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Effects of *Agaricus blazei* Murill Polysaccharide on Cadmium Poisoning on the MDA5 Signaling Pathway and Antioxidant Function of Chicken Peripheral Blood Lymphocytes

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Abstract This experimental study investigated the effect of *Agaricus blazei* Murill polysaccharide (ABP) on cadmium (Cd) poisoning on the melanoma differentiation-associated gene 5 (MDA5) signaling pathway and antioxidant function of peripheral blood lymphocytes (PBLs) in chickens. The experiments were divided into four groups: 7-day-old chickens with normal saline (0.2 mL single/day), Cd (140 mg/kg), ABP (30 mg/mL, 0.2 mL single/day), and Cd + ABP(140 mg/kg/day + 0.2 mL ABP). Peripheral blood was collected on the 20th, 40th, and 60th days for each group, and PBLs were separated. We attempted to detect the expression of MDA5, downstream signaling molecules, and convergence protein (interferon promoter-stimulating factor 1); transcription factors (IRF3 and NF-κB); the content of cytokines (IL-1β, IL-6, TNF-α, and IFN-β) in PBLs; and the antioxidant index of superoxide dismutase (SOD), malondialdhyde (MDA), and glutathione peroxidase (GSH-Px). The results showed that ABP can reduce the accumulation of Cd in the peripheral blood of chickens; reduce the expression of MDA5 and downstream signaling molecules; and reduce the content of IL-1β, IL-6, TNF-α, and IFN-β in PBLs of chickens. The activity of antioxidant enzymes (SOD and GSH-Px) significantly increased, and the content of MDA decreased. These results showed that they have a certain protective effect of ABP on Cd poisoning in chicken PBLs caused by injury.

Keywords Cadmium · *Agaricus blazei* Murill polysaccharide · Chicken peripheral blood lymphocyte · MDA5 signaling pathway · Antioxidant activity

Introduction

Cadmium (Cd) is one of the main pollutants in the environmental and biological systems. Several studies have shown that Cd causes various forms of damage to mammalian organs (such as the lungs, liver, kidneys, testes, and cardiovascular system) [1–3]. A previous study found that Cd can also damage the body's immune system [4]. It has been reported that Cd can change the function of many kinds of immune cells by affecting protein expression and normal immune cell function. Many studies have recently demonstrated that Cd affects the immune system by suppressing lymphocyte proliferation and hemagglutination [5]. Other studies have confirmed that Cd can cause damage through oxidative stress and the apoptosis of lymphocytes [4, 6]. In fowl, Cd poisoning changes the antioxidant enzyme systems, results in the oxidative damage of the immune system, and also leads to the increase of lipid peroxidation (LPO) [7]. A study has shown that Cd can induce apoptosis in human peripheral blood lymphocytes (PBLs) [8]. Other research has shown that Cd can change the production of cytokines in chicken spleen lymphocytes [9]. However, almost no experiments are available regarding the effect on the PBLs of chickens due to Cd.

The innate immune system uses pattern-recognition receptors (PRRs) to recognize viral pathogen-associated molecular patterns (PAMPs), activating a series of signaling pathways that lead to the expression of downstream effector molecules, such as antiviral signaling proteins and inflammatory cytokines. These reactions can occur naturally and effectively, and can quickly control pathogens and remove their threat to

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the body before adaptive immunity is activated; however, at the same time, they can induce the damage of tissue and cells. Pattern-recognition receptors consist of toll-like receptors, nucleotide-binding oligomerization domain protein-like receptors, and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) [10, 11]. The RLR family is composed of RIG-I, melanoma differentiation-associated gene 5 (MDA5), and LGP2, which play important roles in recognizing viral RNA in the cytoplasm [12]. RIG-I and MDA5 both harbor a central DExD/H-box RNA helicase domain, two N-terminal caspase activation and recruitment domains (CARDs), and a C-terminal regulatory domain. The C-terminal regulatory domain is essential for binding viral RNA [13, 14]. The two CARDs function through CARD–CARD interactions with interferon promoter-stimulating factor 1 (IPS-1), leading to the activation of interferon regulatory factor 3 (IRF3) and nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB) [15], which finally induces type I interferon (IFN-I) production and triggers a series of inflammatory reactions. The study found that a lack of RIG-I receptors in chicken cells [16] and the laboratory study found that the MDA5 signaling pathway of chicken PBLs can be activated by double-stranded RNA (dsRNA) virus (IBDV) [17]. Studies have reported that nickel and cobalt can activate TLR4, and then activate the signaling pathway [18]. Both MDA5 and TLR4 belong to the natural pattern recognition receptors. Presently, there are no reports about the effect of heavy metals on MDA5 and its signal transduction pathways in animals.

Agaricus blazei Murrill (ABM), generally known as the "sun mushroom," has received attention in folk medicine because of its use in treating ailments [19]. In Japan, researchers have experimentally demonstrated immunostimulation and anticancer effects of ABM extracts [20]. It has been proved that *A. blazei* Murill polysaccharides (ABPs) are effective in the treatment of diseases including cancer, diabetes, arteriosclerosis, heart disease, and chronic hepatitis [21]. The polysaccharides phytocomplex of ABM is thought to be responsible for its immunostimulant and antitumor properties [22, 23]. It has been proven that ABPs, for example, have antioxidant, immunostimulant, and antitumor properties. According to reports, ABPs function as antioxidants can remove excess free radicals in the body. Another study found that bupleurum polysaccharides may exert its antiinflammatory and immune efficacy through the TLR4 signaling pathway [24]. Therefore, further research is needed to confirm whether ABPs can affect the pattern-recognition receptors and signal transduction pathways.

In summary, this experiment studied the influence of ABP on Cd poisoning in chicken PBLs by establishing of a model of chronic Cd poisoning in chickens. Chickens were given daily ABP, and Cd poisoning was detected through detection of MDA5 and its signaling pathway activation, cytokine content, and changes in antioxidant function in PBLs.

Materials and Methods

Experimental Design

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

A total of 80 Hyline egg-laying chickens (7 days old) were randomly assigned to 4 groups ($n = 20$/group). Group I served as the control group; each chicken was supplemented with 0.2 mL saline every day (control). Each chicken in group II was administered Cd at a dose of 140 mg/kg $CdCl_2$ (Guangfu Technology Co., Ltd., Tianjin, China) in its feed every day for 2 months (Cd). Each chicken in group III was supplemented with 0.2 mL ABP (30 mg/mL) [25] (Department of Veterinary Medicine, Northeast Agricultural University) in its feed every day for 2 months (ABP). Each chicken in group IV was administered Cd in the same way as those in group II, followed by supplementation with ABP in its feed every day for 2 months (ABP + Cd).

Sampling

Animals were kept for 60 days. During the experiment, animals were given water freely. Peripheral blood was collected from the heart at the 20th, 40th, and 60th days for each group after anesthesia, respectively. Samples were kept at 37 °C for 1 h, 4 °C overnight, centrifuged at 3000 rpm for 15 min at 4 °C, and then stored at −20 °C.

Determination of Cadmium Content

Following the instrument operating manual for ICP-MS (Agilent 7800, Agilent Technologies, Beijing, China), we adjusted the instrument to its optimal working condition; then, we tested the serum (0.5 mL) of each group according to the operating conditions noted in Table 1.

Table 1 ICP-MS operating conditions

	Parameter	Cd
Tuning	Nebulizer gas flow (L/min)	0.96
	Auxiliary gas flow (L/min)	1.4
	Plasma gas flow (L/min)	18
	ICP RF power	1400
Timing	Sweeps/reading	30
	Readings/replicate	1
	Number of replicates	3

Isolation and Culture of Peripheral Blood Lymphocytes

Anticoagulant blood samples were collected, carefully placed in a tube containing the 5 mL of lymphocyte in a separation medium (Tian Jin Hao Yang Biological Manufacture Co. Ltd., China), and then centrifuged (2000×*g*) for 20 min. The cells were collected, resuspended, and washed two times in 5 mL of a cell culture medium (RPMI1640, Thermo Fisher, USA), and centrifuged for 15 min. The cells were then suspended in a complete cell culture medium (RPMI 1640 supplemented with 10% fetal bovine serum and 1% antibiotic–antimycotic solution), and cultured in six-well plates. The density of PBLs was adjusted to 1.5×10^6 cells/mL, and the viability of the freshly isolated cells was always above 95% (trypan blue exclusion test) [9]. The cells were cultured in a 37 °C 5% CO_2 incubator for 24 h, centrifuged for 10 min at 2500 rpm, and then stored at −80 °C. The remaining lymphocytes were used for the extraction of RNA and protein.

RNA Extraction and Real-Time PCR

Total RNA was isolated from lymphocytes using a RNApure Total RNA Fast Isolation Kit (Bio Teke, Beijing, China). The reverse transcription (TaKaRa, Japan) reaction (20 μL) is reported in Table 2. The cDNA were stored at −20 °C for real-time PCR.

To design primers, we used the chicken MDA5, IPS-1, IRF3, NF-κB, and β-actin messenger RNA (mRNA) GenBank sequences. We used Primer 5.0 software to design the primers (Table 3), which were synthesized by Invitrogen Biotechnology Co., Ltd. (Shanghai, China). Real-Time PCR was used to detect the mRNA expression of MDA5, IPS-1, IRF3, NF-κB, and the β-actin gene in cells by using Power SYBR Real-time PCR Premixture (BioTeke, China). For each sample, all reactions were carried out using the Light Cycler 480 Real-Time PCR Machine (Roche Applied Science, China). The program consisted of 1 cycle for 2 min at 95 °C, 40 cycles for 20 s at 95 °C and for 20 s at 60 °C, and a final cycle for 30 s at 72 °C (following the manufacturer's instructions).

Table 2 Reverse transcription system

Composition	System
RNA	2 μL
Oligo (dT)	2 μL
DEPC	8 μL
70 °C 10 min, incubated on ice for 2 min	
5 × M-MLV buffer	4 μL
dNTP (10 m/N)	1 μL
Rnase (RRI)	0.5 μL
M-MLV	0.5 μL
DEPC	2 μL
42 °C 1 h, 70 °C 15 min, chilled on ice	

Table 3 Gene specific primers used in the real-time quantitative reverse-transcription PCR

Primer name			Primer length(bp)
MDA5	Forward	TCAGGAGGAGGACG ACCACGAT	22
	Reverse	TTCCCACGACTCTC AATAACAG	22
IPS-1	Forward	TGCAGGGAGGCCATA CACCAGTG	23
	Reverse	TCCACCTCCCAAGGTG ACCCGTG	23
IRF3	Forward	CTCTCTGACTCTTTCA ACCTCTTCG	25
	Reverse	TGCTGCCTGCTCCTGTGG	18
NF-κB	Forward	TCTGAACAGCAAGTC ATCCATAACG	25
	Reverse	AAGGAAGTGAGGTTG AGGAGTCG	23
β-actin	Forward	ATTGCTGCGCTCGTTGTT	18
	Reverse	CTTTTGCTCTGGGCTTCA	18

Western Blot Analysis of MDA5

The proteins were isolated from the lymphocytes, which were added to 600 μL cell lysis buffer for Western and IP (Beyotime Institute of Biotechnology, China), completely lysed, and centrifuged at 12000 rpm for 10 min at 4 °C; the supernatant was then mixed with an equal amount of 2× SDS buffer, and placed in boiling water for 10 min. Protein from the lymphocytes was added to 8% SDS-PAGE, and then transferred to NC membranes (15 V for 2 h) by using a Trans-Blot SD (Bio-Rad, USA). The membranes were blocked with 5% skim milk in TBST at 37 °C for 1.5 h and incubated overnight at 4 °C with the primary antibodies MDA5 (laboratory preservation [26]) and β-actin (Beyotime Biotechnology), which were diluted to 1:2000 and 1:1000, respectively. Then, the membranes were incubated for 1 h at room temperature with Peroxidase-Conjugated AffiniPure Goat Anti-Mouse IgG (1:5000, ZSGB-BIO, China), respectively. An appropriate amount of high-sig enhanced chemiluminescence western blotting substrate (ECL, Tanon, China) was added to the membranes for imaging. ImageJ software was used to detect the integrated density (IntDen) of every band, and the MDA5 expression levels were expressed as the ratio of the IntDen of MDA5 to the IntDen of β-actin.

ELISA

The selective immunological parameters interleukin 1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and beta interferon (IFN-β) were determined by an ELISA Kit (Elabscience Biotechnology Co., Ltd., China).

Measurement of Oxidative Stress

The content of MDA and the activities of SOD and GSH-Px were assayed using kits following the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

Statistical Analyses

Statistical analyses of all data were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.01 (GraphPad Inc., La Jolla, CA, USA). When a significant value ($P < 0.01$) was obtained according to one-way analysis of variance, further analyses were carried out. All data exhibited a normal distribution and passed equal variance testing.

Results

Determination of Cadmium Content

The serum Cd content was significantly increased in animals exposed to Cd when compared with other treated groups, while the Cd concentration was significantly lower in the Cd + ABP-treated group, than in the Cd-treated group (Fig. 1).

Effect of ABP on the Expression of MDA5 in Peripheral Blood Lymphocytes of Chickens with Cd Poisoning

The MDA5 mRNA level in chicken PBLs was measured by real-time PCR (Fig. 2a). In the present study, the Cd-administered group expression of MDA5 showed elevation in PBLs compared with those of the control group. The expression of MDA5 in the Cd + ABP group was lower than that in the Cd group but higher than that in the control group and ABP group. The expression of MDA5 in the ABP group was higher than that in the control group but not significant.

The MDA5 protein level in chicken PBLs was measured by western blot (Fig. 2b**)**. From the figures, the protein expression of MDA5 in the chicken PBLs was elevated in the Cd-administered group, while the Cd + ABP group showed significantly lower MDA5 protein expression than the Cd group. The difference between the control group and the ABP group was not significant, but the ABP group was still higher than the control group. The expression of MDA5 showed an overall upward trend.

Effects of ABP on the mRNA Levels of MDA5's Downstream Signaling Molecules (IRF3 and NF-κB) in Chicken PBLs with Cd Poisoning

The IPS-1, IRF3, and NF-κB mRNA levels in chicken PBLs were measured by real-time PCR (Fig. 3a–c). The expression of IPS-1, IRF3, and NF-κB showed elevation in the chicken PBLs of the Cd-administered group compared with those of the control group. The expression of IPS-1, IRF3, and NF-κB in the Cd + ABP group was lower than that in the Cd group but higher than that in the control group and the ABP group. Compared with the control group, the expression of IPS-1, IRF3, and NF-κB in the ABP group was not significant. As Cd poisoning time increased, the relative expression of mRNA also increased.

Effect of ABP on Cytokines in Chicken PBLs

Results from this study indicated that the cytokines IL-1β, IL-6, TNF-α, and IFN-β in the cell supernatants were significantly higher in the Cd-treated group. The levels of IL-1β, TNF-α, IL-6, and IFN-β increased with the time of Cd poisoning.

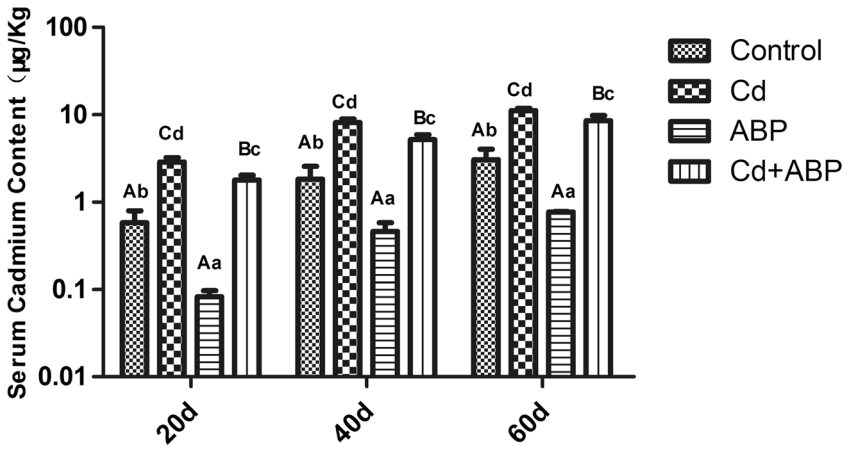

Fig. 1 The serum Cd content was significantly increased in animals exposed to Cd when compared with other treated groups, while the Cd concentration was significantly lower in the Cd + ABP-treated group, than in the Cd-treated group

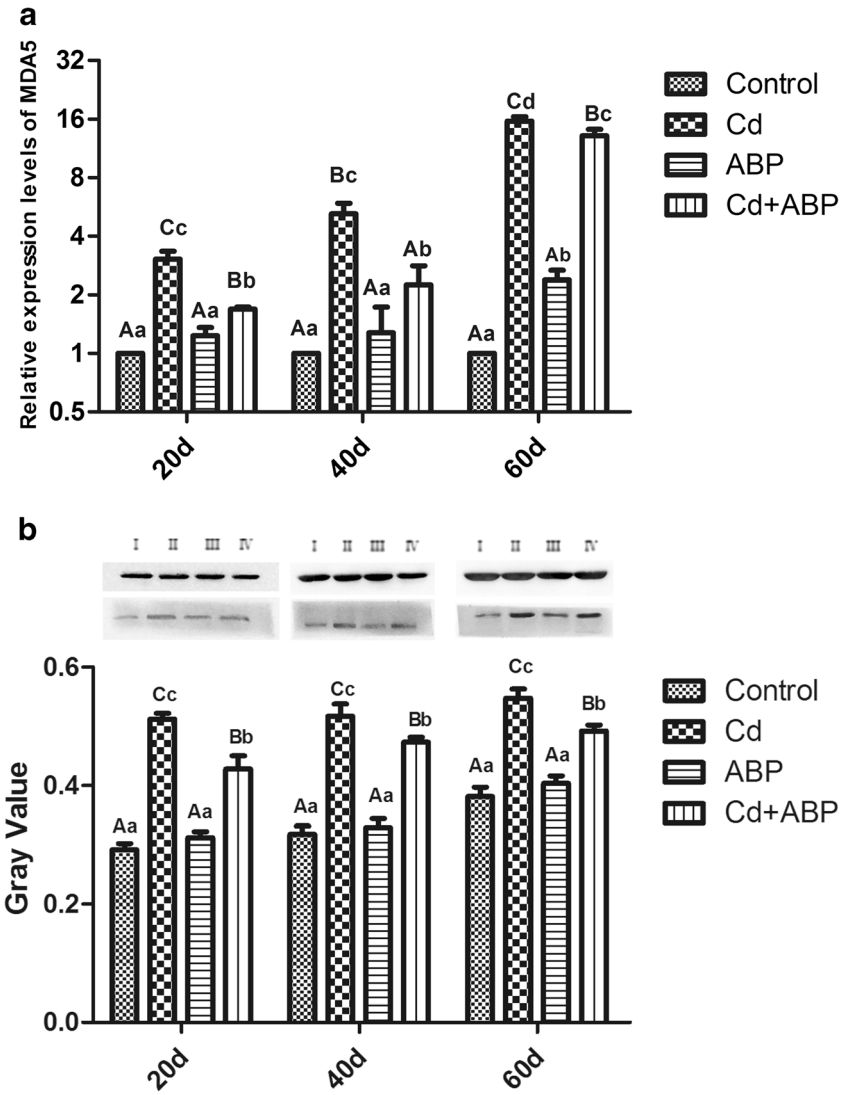

Fig. 2 **a** The MDA5 mRNA level in chicken PBLs was measured by real-time PCR. **b** The MDA5 protein level in chicken PBLs was measured by western blot

Compared with the control group, the four kinds of cytokines were extremely significantly increased in the Cd group. The Cd + ABP group was significantly lower than that in the Cd group. The ABP-treated group of chickens showed no significance in IL-1β, these were significantly increased at the 40th day; these were increased at the 20th day and the 60th day in IL-6 and TNF-α, but not significantly, compared with the control group (Fig. 4a–d).

Antioxidant Index Detection Results

SOD is considered one of the primary antioxidant enzymes in living systems. Treatment of chickens with ABP caused a mild increase in its activity, but the Cd-treated group showed a marked decline in its activity in PBL samples. Treatment of ABP caused a significant recovery in its activity compared to that in the Cd-treated group (Fig. 5a). With the increase in Cd poisoning time, the activity was not changed. GSH-Px is considered one of the main indicators of oxidative stress in the biological system. Hence, its level was estimated in PBLs to assess the burden of oxidative stress after their treatment with ABP, Cd, and their combination. The GSH-Px activity was significantly decreased ($P < 0.01$) in the Cd-treated group compared with the corresponding control group and the ABP-treated group (Fig. 5b). The activity of GSH-Px increased with the time of Cd poisoning. Estimation of MDA was carried out to assess the extent of lipid peroxidation in PBL samples after treatment. ABP demonstrated a decrease in the MDA level in lymphocytes, whereas Cd showed an increase in the samples. In the combination group (Cd + ABP), MDA level decreased in the samples, indicating the ameliorative effect of ABP on Cd toxicity (Fig. 5c).

Discussion

In organisms, Cd stimulation can cause a series of immune responses, maintain the physiological balance of cells, and

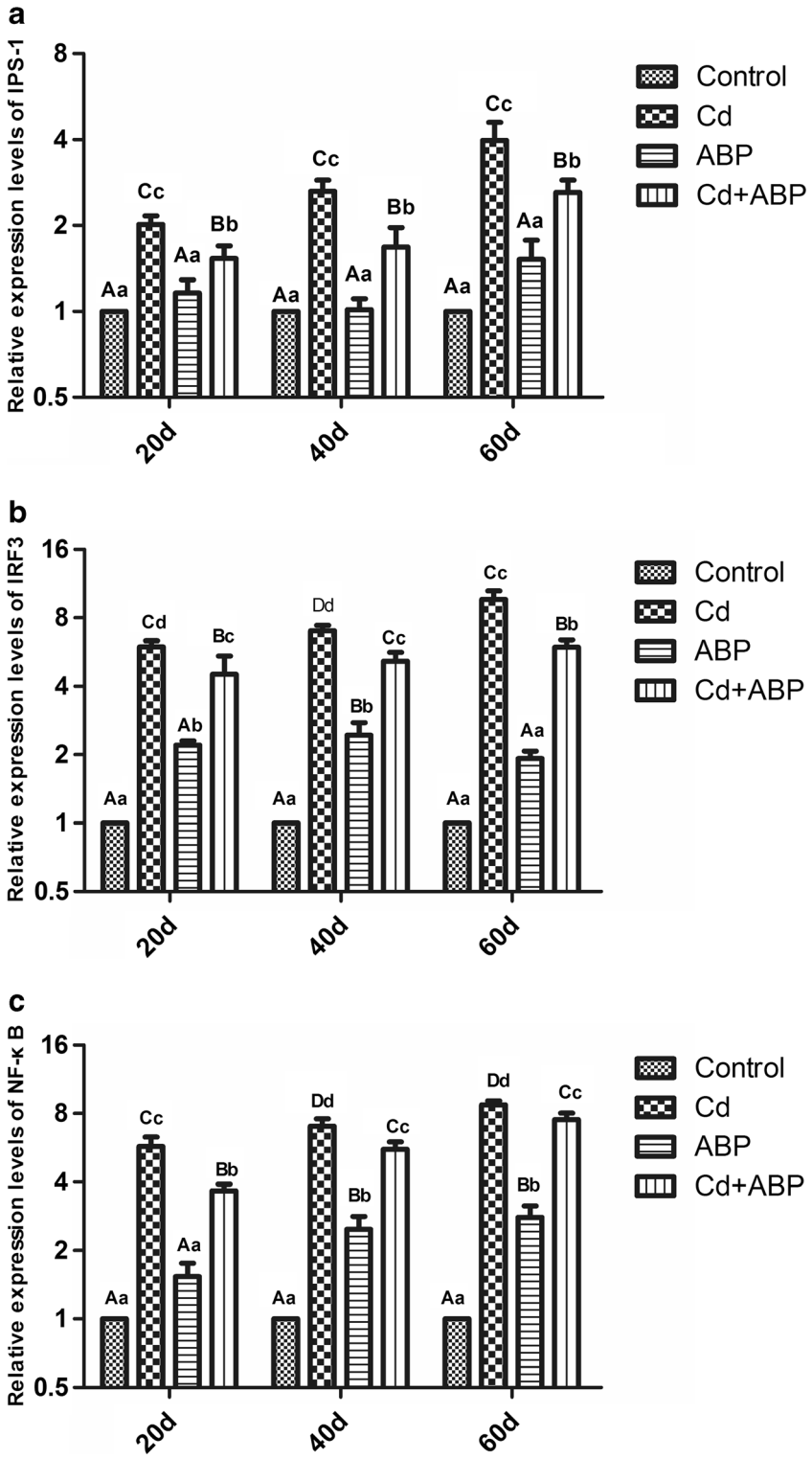

Fig. 3 The **a** IPS-1, **b** IRF3, and **c** NF-κB mRNA levels in chicken PBLs were measured by real-time PCR

maintain the normal signal transduction and immune-related gene expression to improve the ability of biological resistance to external damage. Cd can directly cause tissue damage initially, and subsequent injury can be produced by the inflammatory response [27]. Cd is an immunosuppressive metallic element. It alters a variety of immune cell functions and causes oxidative stress and apoptosis in animals [28]. It has been shown that long-term exposure of animals to Cd induces lesions in many organs [29, 30], including the immune system, which is also a target system for Cd toxicity [31]. Many studies have indicated that Cd has an immunosuppressive effect on the immune system, in which Cd will cause inflammatory

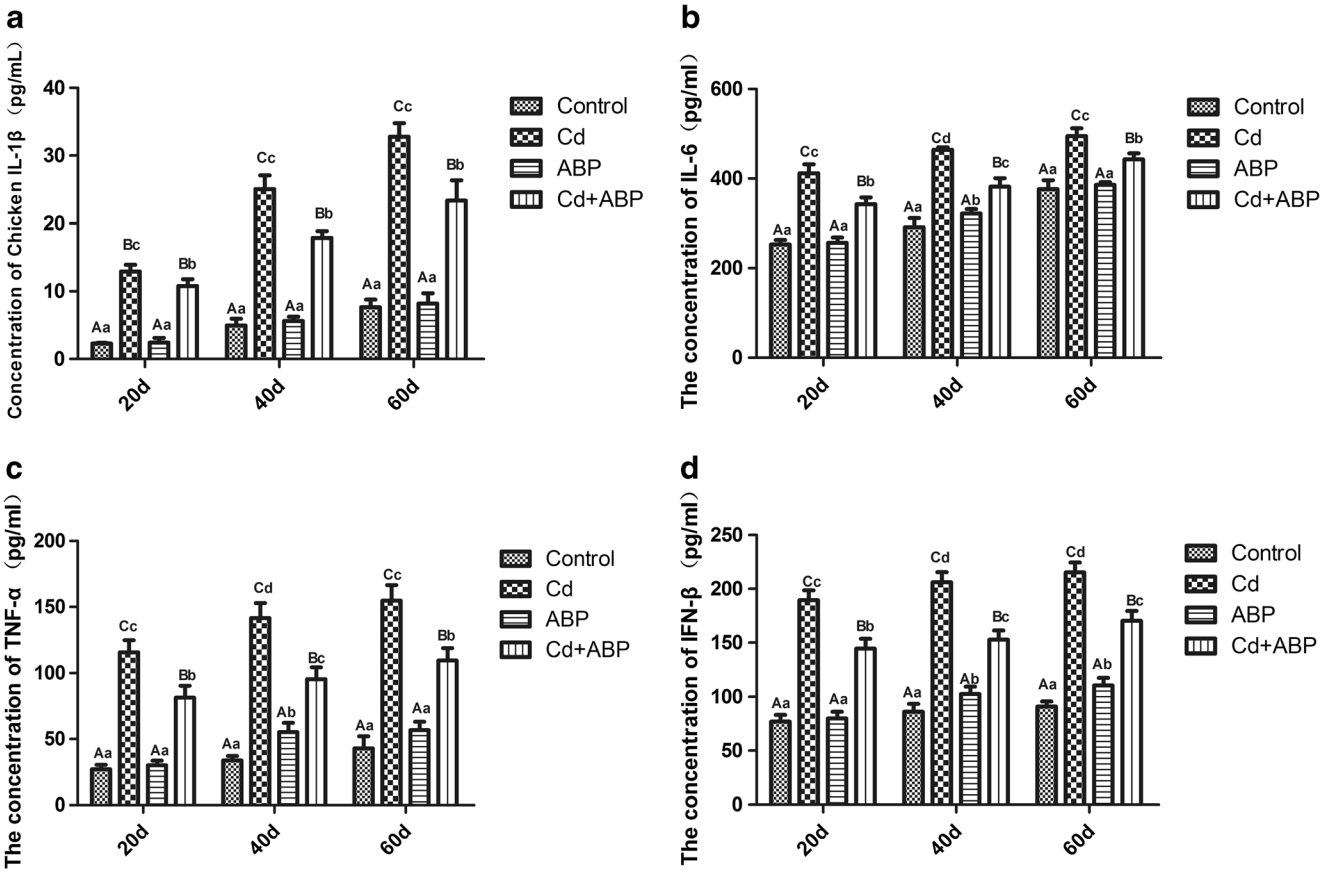

Fig. 4 The ABP-treated group of chickens showed no significance in IL-1β(**a**); these were significantly increased at the 40th day(**b**,**c**), and these were increased at the 20th day and the 60th day in IL-6 and TNF-α (**b**, **c**), but not significantly, compared with the control group (**d**)

damage. Pathak [28] showed that Cd exposure reduced the cell viability of lymphocytes in rodents. In vivo and in vitro studies in mice also have indicated that splenocytes are very sensitive to Cd exposure [28, 32, 33]. The Fengping Xu [34] study showed that Cd can increase the mRNA expression of IL-1β in chicken spleens. Cd can enter human and animal bodies through the digestive tract, respiratory system, skin, and other pathways and then gain entry into the blood cells. When metallothionein (MT) cannot sufficiently bind to Cd^{2+}, free Cd^{2+} causes damage to body tissues and organs. Cd^{2+} through combining with mercaptoethanol or by a competitive or noncompetitive alternative role, it replaces intracellular metal-dependent enzymes, in particular antioxidant metal-based enzymes thus reducing the body's antioxidant enzyme activity. Oxidative damage is caused by the decrease in the free radical scavenging ability. Wen Ming et al. [35] showed that Cd lead to a significant increase in MDA in the serum of mice, while GSH-Px was significantly decreased. Xufang Feng et al. [36] showed that SOD and GSH-Px significantly decreased and MDA content was significantly increased in the serum of ducks after Cd poisoning. Researchers have begun to expand their research to investigate how drugs might reduce the damage caused by Cd poisoning. Over time, drugs with health maintenance and disease management properties have been extracted from plants. ABM and its derivatives have been studied with regard to their safety in terms of biological toxicity and carcinogenicity, and treatment with ABP has been proved to be effective. The immune-protective effect of ABP can be partly attributed to its antiinflammatory and antioxidant properties. A previous study showed that ABP has a scavenging effect on free radicals, which can reduce damage to the body [37]. Haijie Li et al. [38] showed that ABP can reduce the mRNA expression of NF-κB in rats; their results indicated that ABP has a protective effect on the rats' immune injury induced by plumbum. The present study investigated the effect of ABP on PBLs in chickens exposed to Cd. Our results showed that ABP can reduce the accumulation of Cd in serum of chickens and inhibit the activation of the MDA5 signaling pathway in PBLs and the oxidative damage caused by Cd in chickens.

LPO is thought to be an important mechanism for cell membrane injury; MDA is one of its end-products, which is generated during the oxidative degradation of lipids [39]. The system of enzymatic antioxidants includes mainly SOD and GSH-Px. This system can protect the cells from the damage of reactive oxygen species (ROS) and LPO. SOD converts the superoxide anion radical to hydrogen peroxide [40]. GSH-Px catalyzes the oxidation of GSH to GSSG and then eliminates

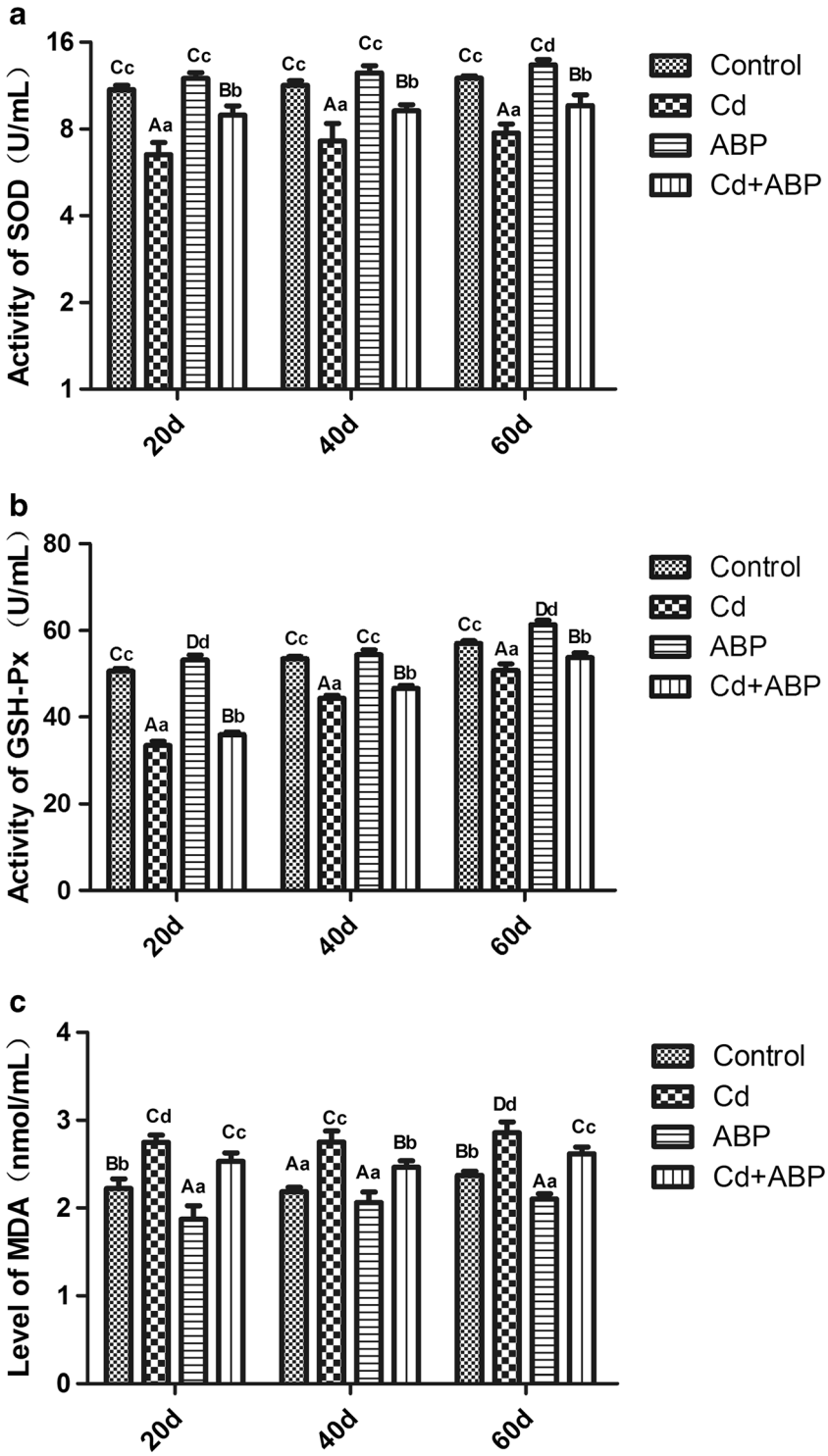

Fig. 5 **a** Treatment of ABP caused a significant recovery in its activity compared to that in the Cd-treated group. **b** GSH-Px activity was significantly decreased ($P < 0.01$) in the Cd-treated group compared with the corresponding control group and the ABP-treated group. **c** In the combination group (Cd + ABP), MDA level decreased in the samples, indicating the ameliorative effect of ABP on Cd toxicity

H_2O_2 [41]. The enhanced LPO may result from the reduction in the activities of SOD and GSH-Px observed in our work, as these antioxidant enzymes provide protection through the elimination of ROS. Cd-induced cell damage is associated with increased LPO. Peng Wang [42] demonstrated that water-soluble polysaccharide ABM can reduce the content of MDA in rat serum. Other studies have shown that the polysaccharides from *Ganoderma lucidum* (PGL) and Angelica and Astragalus (AAP) can increase the activity of SOD and GSH-Px and reduce the content of MDA [43, 44]. This study observed a high level of MDA production after Cd exposure in chicken PBLs; the activities of antioxidant enzymes (SOD

and GSH-Px) were significantly lower in the Cd group than in the control group. These results have shown that treatment with ABP alone caused a significant increase in SOD and GSH-Px activity. ABP can prevent or decrease the harmful effects of oxidants and reactive oxygen species, thus decreasing the MDA concentration in the Cd + ABP group. These results were consistent with the results of previous studies. In this study, ABP played a protective role in the oxidation of the immune cells induced by Cd. Songhai Wu et al. [45] showed that the extraction of polysaccharide from *A. blazei* can remove the free radicals and has antioxidant activity. Hui Zhang et al. [46]. indicated that ABP is directly involved in the quenching of superoxide and hydroxyl radicals, and endogenous cellular oxidant activity and that the organism itself contains large amounts of antioxidants in the active state; the antioxidant activity increased to a level that reduced free radical damage in the body. Therefore, these results indicate that Cd-suppressed immune function and ABP partly attenuate immune toxicity induced by Cd in chicken PBLs. The information presented in this study helps to illuminate the mechanism of Cd-induced immunotoxicity and ABP's protective role.

Cd stimulates the body to produce a series of immune responses in vivo. Cd can affect the cellular immune function by affecting the number of T lymphocytes and the transformation of immune function and cytokines. Some studies [18] have reported that nickel and cobalt can activate the human TLR4, thereby activating pathway. Other studies [47] have reported that copper can increased the expression of NOD2, while the expression of TLR2–5, TLR7, and TLR8 was significantly changed in the digestive gland tissue of the Japanese scallop. MDA5, like TLR and NOD, belongs to the PRRs; therefore, it is speculated that the heavy metal Cd has an effect on MDA5 and its downstream signaling molecules. There are no reports on the effect of heavy metals on MDA5 at present. MDA5 recognizes the dsRNA virus [17], and through a combination of protein-protein contact, it activates its downstream adaptor protein molecule IPS-1, and IκB and IκB kinase. These two kinases activate NF-κB and IFN, thus forming transcription complexes that bind to the promoter region of IFN-β and promote the transcription and expression of downstream cytokines. In this study, the mRNA expression of MDA5 was significantly higher in the Cd treatment group than in the control group, and the protein level of MDA5 also increased in the Cd group. Moreover, the adapter protein (IPS-1) and downstream signaling molecules (IRF3, NF-κB) were also significantly higher in the Cd treatment group than in the control group. In the Cd + ABP group, the expression of MDA5, IPS-1, IRF3, and NF-κB decreased. This suggests that Cd could activate the MDA5 signaling pathway in PBLs of chickens, and ABP could inhibit the activation. Studies have reported that [48] $CdCl_2$ can induce single-strand and double-strand breaks in DNA, causing damage in crucian carp lymphocytes, with an increased toxic dose causing more serious damage to DNA. Study has shown that Cd damages spleen lymphocyte DNA in rats [49], and another showed that Cd can produce oxidative DNA damage to lymphocytes [50]. In this experiment, the correlation analysis of MDA5 protein levels and antioxidant indexes (SOD, GSH-Px, and MDA) of PBLs in chickens was performed (the results are not shown). The results showed that the expression of MDA5 protein in PBLs was negatively correlated with GSH-Px and SOD and positively correlated with MDA. This means that as the expression of MDA5 increased, the activity of SOD and GSH-Px decreased, and the content of MDA increased. Therefore, the peroxidation of Cd poisoning and other factors on chicken PBL DNA damage affected DNA transcription, destabilized the RNA structure and activation of the MDA5 signaling pathway, and stimulated the downstream signaling molecules. The specific mechanism of the MDA5 signaling pathway needs to be studied further.

Marit et al. [51] showed a Cd-induced reduction in IL-1β and IL-6 in human fibroblasts after exposure to 0.098 μg/L Cd^{2+} for 7 h. Other studies found that Cd also induced the release of TNF-α and IL-6 [52–54]. They concluded that Cd could reduce the cytokines in monocytes through signal transduction. In the MDA5 signaling pathway, IRF3 and NF-κB can induce cytokine I-IFN (IFN-α, IFN-β), IL-1 β, IL-6, TNF-α, and NF-κB; therefore, we detected the changes in IL-1β, IL-6, TNF-α, and IFN-β in the lymphocytes of each group. We found that the expression of IL-1β, IL-6, TNF-α, and IFN-β in the Cd group was higher than those in the control group. This was consistent with the results noted above. Because the expression levels of IL-1β, IL-6, TNF-α, and IFN-β in the Cd + ABP group were significantly lower than those in the control group, our results suggested that Cd induced the synthesis and secretion of cytokines in chicken lymphocytes through the MDA5 signaling pathway. ABP can inhibit the activation of the MDA5 signaling pathway to a certain extent and affect the levels of the abovementioned cytokines. Another study showed that ABM has an antiinflammatory effect on the inflammatory factors in the blood and liver [55]. Shaoyi Jia et al. [56] showed that the products (polysaccharides) extracted from the ABM fruiting bodies have a free radical scavenging level. It is suggested that ABP can affect the activation of the MDA5 signaling pathway caused by Cd and affect the level of cytokines by enhancing the body's antioxidant function. In summary, ABP has a protective effect on the lymphocyte damage caused by Cd poisoning.

Conclusion

ABP can reduce the accumulation of Cd in the blood of chickens. Inhibition of Cd-induced activation of the MDA5 signaling pathway reduced the expression of cytokines (IL-

1β, IL-6, TNF-α, and IFN-β), significantly improved the antioxidant enzyme (SOD, GSH-Px) activity, and reduced the level of MDA in PBLs of chicken. ABP has a protective effect on the PBLs of chickens affected by Cd poisoning.

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Compliance with Ethical Standards All procedures used in the current study were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University in China.

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