cd group (**b**), the long arrow shows a decreased number of lymphocytes and the front arrow shows a large number of red blood cells. (**c**) Spleen of the triterpenoid group. In the spleen of the Cd–triterpenoid group (**d**), the anterior arrow shows the red intramedullary red blood cells

and prevent apoptosis during the apoptotic phase. Therefore, *Ganoderma lucidum* triterpenoids can reduce tissue apoptosis induced by cadmium and protect spleen tissue cells.

Conclusion

In conclusion, *Ganoderma lucidum* triterpenoids can reduce the cadmium content of spleen cadmium, enhance the activity of antioxidant enzymes (GSH-Px, SOD), reduce the expression of MDA and inflammatory factors (TNF-α, IL-1β, and IL-6), regulate the expression of apoptotic proteins (Bax, caspase-3, Bcl-2), and alleviate the morphological damage of spleen. *Ganoderma lucidum* triterpenoids can be considered as a natural drug that has protective effects against cadmium-induced spleen toxic damage.

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Compliance with ethical standards

All procedures used in this study were approved by the agency for the Northeast Agricultural University Animal Protection and Use Committee.

Conflict of interest The authors declare that there are no conflicts of interest.

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