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Protective Effect of Ganoderma Triterpenoids on Cadmium-Induced Testicular Toxicity in Chickens

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Abstract

Studies have shown that cadmium can cause chicken testicular damage, but a protective effect of Ganoderma triterpenoids on cadmium-induced testicular damage in chickens has not yet been reported. The present study was designed to research the protective effect of Ganoderma triterpenoids on cadmium-induced testicular damage in chicken. Eighty healthy 7-day-old Hyline egg laying chickens were randomly divided into four groups with 20 in each group. The control group was fed with normal full-fodder, the model group was fed with normal full-fodder with 140 mg/kg of $CdCl_2$, the Ganoderma triterpenoid treatment group was fed with a full-fodder diet containing 140 mg/kg of $CdCl_2$ and 0.5 mL of Ganoderma triterpenoid solution (20 mg/mL), and the Ganoderma triterpenoid group was fed normal full-fodder and 0.5 mL of Ganoderma triterpenoid solution (20 mg/mL) gavage. The chickens were euthanized at 20, 40, and 60 days, respectively, and the testes were harvested. The changes of cadmium contents, the antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px)), peroxide (malondialdehyde (MDA)), inflammatory factors (interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNF-α)), and apoptosis-related proteins (Bax, Bcl-2, and Caspase-3) were detected. The pathological sections of the testes were made at the same time. The results suggested that Ganoderma triterpenoids could reduce the accumulation of cadmium in testis tissue; reduce the content of IL-1β, IL-6, and TNF-α in cadmium poisoning testis; significantly increase the activity of SOD and GSH-Px; decrease the content of MDA; regulate the expression of Bax, Caspase-3, and Bcl-2; and reduce the damage of testicular tissue. The results showed that Ganoderma triterpenoids have a protective effect on cadmium-induced testicular injury in chicken.

Keywords Ganoderma triterpenoids · Cadmium · Chicken testis · Antioxidation · Inflammatory factors · Apoptosis

Introduction

Cadmium (Cd) is a highly toxic heavy metal pollutant. Ingesting even small amounts of Cd can cause toxicity in animals and humans [1–3]. The environmental pollution produced by industry and agriculture is an important source of the harm of Cd [4, 5]. Food and water contaminated with Cd can lead to animal poisoning. Oral intake of Cd reaches the whole body with blood circulation. Since Cd has a half-life of 40 years, resulting in slow metabolism, long-term poisoning can affect the health status of animals [6–8]. Cd poisoning has many target organs in the animal body, including the kidney [9], liver, testis, lung, placenta, bone, and pancreas [10–12]. At the same time, cadmium can cause oxidative stress [13], autophagy [14], inflammation [15], apoptosis [16], and cancer in animals. Cd also can inhibit the production of nitric oxide in endothelial cells and induce apoptosis by inhibiting the phosphorylation of endothelial nitric oxide synthase [17]. Testicles are one of the three major target organs of Cd in the animal body [18–20]. The number of sperm has been found to decrease, and oligospermia or no semen appears in the testes and epididymis of Cd poisoned rats [4]. Cd-induced damage to the testis can be found at the level of stroma and tubule after acute exposure [21]. Dehao et al. [22] proved that Cd-induced testicular toxicity of quail, with the dose increased, smaller testicular volume significantly. These results demonstrate that Cd can cause toxic effects on the reproductive system of many animals [23]. Exposure to cadmium can alter the

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antioxidant activity of antioxidant enzymes such as GSH and SOD in the antioxidant system, leading to the occurrence of oxidative stress. Cadmium toxicity depends on the production of reactive oxygen species, and cadmium can cause an imbalance between oxidation and oxidation, thereby increasing the production of reactive oxygen species. Studies have shown that the addition of 100 mg/L of $CdCl_2$ to drinking water in 8-week-old mice significantly inhibits the activity of superoxide dismutase and can significantly increase renal lipid peroxidation. Previous studies have shown that cadmium mainly induces apoptosis in the kidney and changes cell signaling pathways and other toxic effects [24].

Ganoderma is a medicinal mushroom that has nutritional health benefits and has been shown to promote health and longevity [25], such as effective treatment of chronic liver disease, hypertension, and hyperglycemia [26], and enhance the antioxidant capacity and anti-inflammatory effect [27]. The rich variety of bioactive compounds in Ganoderma lucidum mainly consists of polysaccharides and triterpenoids [28]. In 1982, Ganoderma triterpenoids were isolated for the first time [29]. The mother nucleus made of isoprene is an important component of triterpenoids. With the development of separation technology, the biological activity of Ganoderma triterpenoids has been extensively studied, including antitumor [30], anti-acetylcholinesterase [31], antioxidant [32], sedative [33] activity, enhancement of learning and memory [34], and anti-inflammatory activity [35].

The exposure of heavy metals to cadmium can cause great harm to human and animal health. As an important active ingredient of Ganoderma lucidum, Ganoderma triterpenoids has many pharmacological activities. However, research was lacking on the effect of Ganoderma triterpenoids on Cd poisoning in chicken testes. The Cd poisoning model of chickens was created in this experiment, and then the Ganoderma triterpenoids were administered to Cd-poisoned chickens every day. The protective effects of Ganoderma triterpenoids on Cd induced testicular injury were determined by measuring the content of Cd, oxidative index, inflammatory cytokines, apoptotic protein, and histopathological changes in chicken testes. This experiment provided experimental basis for exploring the protective effect of Ganoderma triterpenoids on cadmium-induced bird testicular damage and also provided theoretical basis for the prevention and treatment of cadmium poisoning in humans and animals.

Experimental Animals and Design

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

After the Ganoderma fruiting bodies are crushed, they are first extracted with 95% ethanol (Changsha Aetna Fine Chemical Reagent Company, Changsha, China) and then extracted with chloroform (Sinopharm Chemical Reagent Company, Beijing, China) to obtain a neutral extract, which is then subjected to acidification by alkali and extracted with chloroform to obtain an acidic extract. The crude extracts of Ganoderma triterpenoids were separated and purified by thin layer chromatography (Qingdao Ocean Chemical Plant, Qingdao, China), silica gel column chromatography, and preparative high-performance liquid chromatography (Waters Corporation, Shanghai, China). A total of 100 μg/mL of oleanolic acid reference solution (Aladdin Reagent Company, Shanghai, China) was weighed, and 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL portions were placed in test tubes. A blank was used as a control. All tubes were heated in a water bath until the solvent evaporated to dryness, with 0.4 mL of 5% vanillin, a glacial acetic acid, and 1 mL of perchloric acid (Chengdu Jinshan Chemical Reagent Company, Sichuan, China) rapidly mixed after a 15-min water bath at 65 °C. When the solution was cooled to a constant volume of 5 mL, the wavelength was set, and the average absorbance value was measured three times. Absorbance and standard concentration were set as the standard curve of the vertical and horizontal coordinates, based on the relationship between the two established regression equations. The resulting Ganoderma triterpenoids extract volume was 250 mL. The water bath was heated to evaporate the test tube extract, and then 0.4 mL of 5% vanillin, a glacial acetic acid, and 1 mL of perchloric acid were quickly mixed at 65 °C, with 15 min of water bath heating, followed by natural cooling until reaching a constant volume of 5 mL. The control blank reagent was measured at the same wavelength three times. The average absorbance, combined with the regression equation, was calculated by the extract Ganoderma triterpenoids content of 20 mg/mL. Eighty 1-day-old Hyline egg laying chickens were fed to 7 days and were randomly divided into four groups of 20. The first group was the Cd poisoning model group, fed with normal full-fodder and 140 mg/kg of $CdCl_2$ daily [36] (Tianjin Guangfu Science and Technology Development Company, Tianjin, China); the second group was the control group fed normal full-price feed everyday; the third group was treated with Ganoderma triterpenoids, fed with normal full-fodder containing 140 mg/kg of $CdCl_2$ per day and with 0.5 mL (20 mg/mL) of Ganoderma triterpenoid solution; and the fourth group of Ganoderma triterpenoids was fed normal full-price feed, and 0.5 mL (20 mg/mL) of Ganoderma triterpenoid solution was administered daily. The animal test lasted 60 days, during which time all experimental animals were allowed water ad libitum. The experiment was divided into three time points, respectively, at 20, 40, and 60 days when sampling. Each group of five randomly selected chickens were euthanized. The testis tissue collected was divided into two parts. Part of the tissue was placed into liquid nitrogen precooling for the detection of antioxidant enzymes, inflammatory factors, and apoptosis

protein expression levels, and part of the tissue sample was fixed in paraformaldehyde solution at 4 °C for pathological histological examination.

Determination of Cd in Testis

A 0.5-g sample of testis tissue was collected and pretreated with Polytech ST60. The testicular Cd content was measured by ICP-MS (inductively coupled plasma mass spectrometry, Agilent 7800). The test conditions are shown in Table 1.

Table 1 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

Antioxidant Index Test

Testicular tissues were ground in physiological saline with an ice bath and centrifuged at 3000 r/min. The supernatant was collected, and the protein concentration in the supernatant was measured using a Coomassie brilliant blue kit. The activities of glutathione peroxidase and superoxide dismutase were determined by the colorimetric method and hydroxylamine method, respectively. The MDA content was determined by the thiobarbituric acid (TAB) method. All kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Inflammatory Factors and Apoptosis Protein mRNA Levels Were Detected

Testicular samples (0.1 g) were collected according to the instructions to extract total RNA (Beijing Leagene Biotechnology Company, Beijing, China). RNA concentration was detected and reverse transcribed into cDNA, and synthesized cDNA was stored in a − 80 °C refrigerator. β-Actin was used as the as internal reference. Samples were collected at 20, 40, and 60 days by the real-time quantitative PCR (polymerase chain reaction) detecting system method. The mRNA levels of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, Bcl-2, Caspase-3, and Bax were detected in a 20-μL system for each sample and detected with a Light Cycler 480 real-time PCR machine (Roche Light Cycler 480, Basel, Switzerland) at 95 °C for 1 cycle, 2 min at 95 °C for 40 cycles, 20 s at 60 °C, 20 s at 72 °C, and 30 s, using the primer design shown in Table 2.

Apoptotic Protein Expression

Testicular samples (0.1 g) were collected, and 1 mL of protein lysis solution (Beyotime Biotechnology, Shanghai, China) was added to extract testicular tissue total protein. One hundred microliters of tissue protein were added to an equal volume of 2× sample buffer after boiling sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The first polyclonal antibodies were prepared in this laboratory, incubated at a 1:1000 concentration; the second antibodies (Jinqiao Company, Beijing, China) were purchased from the corresponding antibody and incubated to a 1:5000 concentration. To correct the sample load, it was incubated at 1:1000 with β-actin antibody (Beyotime Biotechnology, Shanghai, China) and diluted solution. The second antibody (Jinqiao Company, Beijing, China) purchased the corresponding antibody, and was incubated at the concentration of 1:5000. The exposure was performed using the bio-imaging system of Shanghai Qinxiang Scientific Instrument Company (Shanghai, China).

Histopathological Examination

Testis specimens were fixed in 4% paraformaldehyde solution. Paraffin sections were prepared for hematoxylin and eosin (HE) staining to observe the pathological changes.

Table 2 Gene-specific primers used for qPCR

Gene	Primer (5′-3′)	Accession number
TNF-α	CAGATGGGAAGGGAATGAAC AGAGCATCAACGCAAAAGGG	NM_204267.1
IL-6	ATGGTGATAAATCCCGATGAAG CCTCACGGTCTTCTCCATAAAC	NM_204628.1
IL-1β	TTCCGCTACACCCGCTCACAGT CCGCTCATCACACACGACAT	NM_000576.2
Bcl-2	GGATGCCTTTGTGGAATTGT ATAAGCGCCAAGAGTGATGC	NM_205339.2
Caspase-3	GGCTCCTGGTTTATTCAGTCTC ATTCTGCCACTCTGCGATTT	XM_015276123.1
Bax	GTGATGGCATGGGACATAGCTC TGGCGTAGACCTTGCGGATAA	XM_015290061.1
β-Actin	ATTGCTGCGCTCGTTGTT CTTTTGCTCTGGGCTTCA	NM_205518.1

Data Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 17.0 software package, and all data were assessed using one-way analysis of variance (ANOVA). The differences of one group from the others were considered significant when $P < 0.05$.

Result

Cd Content

Figure 1 shows that Cd levels in testes increased with time and dose of Cd poisoning. Cd content in the testes of the Cd group was significantly higher than that in the control and Ganoderma triterpenoid groups; Cd content in the Ganoderma triterpenoid group was significantly lower than that in the Cd group; at the same time, Cd content in the Ganoderma triterpenoid group was lower than that in the control group without significant difference.

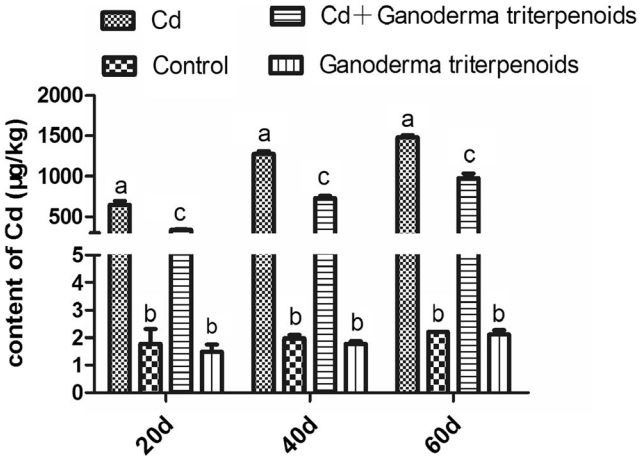

Fig. 1 Effects of Ganoderma triterpenoids on the content of cadmium in testes. At the same time, the blank group was used as a datum, and different letters represent significant differences. ($P < 0.05$)

Results of Antioxidant Index Test

As shown in Fig. 2a, b, the activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in the testes of the Cd group were significantly lower than those of the other three groups at 20, 40, and 60 days. At 20 and 40 days, there were no significant differences between the Ganoderma triterpenoid group and the control group, but these were lower than that in the control group, with significant difference at 60 days. There was no significant difference between the Ganoderma triterpenoid group and the control group in the three points, but both were slightly higher than those in the control group.

As shown in Fig. 2c, the contents of MDA in the testes of the Cd group were significantly higher than those in the other three groups at any time. In three points, the Ganoderma triterpenoid group had no significant difference from the control group but was slightly lower than that of the control group. At the same time, the Ganoderma triterpenoid group and the control group also had no significant differences, but the Ganoderma triterpenoid group was slightly lower than the control group.

Results of Inflammatory Factor

As shown in Fig. 3a–c, the levels of inflammatory factor (TNF-α, IL-1β, and IL-6) mRNA in the Cd group was significantly higher than those in the other three groups. The levels of TNF-α, IL-1β, and IL-6 mRNA in the Ganoderma triterpenoid treatment group were significantly lower than those in the Cd group, and the levels of inflammatory factor (TNF-α, IL-1β, and IL-6) mRNA had no significant difference from the control group.

Results of Apoptotic Protein Expression

As shown in Fig. 4a, b, the transcription levels of Bax and Caspase-3 in Cd group were higher than those in the other three groups at any time point. The transcription level of Caspase-3 and Bax in the Ganoderma triterpenoid treatment group was significantly lower than that in the Cd poisoning model group. The difference between the Ganoderma triterpenoid treatment group and the control group was not obvious.

As shown in Fig. 4c, the transcription levels of Bcl-2 in the Cd group were significantly lower than that in the other three groups, and the transcription levels of Bcl-2 in the Ganoderma triterpenoid treatment group were significantly higher than that in the Cd poisoning group. There was no significant difference between the control group and the Ganoderma triterpenoid group.

The expression of Bax, Caspase-3, and Bcl-2 protein at 60 days was also tested. As shown in Fig. 5, the protein expression of Bax and Caspase-3 in Cd group was higher than that of the other three groups at any time point ($P < 0.05$). The protein expression of Caspase-3 and Bax in Ganoderma triterpenoid treatment group was significantly lower than that in Cd poisoning model group ($P < 0.05$). There was no significant difference between the Ganoderma triterpenoid treatment group and the control group ($P > 0.05$). The protein expression of Bcl-2 was significantly lower in the Cd group than in the other three groups ($P < 0.05$). The protein expression of Bcl-2 was significantly higher in the Ganoderma triterpenoid treatment group than in the Cd poisoning group ($P < 0.05$). There was no significant difference between the control group and the Ganoderma triterpenoid group ($P > 0.05$).

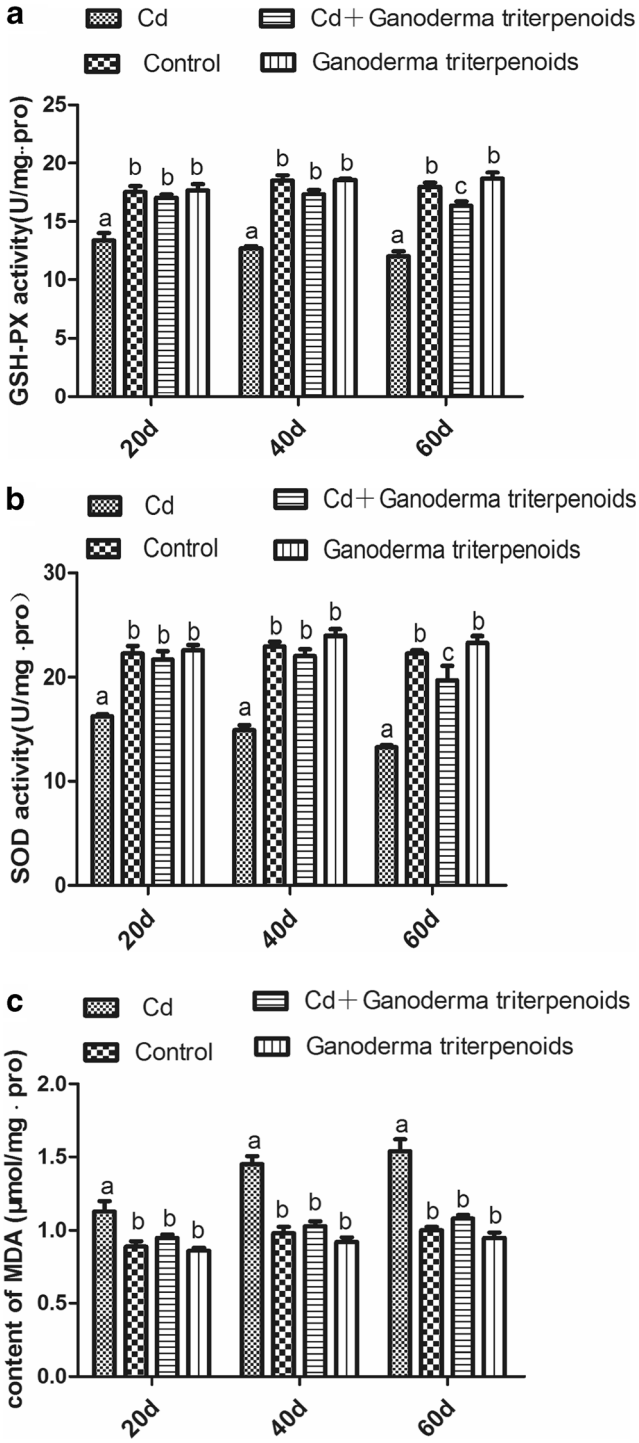

Fig. 2 Effects of antioxidant enzyme activity (GSH-Px and SOD) and MDA content in testes of Ganoderma triterpenoids in Cd-induced injury. At the same time, the blank group was used as a datum, and different letters represent significant differences. **a** The GSH-Px activity. **b** The SOD activity. **c** The MDA content. ($P < 0.05$)

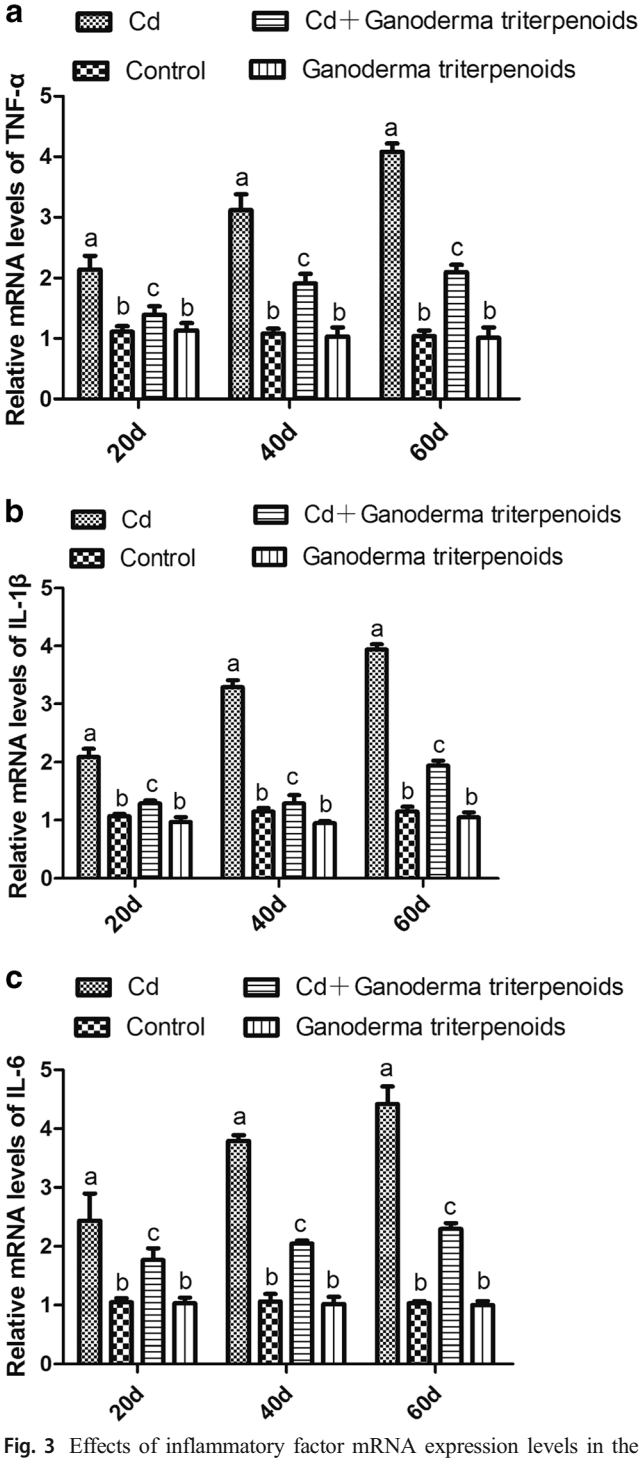

Fig. 3 Effects of inflammatory factor mRNA expression levels in the testes of the Ganoderma triterpenoid group in Cd-induced injury. At the same time, the blank group was used as a datum, and different letters represent significant differences. **a** Relative mRNA levels of TNF-α. **b** Relative mRNA levels of IL-1β. **c** Relative mRNA levels of IL-6. ($P < 0.05$)

Histopathological Changes

This experiment made histopathological sections with testicular samples collected at 60 days. Figure 6a has shown a marked deformation of the seminiferous tubules in the testes of Cd-exposed chickens, with germ cells shedding into the lumen (arrow). Figure 6b is the control group of testicular slices. The control group chickens presented

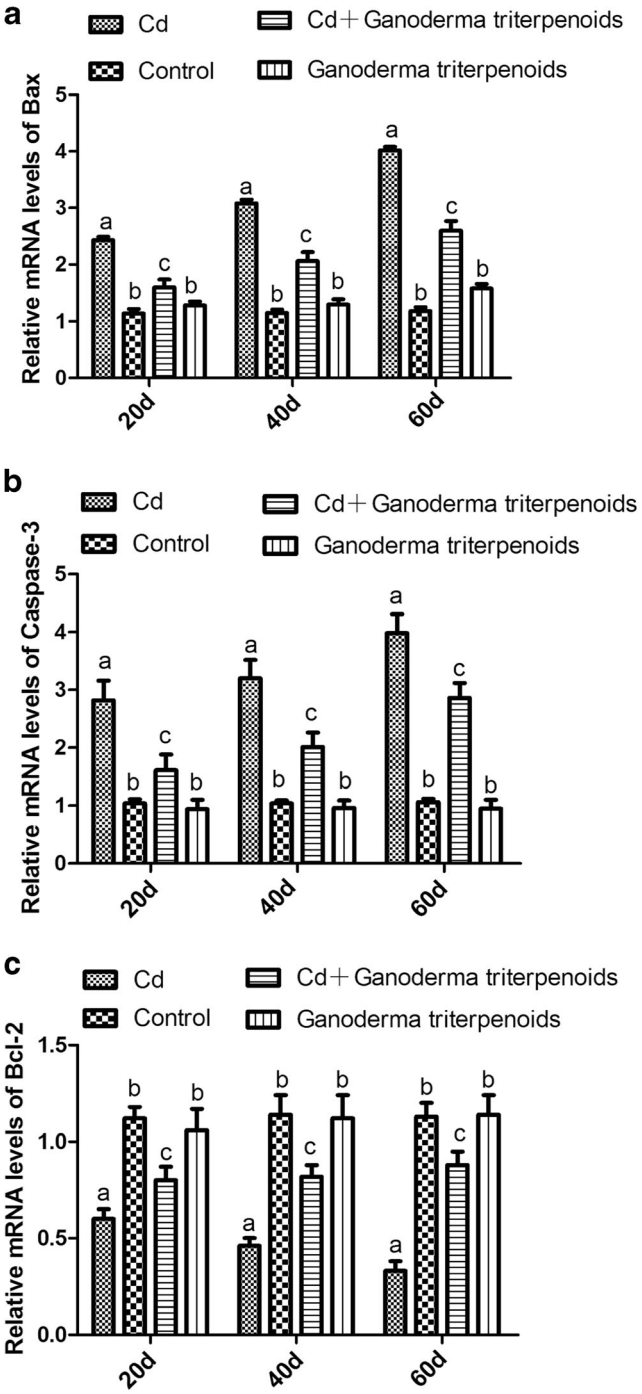

Fig. 4 Effects of Bax, Caspase-3, and Bcl-2 mRNA expression levels in testes of Ganoderma triterpenoids on Cd-induced injury. At the same time, the blank group was used as a datum, and different letters represent significant differences. **a** Relative mRNA levels of Bax. **b** Relative mRNA levels of Caspase-3. **c** Relative mRNA levels of Bcl-2. ($P < 0.05$)

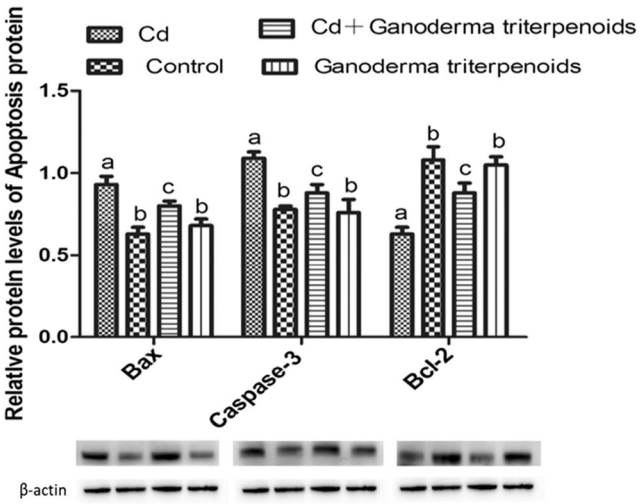

Fig. 5 Effects of Bax, Caspase-3, and Bcl-2 protein expression levels in the testes of Ganoderma triterpenoid on Cd-induced injury. At the same time, the blank group was used as a datum, and different letters represent significant differences. ($P < 0.05$)

normal testes, with seminiferous tubules neatly arranged and structural integrity. Figure 6c shows that the testis tissue has mild lesions compared to the Cd group, and Fig. 6d shows no significant difference from the control group.

Discussion

Cd is a toxic heavy metal, which poisons testes as one of the target organs. The testis is particularly sensitive to cadmium toxicity, and exposure to cadmium in the environment can lead to male infertility and reduced sperm quality [37]. Cd absorbed into the bloodstream soon after the testis can cause accumulation in the testis. Many studies have shown that Cd can not only damage the oxidation system [38] but also cause the body's inflammatory response [39]. Researchers have proven that Ganoderma triterpenoids have the ability to remove and restore 2,2-diphenyl-1-picrylhydrazyl (DPPH) [40]. Additionally, the Ganoderma triterpenoids can reduce the inflammatory response [41]. However, few studies have shown the protective effect of Ganoderma triterpenoids on Cd-induced testicular injury in chicken. The test showed that Cd can accumulate in the testis, inhibit the testis antioxidant enzyme activity, promote the expression of inflammatory cytokines and apoptotic proteins, and cause histopathological injury. However, Ganoderma triterpenoids could alleviate Cd-induced testicular injury by improving antioxidant enzyme activity, reducing inflammatory cytokines and apoptosis protein expression.

Studies have shown that Cd is an oxidant stress inducer. Tremellen [42] reported that oxidative stress is an important inducing factor of infertility. GSH-Px has the function of clearing the harmful metabolites of peroxisomes and protecting the cell structure and function. SOD has the function of clearing superoxide radicals and indirectly inhibiting lipid peroxidation and membrane damage. MDA is a product of lipid peroxidation, indirectly reflecting the degree of oxidative damage [43]. Therefore, the detection of GSH-Px, SOD, and MDA values can reflect the extent of Cd damage to the

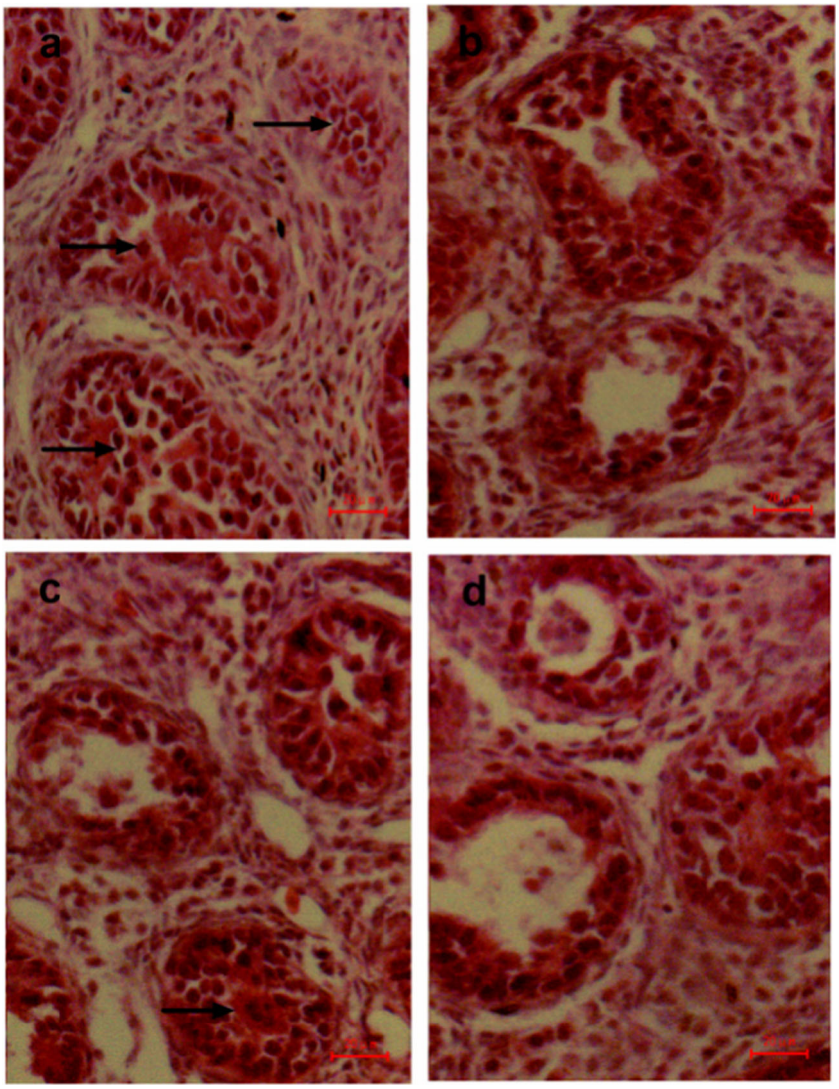

Fig. 6 Sections of the testis stained with H&E. **a** The testicular tissue section of the Cd group. **b** The testicular tissue section of the control group. **c** Testicular tissue section of the Ganoderma triterpenoid treatment group. **d** The testicular tissue section of the Ganoderma triterpenoid group

chicken testes. Some studies have reported that Cd accumulation leads to a decrease in antioxidant enzymes activity in plasma and tissue as well as an increase in MDA content [44]. In this experiment, the activity of GSH-Px and SOD in the Cd group decreased and the content of MDA increased. Compared with the Cd group, the activity of antioxidant enzymes increased, and MDA decreased in the Ganoderma triterpenoid treatment group. In recent years, many studies have shown that triterpenes have anti-oxidant functions. For example, all four methanolic extracts of Ganoderma have significant anti-oxidant activity [45]. Ganoderma triterpenoids could inhibit the production of superoxide anion in rat neutrophils [46]. The experimental results are similar to these results, indicating that Ganoderma triterpenoids can enhance the antioxidative capacity of Cd-induced testicular tissue damage in chickens.

Many studies have shown that Cd can not only damage the body's antioxidant system but also cause the body's inflammatory response. During the course of infection, proinflammatory cytokines play a major role in the inflammatory response process. The proinflammatory cytokines mainly contain TNF-α, IL-1β, and IL-6. This experiment showed that Cd poisoning can significantly increase the expression of TNF-α, IL-1β, and IL-6, which are consistent with the findings of Liu [47]. Increased levels of oxidative stress and inflammatory cytokines play an important role in the pathogenesis of tissue damage by exposure to Cd. Many researchers have shown that triterpenoids can reduce the expression of inflammatory cytokines. For example, triterpenoid acid exerts anti-inflammatory activity by inhibiting the synthesis of nitric oxide (NO) [48]. Ganoderma triterpenoids can reduce cellular inflammatory response induced by lipopolysaccharides (LPS). Ganoderma has a protective effect on LPS/D-GalN-induced acute hepatitis [49–51]. In this experiment, the transcription level of inflammatory cytokines in the Ganoderma treatment group was significantly lower than that in the control group, and the toxic effect of Cd on testes was reduced.

Apoptosis, or programmed cell death, is a normal part of growth and metabolism. Excessive ROS production may result in oxidative stress, loss of cell function, and ultimately apoptosis or necrosis [52]. Cd-induced changes in the mitochondrial environment include changes in mitochondrial membrane potential and decomposition. The excessive ROS according to these changes may promote apoptosis through intracellular acidification and depletion of intracellular antioxidant levels [53]. Pro-apoptotic and anti-apoptotic proteins can mutually regulate the mitochondria-mediated apoptotic pathway [54]. Bcl-2 and Bax alter the permeability of the mitochondrial membrane, resulting in the release of mitochondrial cytochrome C into the cytoplasm, which then activates Caspase-3 [55]. Caspase-3 is a cytoplasmic affinity enzyme which is crucial for the initiation and effector stage of apoptosis. When activated, the process of apoptosis is irreversible [56]. The contamination of Caspase-3 can modulate the mitochondrial pathway-mediated apoptosis [57]. Exposure to low doses of Cd activates Caspase-3 and induces apoptosis [58]. As mentioned earlier, we found in the study that Cd also decreased the transcription of Bcl-2 mRNA and increased the transcription of Bax and Caspase-3. We found that in Cd-poisoned chickens treated with Ganoderma triterpenoids that significantly increased anti-apoptotic protein Bcl-2 mRNA levels, the pro-apoptotic genes Caspase-3 and Bax mRNA levels were significantly reduced. This finding is consistent with the findings that Ganoderma extract significantly inhibits the proliferation of highly metastatic lung cancer cell lines [59] and that the Ganoderma triterpenoids have a protective effect on α-MA induced liver injury by inhibiting apoptosis [60]. This indicates that Ganoderma triterpenoids play an important role in inhibiting Cd-induced apoptosis in testicular cells of chickens.

Histopathology can directly indicate the morphological changes in order to determine whether tissue is healthy. In this experiment, the testicular seminiferous tubules of the Cd group were obviously deformed, and the germ cells shed into the lumen. This result is consistent with previous studies [61, 62]. These lesions were significantly reduced in the Ganoderma triterpenoid treatment group compared with the Cd group, indicating that Ganoderma triterpenoids can alleviate Cd-induced testicular tissue lesions. This result is consistent with the immunomodulatory and anti-inflammatory effects of Ganoderma triterpenoids found by Pu et al. [63]. The LPS-induced lymphocyte proliferation assays and the discovery of Ganoderma Lucidum were reported by Barbieri et al. [64] in melanoma, and triple negative breast anti-inflammatory activity in cancer cells is similar.

Some studies have demonstrated that the *Agaricus blazei* polysaccharides have protective effects in the injured blood system of Cd poisoning mice by inducing the expression of MT. This process will also promote the combination of MT and Cd2+ and reduce the affinity of other major intracellular organelles. Furthermore, it can separate the Cd from other activated proteins or enzymes, which in turn can reduce the poison effects of Cd in the blood system [65–67]. This study found that the concentration of Cd in the Ganoderma triterpenoid treatment group had significantly reduced. However, the mechanism of the modulation of Cd induced by Ganoderma triterpenoids in the animals should be further proven in the future.

Conclusion

Ganoderma triterpenoids can significantly reduce the accumulation of Cd in chickens, enhance the activity of antioxidant enzymes (GSH-Px, SOD), reduce the content of MDA and the expression of inflammatory cytokines (TNF-α, IL-1β, and IL-6) and apoptosis proteins (Bax, Caspase-3, Bcl-2), reduce the damage of testicular tissue morphology, and protect the chickens from testicular toxicity induced by Cd.

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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 conflict of interest.

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