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Agaricus blazei Murill Polysaccharides Protect Against Cadmium-Induced Oxidative Stress and Inflammatory Damage in Chicken Spleens

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Abstract *Agaricus blazei* Murill polysaccharide (ABP) has exhibited antioxidant and immunoregulatory activity. The aim of this study was to investigate the effect of ABP on cadmium (Cd)-induced antioxidant functions and inflammatory damage in chicken spleens. In this study, groups of 7-day-old chickens were fed with normal saline (0.2 mL single/day), $CdCl_2$ (140 mg/kg/day), ABP (30 mg/mL, 0.2 mL single/day), and Cd + ABP (140 mg/kg/day + 0.2 mL ABP). Spleens were separated on the 20th, 40th, and 60th day for each group. The Cd contents, expression of melanoma-associated differentiation gene 5 (MDA5) and its downstream signaling molecules (interferon promoter-stimulating factor 1 (IPS-1), transcription factors interferon regulatory factor 3 (IRF3), and nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB)), the content of cytokines (interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) and beta interferon (IFN-β)), protein levels of heat shock proteins (HSPs), levels of malondialdehyde (MDA), activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), and histopathological changes of spleens were detected on the 20th, 40th, and 60th day. The results showed that ABP significantly reduced the accumulation of Cd in the chicken spleens and reduced the expression of MDA5, IPS-1, IRF-3, and NF-κB; their downstream inflammatory cytokines, IL-1β, IL-6, TNF-α, and IFN-β; and the protein levels of HSPs (HSP60, HSP70, and HSP90) in spleens. The activities of antioxidant enzymes (SOD and GSH-Px) significantly increased, and the level of MDA decreased in the ABP + Cd group. The results indicate that ABP has a protective effect on Cd-induced damage in chicken spleens.

Keywords Cadmium · ABP · Chicken spleens · MDA5 signaling pathway · HSPs · Antioxidant activity

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Introduction

Cd is a common toxic heavy metal in the environment. It greatly affects the growth and development of plants and can be absorbed into human and animals bodies via food chains or environmental exposures, resulting in the occurrence of many diseases [1, 2]. Cd can damage organs differentially in different species. The immune system is one of the targets of Cd. The spleen is an important peripheral immune organ in the animal body. It has been suggested that toxic effects such as morphological changes, oxidative stress, and ion disorder of the spleen can be induced by Cd among mammals and poultry [3–5].

The innate immune response is the body's first line of defense against potentially harmful organisms [6]. Germline-encoded pattern recognition receptors (PRRs) are appointed to sense pathogen-associated molecular patterns (PAMPs), activate related signaling pathway to resist pathogens, and subsequently induce the production of inflammatory cytokines [7, 8]. PRRs consist of Toll-like receptors (TLRs), nucleotide-binding oligomerization domain protein-like receptors, and retinoic acid-inducible gene I-like receptors (RLRs) [9]. Among them, the cytosolic RNA helicase MDA5, belonging to the RLRs, contains two N-terminal caspase activation and recruitment domains that serve as signaling domains, including a central DExD/H RNA helicase domain that promotes RNA binding and a C-terminal domain that assists in RNA ligand recognition and binding specificity [10]. MDA5 plays fundamental roles in interferon responses after viral infection and acts as intracytoplasmic sensors of double-stranded RNA (dsRNA) and uncapped 5′-triphosphates on viral RNA. Upon activation, MDA5 can activate downstream IRF3 and NF-κB to induce pro-inflammatory cytokine genes to produce a series of inflammatory reactions through interacting with a mitochondrion-anchored adaptor molecule (IPS-1) [11]. Recent studies demonstrated that Cd induced oxidative DNA lesions [12]. Oxidative DNA damage adversely affected DNA transcription and resulted in damage of RNA structure [13]. We previously showed that high level of MDA5 could be detected in normal chicken spleens. However, no mechanistic research has been done on whether the Cd-induced damage in chicken spleens is related to PRR MDA5, the activation of the MDA5 signaling pathway, and the subsequent production of inflammatory cytokines.

Agaricus blazei Murill, a traditional Chinese fungus, possesses numerous biological properties and various pharmacological activities, including antioxidant and anti-inflammatory effects, anticancer activities and immune system enhancement, the modulation of biological homeostasis, and the ability to counteract diseases and prevent cancer [14, 15]. Polysaccharides are one of the main bioactive constituents of *Agaricus blazei* Murill with health functions [16]. *Agaricus blazei* Murill polysaccharide (ABP) is one of the most commonly used mushroom extracts and has been effectively used as an immune modulator and an antioxidant [17, 18]. It has been used as a non-prescription remedy in traditional medicines for cancer, diabetes, hyperlipidaemia, arteriosclerosis, and chronic hepatitis [19]. ABP can strengthen and modulate the immune system, which displays a reduction in the ratio of IL-1β/β-actin and the IL-1β level of a burn wound in rat skin after being fed with ABP [20]. Wu et al. demonstrated that *Agaricus blazei* Murill polysaccharides are natural antioxidants and should be used to produce antioxidants in the food industry [21]. Studies have also shown that ABP has a protective effect on Cd poisoning in chicken peripheral blood lymphocytes caused by injury [22].

In this study, a chicken-based model for exposure to Cd following a daily treatment with ABP was created. Moreover, the Cd content, the histopathological changes, the expression of HSPs, the levels of antioxidant indices, the activation of MDA5 signaling pathway, and the contents of inflammatory cytokines were examined in chicken spleens to determine whether ABP supplementation had a protective effect against the Cd-induced toxic effect in chicken spleens.

Materials and Methods

Experimental Design

$CdCl_2$ (10^{-6} mol/L, Guangfu Technology Co. Ltd., Tianjin, China) and ABP (30 mg/mL, Department of Veterinary Medicine, Northeast Agricultural University) selections were based on our previous work [14]. A total of 80 healthy 7-day-old Hyline egg-laying chickens were randomly divided into four groups of 20 chickens. In Group I, the chickens were maintained on a basic diet containing 0.2 mL saline every day (the control group). In Group II, the chickens were fed with a supplemented diet containing 140 mg/kg $CdCl_2$ (Cd-treated group). In Group III, the chickens were administered with a supplemented diet containing 0.2 mL ABP (ABP-treated group). In Group IV, the chickens were fed with a supplemented diet containing 140 mg/kg $CdCl_2$ and 0.2 mL ABP solution (Cd + ABP-treated group). The feeding experiment lasted for 60 days. During the experiment, the animals were given water freely.

Sampling

Spleens were dissected immediately from five chickens on the 20th, 40th, and 60th day for each group after euthanizing via an overdose intravenous injection of sodium pentobarbital (Nembutal, l50 mg/kg; Abbot Labs, USA). Portions of the

spleen samples were used for the determination of cadmium content. The other portions of the spleen samples were used for detections of levels of proteins and mRNAs. Another portion of the spleen samples were used for the ELISA and antioxidant index analyses. The remaining portion of each spleen specimen fixed in 4% paraformaldehyde solution was routinely processed for histopathological examination.

Cd Content Analysis

The Cd level of a spleen (0.5 g) was determined using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7800, Agilent Technologies, Beijing, China) after pretreating in a graphite digestion system (Polytech ST60, Polytech Instrument Ltd., Beijing, China). The operating conditions are shown in Table 1.

Pathological Lesions

The spleen tissue samples obtained at necropsy were fixed in 4% paraformaldehyde solution, and a routine technology of paraffin section was used to prepare pathological sections, followed by staining with hematoxylin and eosin (H&E) using a light microscope (Leica, German).

Evaluation of Oxidative Stress Markers

Spleen samples were homogenized in physiological saline on ice and centrifuged at 12,000 rpm in 4 °C for 10 min. The supernatants were then collected for the analysis of antioxidation activity. The concentrations of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were measured at 532, 550, and at 412 nm, respectively, using kits following the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

Western Blotting Analysis

The expressions of MDA5 and HSPs in spleens were measured using the Western blotting method. First, the spleen (0.2 g) was cut into pieces at 4 °C in a 600 μL RIPA tissue lysis buffer (Beyotime Institute of Biotechnology, China) containing a mixture of protease inhibitors. The homogenates were centrifuged at 12,000*g* at 4 °C for 10 min. Resulting supernatants were mixed with an equal amount of 2× SDS buffer and placed in boiling water for 10 min. Samples were analyzed under reducing conditions on 8% SDS-PAGE electrophoresis. The protein bands were transferred (15 V for 2 h) onto NC membranes by using a Trans-Blot SD (Bio-Rad, USA). Membranes were blocked (60 min) with skimmed milk in TBST. Blots were then probed with the primary rabbit antibodies anti-HSP60, anti-HSP70, and anti-HSP90 (1:1200 [20]); primary mouse monoclonal anti-chicken MDA5 antibody (1:1000 [22]); or mouse monoclonal anti-beta-actin antibody (1:1000, Beyotime Institute of Biotechnology, Shanghai, China), and then incubated with secondary antibodies horseradish peroxidase-conjugated goat anti-rabbit IgG (1:5000, ZSGB-BIO, China) or goat anti-mouse IgG (1:5000, ZSGB-BIO, China). Blots were revealed using the chemiluminescence Western blotting substrate (ECL, Tanon, China). The band intensities were quantified using ECL accompanying ImageJ software. The MDA5 or HSP (HSP60, HSP70, and HSP90) expression levels were expressed as the ratio of the IntDen of MDA5 or HSPs to the IntDen of β-actin.

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

Quantitative Real-Time Polymerase Chain Reaction

The mRNA expression levels of MDA5, IPS-1, IRF3, and NF-κB in spleens were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR).

First, the appropriate amount of chicken spleen tissue was taken into the mortar and then poured into liquid nitrogen immediately. Then, spleen samples were grinded to homogenates after the liquid nitrogen evaporated. Afterwards, the homogenates were added to 1 mL lysate RL (BioTeke, Beijing, China) and then grinded to homogenates for RNA extraction. Total RNA was isolated from spleens using an RNA-pure Total RNA Fast Isolation Kit (BioTeke, Beijing, China). The concentration of the total RNA was determined using Thermo Nano Drop 2000 (Thermo Fisher Scientific Inc., Waltham, USA). The complementary DNA was then synthesized. Primers for the amplification of genes MDA5, IPS-1, IRF3, and NF-κB were designed using Primer 5.0 software. The primer sequences (Invitrogen Biotechnology Co., Ltd., Shanghai, China) and GenBank accession numbers are shown in Table 2. Gene expression levels of MDA5, IPS-1, IRF3, and NF-κB genes in spleens were assessed by qRT-PCR by using Power SYBR Real-time PCR Premixture (BioTeke, China). The PCR cycles for each reaction were as follows: denaturation at 95 °C for 2 min and 40 cycles of 95 °C for 20 s, 60 °C for 20 s, and a final cycle for 30 s at 72 °C (following the manufacturer's instructions). All reactions were carried out using the Light Cycler 480 qRT-PCR machine

Table 2 Gene-specific primers used in the real-time quantitative chain reaction

Primer name		Primer sequence	Primer length (bp)
MDA5	Forward	TCAGGAGGAGGACGACCACGAT	22
	Reverse	TTCCCACGACTCTCAATAACAG	22
IPS-1	Forward	TGCAGGGAGGCCATACACCAGTG	23
	Reverse	TCCACCTCCCAAGGTGACCCGTG	23
IRF-3	Forward	CTCTCTGACTCTTTCAACCTCTTCG	25
	Reverse	TGCTGCCTGCTCCTGTGG	18
NF-κB	Forward	TCTGAACAGCAAGTCATCCATAACG	25
	Reverse	AAGGAAGTGAGGTTGAGGAGTCG	23
β-actin	Forward	ATTGCTGCGCTCGTTGTT	18
	Reverse	CTTTTGCTCTGGGCTTCA	18

(Roche Applied Science, China). All data from the qRT-PCR experiments were analyzed by the $2^{-\Delta\Delta Ct}$ method [23].

ELISA Analysis

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

Statistical Analyses

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 18.0 software package (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.01 (GraphPad Inc., La Jolla, CA, USA). Differences between means were assessed by using one-way analyses of variance (ANOVAs). All data exhibited a normal distribution and passed equal variance testing.

Result

The Cd Content of Chicken Spleens

As shown in Fig. 1, the Cd content in the Cd group was significantly higher than that in the other groups ($P < 0.01$). The content of Cd in the Cd + ABP group was significantly lower than that in the Cd group ($P < 0.01$) but was significantly higher than that in the control group ($P < 0.01$). Cd concentration was significantly lower in the ABP group than in the Cd group ($P < 0.01$). As the duration of Cd poisoning increased, the relative content of Cd in spleen tissue also increased.

Histopathology of the Spleen

The spleen tissues for pathological sections in this paper were collected at 60 days. As shown in Fig. 2, the chicken spleens in the control group (Fig. 2a) and the ABP group (Fig. 2c) displayed the normal architecture. The red pulp and white pulp of the spleens showed clear boundaries and a high quantity of well-arranged lymphocytes were uniformly distributed in the red pulp and white pulp of the spleen. Exposure to Cd poisoning (Fig. 2b) caused some histological changes to spleen structure. The chicken spleens in the Cd group were characterized by unclear boundaries between the red pulp and white pulp, reduced and loosely arranged lymphocytes, and many erythrocytes full of red pulp. However, in the Cd + ABP group (Fig. 2d), splenic lymphocytes also appeared in a relatively loose arrangement, but the number of spleen lymphocytes exhibited was significantly higher in comparison to that in the Cd group.

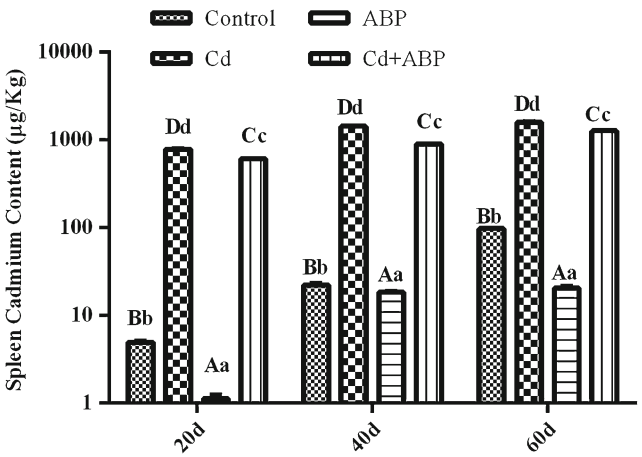

Fig. 1 Effects of Cd, ABP, and ABP + Cd treatment on Cd content in chicken spleens. The values in the control group were used as the reference values. Bars with different capital letters were statistically significantly different between the groups ($P < 0.01$). Bars with different small letters were statistically significantly different between the groups ($P < 0.05$). Bars with same letters indicate no significant differences ($P > 0.05$)

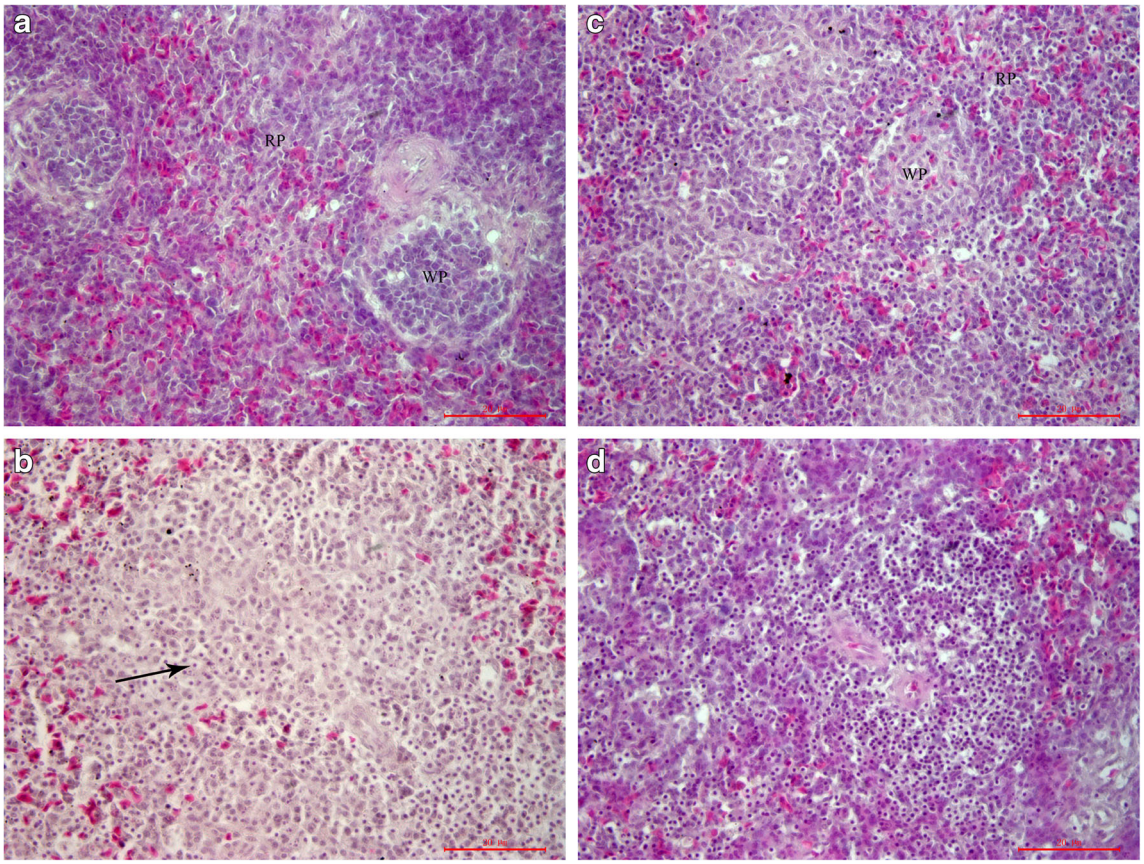

Fig. 2 Effects of Cd, ABP, and ABP + Cd treatment on the histological analysis on day 60 in chicken spleens. **a** Control group (HE, 40×); the red pulp (RP) and white pulp (WP) of the spleen showed clear boundaries. **b** Cd poisoning group (HE, 40×); unclear boundaries between the red pulp and white pulp were shown, lymphocytes showed reduced and arranged relatively loose arrangement (black arrow), and many erythrocytes were full of the red pulp. **c** ABP group (HE, 40×); clear boundaries between the red pulp (RP) and white pulp (WP) were shown. **d** Cd + ABP group (HE, 40×) splenic lymphocytes showed a relatively loose arrangement, but the number of spleen lymphocytes exhibited was significantly higher in comparison to the Cd group

The Protein Expression of Hsp60, Hsp70, and Hsp90

As shown in Fig. 3, the protein expression of HSPs were examined on day 60 using the methods described previously. The levels of HSPs (HSP60, HSP70, and HSP90) were markedly elevated after the Cd treatment ($P < 0.01$). However, the ABP treatment effectively prevents Cd-induced high levels of HSPs (HSP60, HSP70, and HSP90) in chickens ($P < 0.01$). The levels of HSPs (HSP60, HSP70, and HSP90) in the ABP group, without exception, showed no significant difference from those in the control group ($P > 0.05$).

The Antioxidant Levels

As shown in Fig. 4a, b, the activities of SOD and GSH-Px were significantly higher in the ABP groups on days 20, 40, and 60 ($P < 0.01$). The activities of SOD and GSH-Px in the Cd group were significantly lower ($P < 0.01$). However, there was a significant difference in the SOD and GSH-Px levels between the Cd + ABP group and the Cd group ($P < 0.01$), with the Cd + ABP group having significantly higher values.

As shown in Fig. 4c, MDA content was significantly higher in the Cd group compared to the control group ($P < 0.01$) and the ABP group ($P < 0.01$). However, Cd exposure in combination with ABP sharply up-regulated the content of MDA ($P < 0.01$). Additionally, MDA content was significantly lower in the ABP group compared with the control group at days 20 and 40 ($P < 0.05$), and there was a significant down-regulation on day 60 ($P < 0.01$) in the ABP group compared to the control group.

The Expression of MDA5

As shown in Fig. 5, Cd exposure up-regulated the mRNA (Fig. 5a) and protein (Fig. 5b) levels of MDA5 in a time-dependent manner in the chicken spleens. A higher MDA5 level was observed under chronic Cd exposure alone ($P < 0.01$) or in combination with ABP ($P < 0.01$) compared with the control. MDA5 levels were lower in the combined group compared with the single Cd-exposed group ($P < 0.01$). ABP given alone did not affect the mRNA and protein levels

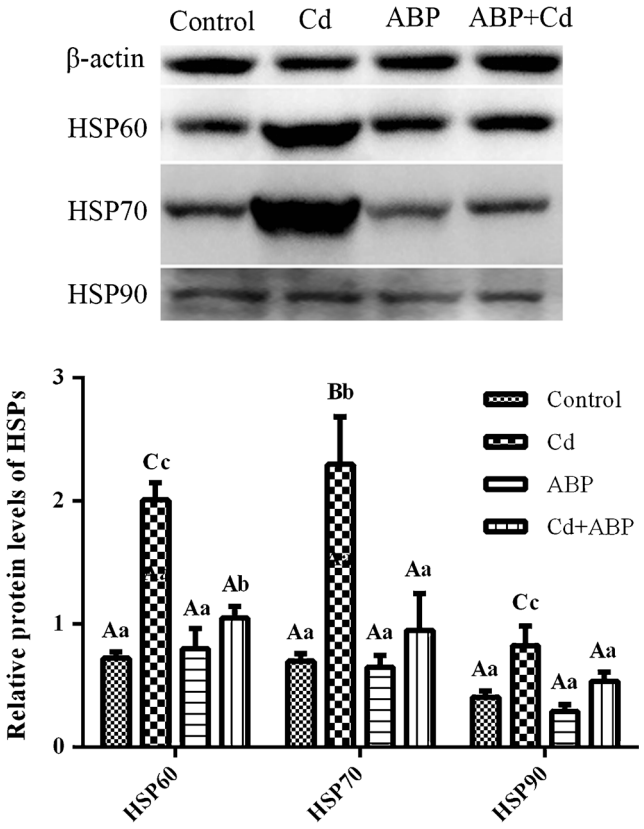

Fig. 3 Effects of Cd, ABP, and ABP + Cd treatment on protein expression of HSP60, HSP70, and HSP90 levels on day 60 in chicken spleens. The values in the control group were used as the reference values. Bars with different capital letters were statistically significantly different between the groups ($P < 0.01$). Bars with different small letters were statistically significantly different between the groups ($P < 0.05$). Bars with same letters indicate no significant differences ($P > 0.05$)

of MDA5 ($P > 0.05$). The relative expression of Cd in spleen tissue increased with the duration of Cd exposure.

The mRNA Expression of IPS-1, IRF3, and NF-κB

To identify the effect of ABP on Cd-induced downstream targets of the MDA5 signal transduction pathway, we examined the mRNA expression of convergence protein IPS-1 (Fig. 6a) and transcription factors IRF3 (Fig. 6b) and NF-κB (Fig. 6c). When compared with the control, Cd treatment significantly up-regulated the expression of IPS-1, IRF3, and NF-κB ($P < 0.01$). In addition, treatment with ABP significantly lowered the expression of IPS-1, IRF3, and NF-κB in spleens when compared with the Cd groups ($P < 0.01$). However, no significant differences were detected between the ABP group and the control group ($P > 0.05$). As the duration of Cd poisoning increased, the mRNA expression of IPS-1, IRF3, and NF-κB in spleens also increased.

The Contents of IL-1β, IL-6, TNF-α, and IFN-β

To further demonstrate the effect of ABP on the Cd-induced MDA5 signal transduction pathway, levels of inflammatory cytokines were examined using the methods described previously. The IL-1β (Fig. 7a), IL-6 (Fig. 7b), TNF-α (Fig. 7c), and IFN-β (Fig. 7d) levels were significantly higher in the groups treated with Cd than in the control group ($P < 0.01$). Levels of the four inflammatory cytokines were significantly lower in the groups treated with ABP than in the Cd group ($P < 0.01$). The expression of the four inflammatory cytokines in the ABP group was slightly higher than that in the control group, but there were no significant differences ($P > 0.05$).

Discussion

With the development of economy and technology, heavy metal pollution, which can pollute agricultural soils and vegetables and is one of the most severe ecological problems worldwide, has become progressively worse. Unlike organic substances, heavy metals are essentially non-biodegradable and therefore accumulate in the environment and the animal body [24]. The toxicity mechanism of heavy metal lead (Pb) may be described as increasing the oxidative stress, promoting apoptosis and activating the NF-κB pathway [25, 26]. The toxicity mechanism of Cd has also been reported in many kinds of cell and animal experiments [27, 28]. However, the mechanism of Cd damage to the spleens is not entirely clear. Great efforts have been made to prevent the adverse effects of Cd [29, 30]. The beneficial effects of ABP have extended to every organism and every organ. Within the past few years, the beneficial effects of ABP in improving oxidation function in the rats [31] and regulating inflammatory damage in the spleen cells of BALB/c mice [32] and in human monocytes and vein endothelial cells in vitro [33] have been widely reported. Recently, in many systems, ABP has been successfully used to reduce oxidative stress caused by excessive Cd exposure in chickens [14, 34]. In this study, we examined the effect of supplemented ABP on Cd-induced oxidative stress and inflammatory damage in chicken spleens. The protective effect of ABP after Cd poisoning could be explained by three mechanisms. First, ABP ameliorated the accumulation of Cd and Cd-induced structural damage in chicken spleens, as reflected by changes in histology. Second, ABP mitigated Cd-induced oxidative damage. For example, ABP reduced MDA level and enhanced antioxidant enzyme activities under Cd stress [14]. Finally, ABP mitigated Cd-induced inflammatory response. For example, Liu et al. suggested that the administration of ABP clearly inhibited Cd-induced levels of inflammatory cytokines on chicken PBLs [34]. However, the protective effects of ABP are not simply associated with the direct scavenging of free radicals or increasing the expression

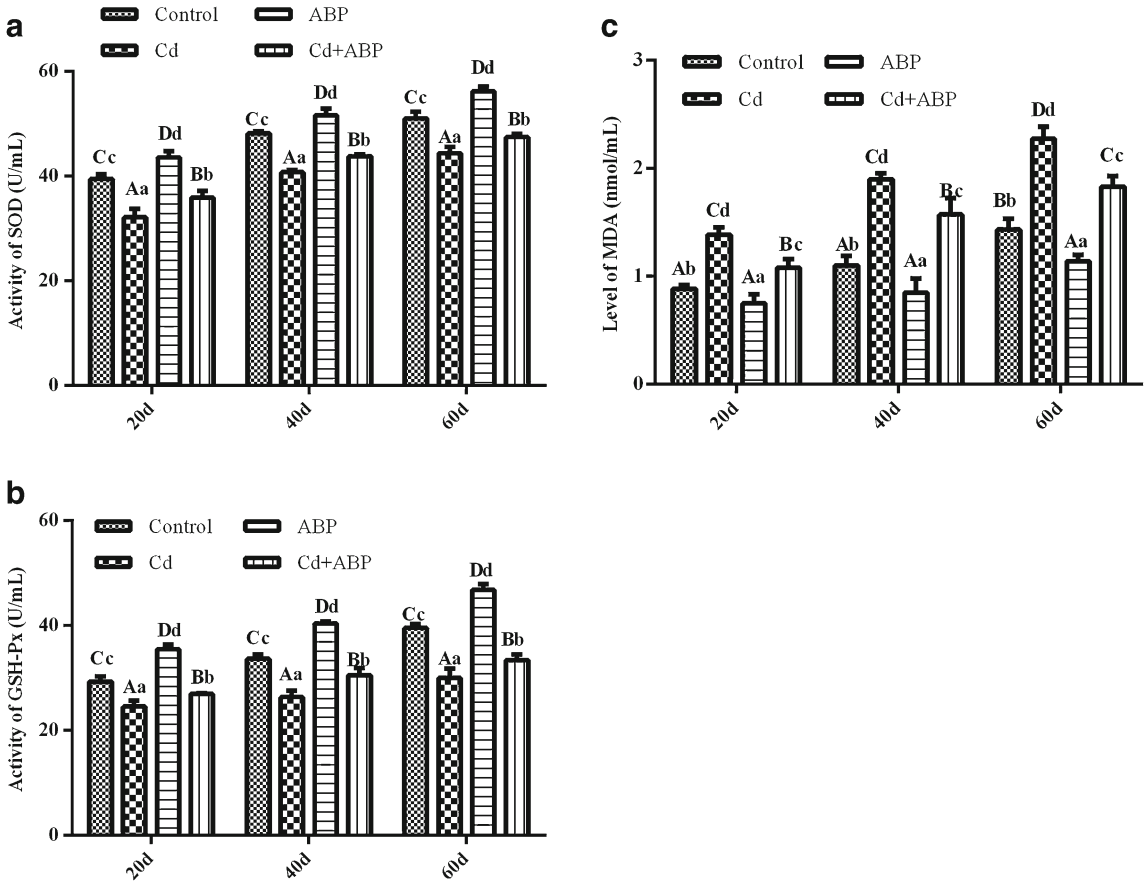

Fig. 4 Effects of Cd, ABP, and ABP + Cd treatment on antioxidant activities of SOD and GSH-Px and MDA content on days 20, 40, and 60 in chicken spleens. The values in the control group were used as the reference values. Bars with different capital letters were statistically significantly different between the groups ($P < 0.01$). Bars with different small letters were statistically significantly different between the groups ($P < 0.05$). Bars with same letters indicate no significant differences ($P > 0.05$)

and activity of antioxidant enzymes and reducing inflammatory activities of cytokines but also involve other mechanisms.

Cd is a heavy metal that is toxic to many organs, including the immune system, which is also a target system for Cd toxicity [35]. In this study, a chicken-based model for exposure to Cd following treatment with ABP was created. We detected that the Cd content of chicken spleens in the ABP group was significantly lower than that in the Cd group. ABP treatment effectively reserved Cd-induced histology characterized by unclear boundaries between the red pulp and white pulp, lymphocyte reduction, and dispersed lymphocytes. HSPs are a family of stress-inducible proteins that help to preserve cellular homeostasis by preventing protein aggregation and apoptosis [36]. The synthesis of constitutive forms of HSPs is significantly up-regulated by a variety of stressful factors, including heat shock, heavy metals, and oxidative radicals [37]. As stress response proteins, HSPs act as a "danger signal" to immune cells and promote immune responses [38]. Other studies have shown that increased heat shock response tried to protect chicken brain tissues from tissue damage caused by oxidative stress [39]. It was previously reported that exposure to heavy metals such as Cd raised the expression levels of Hsp60 and HSP90 [40, 41]. However, Hu et al. indicated that ABP could serve as immune regulators under Cd stress, which inhibited the expression of HSPs in chicken livers [14]. These results were consistent with our findings that ABP treatment effectively prevented Cd-induced high levels of HSPs (HSP60, HSP70, and HSP90) in chicken spleens. One possible explanation is that ABP promoted the metabolism of Cd, resulting in the alleviation of Cd-induced stress, and decreased the levels of HSPs in spleens. Other research showed that the cellular oxidative stress induced by Cd is most likely mediated by the disruption of redox homeostasis and causes oxidative DNA damage [42, 43]. As a consequence, one of the ABP extracts has antigenotoxic, antimutagenic, and antioxidative properties [44–46] that may protect against DNA damage. These vast amounts of evidence confirm that ABP has protective effects on Cd, by preventing the triggering of a range of adverse physiological and pathological effects in spleens. However, the exact mechanism requires further studied.

As a stressor, heavy metals such as Cd can upset the balance of the oxidant/antioxidant system by affecting the regulation of enzymatic oxidation, protein oxidation, and lipid

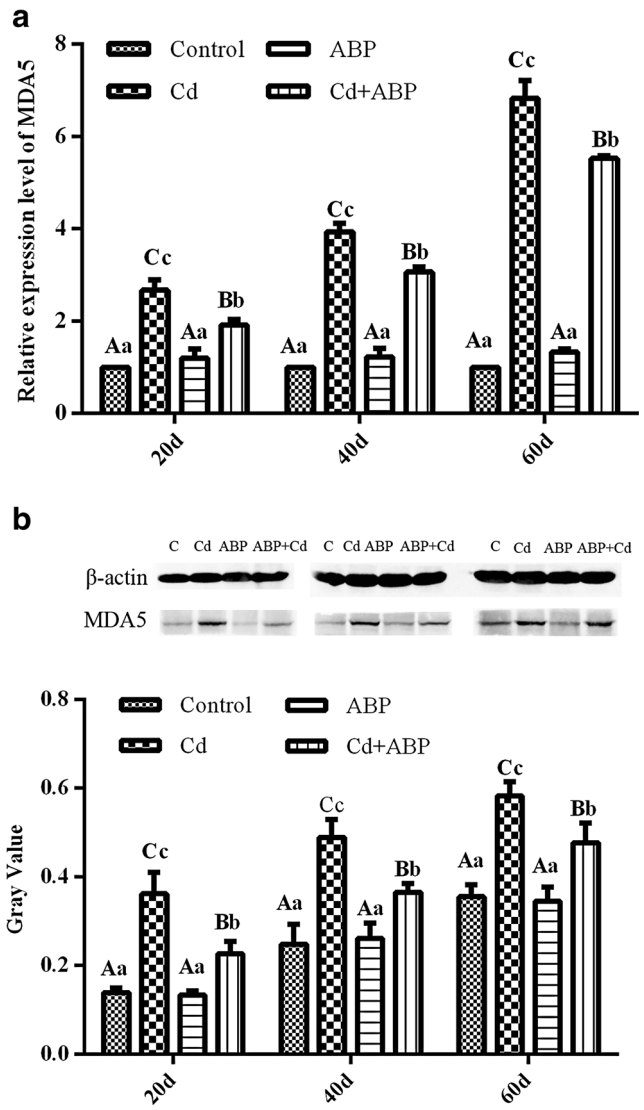

Fig. 5 Effects of Cd, ABP, and ABP + Cd treatment on mRNA and protein expression of MDA5 level on days 20, 40, and 60 in chicken spleens. The values in the control group were used as the reference values. Bars with different capital letters were statistically significantly different between the groups ($P < 0.01$). Bars with different small letters were statistically significantly different between the groups ($P < 0.05$). Bars with same letters indicate no significant differences ($P > 0.05$)

peroxidation activities, and can cause tissue damage [47]. Some studies have reported that Cd accumulation in the body results in a decrease in antioxidant enzyme activities and an increase in MDA level in both plasma and tissues [48]. MDA is an oxidized lipid metabolite and can be used to measure the level of oxidative stress in an organism [9]. GSH-Px and SOD are active in antioxidant defense systems [21]. SOD catalyzes the dismutation of free superoxide anion radicals into hydrogen peroxide and GSH-Px coverts hydrogen peroxide into water and disulfide glutathione, protecting tissues from oxidative damage [49]. Hence, their levels were estimated in chicken spleens to assess the burden of oxidative stress after treating spleens with ABP, Cd, and a combination. Previous studies have connected Cd with oxidative stress because Cd is able to alter the antioxidant defense system [50, 51]. ABP can improve the enzyme activity of SOD, GSH-Px, and CAT in rat liver, kidney, heart, and serum after heavy metal poisoning [34]. Recent research has found that ABP can protect the liver against oxidative damage in rats and is an efficient antioxidant agent against carbon tetrachloride-induced liver injury [31]. Our results showed that excessive Cd intake induced lower GSH-Px and SOD activities, but a higher level of MDA in a time-dependent manner. However, there was an extremely significant increase in SOD and GSH-Px levels but a significant reduction in MDA content in the Cd + ABP group in comparison with the Cd group, which indicated that ABP can protect the spleen against oxidative damage in chickens. However, further studies are needed to address whether other mechanisms play roles in mitigating Cd-induced oxidative damage.

Metals such as Cd can damage numerous biochemical pathways, even at low concentrations [52]. Lee et al. indicated that metals induced many types of protein kinases via the signal transduction within cells, including critical kinases mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI3K)/Akt pathway, and Rous sarcoma oncogene cellular homolog (Src) [53]. It was previously reported that oxidative stress could trigger several types of physical or pathological signal cascades, such as the inflammation response and cytokine responses, and ROS act directly or as signaling molecules in innate immune responses [54]. Oxidative stress triggers a range of physiological and pathological responses either as a result of cellular damage or through specific signaling molecules [55]. Therefore, in the present study, Cd is likely to produce oxidative damage by triggering specific signaling molecules. The MDA5 gene is the member of the RLR family of innate immune responses. Lv et al. suggested that ABP protected against oxidative stress via the MDA5 signaling pathway and indicated that the expression of MDA5 proteins in PBLs was negatively correlated with GSH-Px and SOD and positively correlated with MDA [22]. These results were consistent with our findings that the ABP treatment effectively prevented Cd-induced high levels of MDA, MDA5, IPS-1, NF-κB, and IRF-3 and significantly prevented Cd-induced down-regulation of SOD and GSH-Px in chicken spleens. This indicates that ABP has the capacity to protect against the Cd-induced oxidative stress through MDA5 signaling pathway.

In animals, the induction of the expression of cytokines by environmental chemicals was considered as an inflammation response, which is a key mediator of the host response against microbial pathogens [53, 56]. Fengping Xu et al. proved that Cd significantly increased the expression of IL-1β, TNF-α, and other cytokines in chicken spleen lymphocytes [57]. However, Bernardshaw et al. indicated that ABP modulated the immune system by decreasing the expression of IL-2, IL-4, and

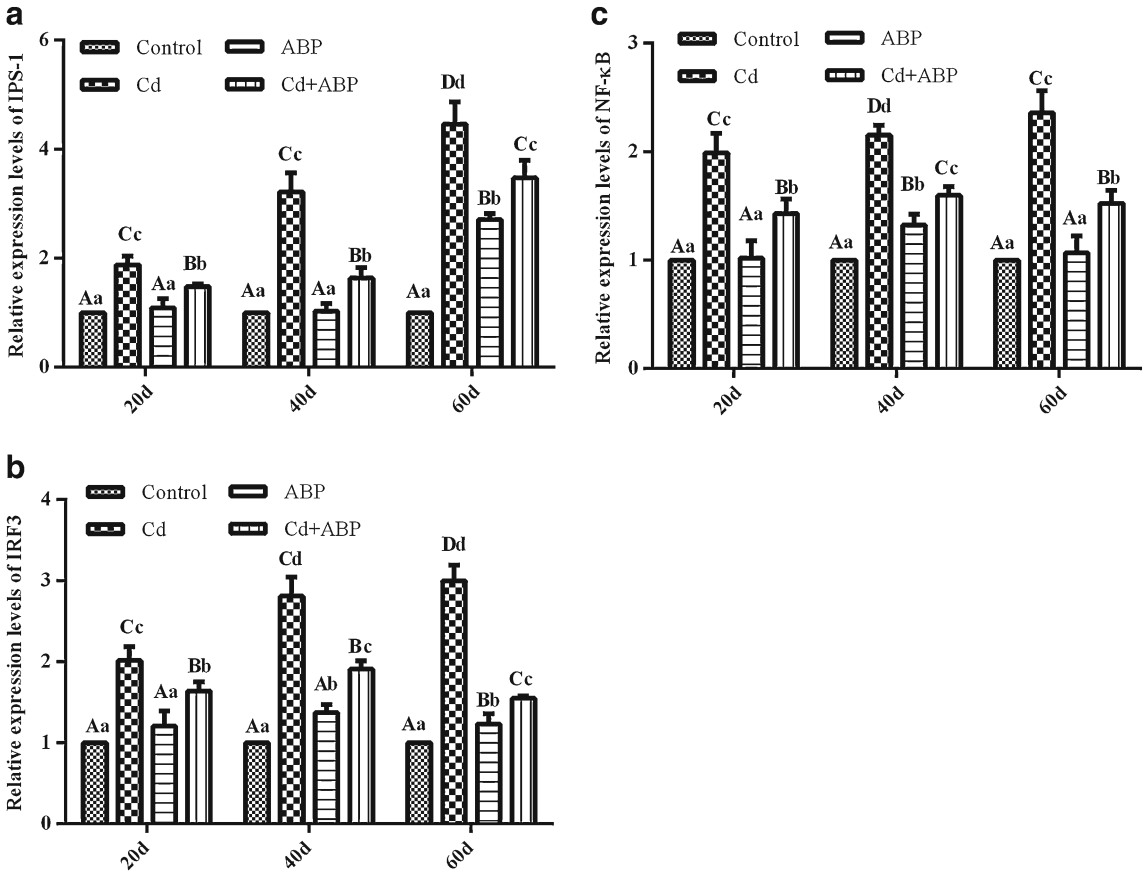

Fig. 6 Effects of Cd, ABP, and ABP + Cd treatment on mRNA expression of IPS-1, IRF3, and NF-κB levels on days 20, 40, and 60 in chicken spleens. The values in the control group were used as the reference values. Bars with different capital letters were statistically significantly different between the groups ($P < 0.01$). Bars with different small letters were statistically significantly different between the groups ($P < 0.05$). Bars with same letters indicate no significant differences ($P > 0.05$)

IFN-γ by peripheral blood mononuclear cells [33]. In this study, Cd significantly increased mRNA levels of IL-1β, IL-6, TNF-α, and IFN-β. However, four inflammatory cytokines were significantly lower in the groups treated with ABP than in the Cd group, suggesting that ABP could protect against Cd-induced inflammatory injury in chicken spleens. However, further studies are needed to address whether other mechanisms are important for ABP to protect against Cd-induced inflammatory injury.

Cd is a potent immunotoxicant that has been reported to affect the immunocytes and immune organs both in humans and in rodents [58]. Research indicates that Cd exposure dramatically enhances the mRNA level of TNF-α via the master inflammatory regulator NF-κB in the brain, ovary, and liver of zebrafish [59, 60]. NF-κB is widely distributed in biological organisms; participates in the regulation of multiple genes, inflammatory responses, and immune responses; and is essential in priming inflammasome activation for the production of cytokines such as TNF-α, IL-6, and pro-IL-1β [61, 62]. Research indicates that the MDA5 gene could interact with IPS-1 to activate the downstream gene IRF3 and NF-κB and influence the production of type I interferon and inflammatory cytokines [11]. In this study, Cd treatment significantly up-regulated the expression of MDA5, IPS-1, IRF3, and NF-κB, indicating that Cd could activate the MDA5 signaling pathway in chicken spleens. Research suggests that the transcription factors IRF and NF-κB are translocated to the nucleus to stimulate expression of interferon (IFN) and pro-inflammatory cytokines [63, 64], which was consistent with our results that Cd promoted the expression of inflammatory factors by the activation of transcription factors IRF3 and NF-κB. It indicated that IRF3 and NF-κB are key transcription factors that regulate the expression of IFN-β and downstream effectors of MDA5. It is also suggested that Cd could induce inflammatory injury in chicken spleens by the activation of the MDA5 signaling pathway. Presently, there are few reports about the effect of ABP on MDA5 and its signal transduction pathways. Cheng et al. indicated that Toll-like receptors (TLRs) might be receptors of ABP and suggested that ABP had anti-inflammatory effects by down-regulating various transcription factors (JNK, ERK, and p38) in MAPK signaling pathways [65]. Both MDA5 and TLR4 belong to the natural pattern recognition receptors. In this study, we showed that ABP could significantly inhibit the Cd-induced mRNA expression of IPS-1, NF-κB, and IRF-3 and decrease the expression of cytokines in chicken spleens. As a consequence, we demonstrate that ABP plays a protective role

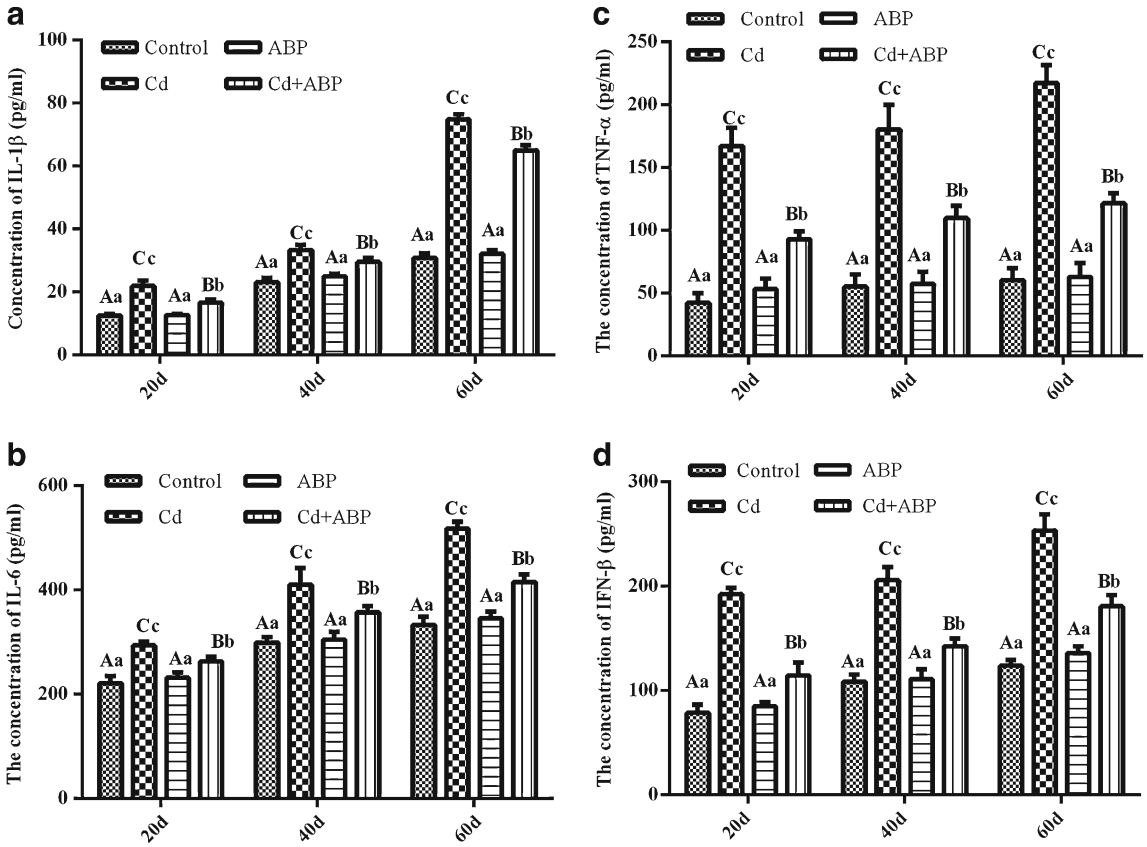

Fig. 7 Effects of Cd, ABP, and ABP + Cd treatment on the expression of IL-1β, IL-6, TNF-α, and IFN-β levels on days 20, 40, and 60 in chicken spleens. The values in the control group were used as the reference values. Bars with different capital letters were statistically significantly different between the groups ($P < 0.01$). Bars with different small letters were statistically significantly different between the groups ($P < 0.05$). Bars with same letters indicate no significant differences ($P > 0.05$)

against Cd-induced inflammatory injury in chicken spleens via the MDA5 signaling pathway.

Conclusion

Our data provide an important insight into the pathway involved in the effects of ABP on Cd-induced immunopathology. ABP can protect against Cd-induced oxidative stress and pro-inflammatory cytokine expression in chicken spleens, and the MDA5 signaling pathway plays a major role in this process.

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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.

Conflict of Interest The authors declare that they have no conflicts of interest.

References

1. Van Maele-Fabry G, Lombaert N, Lison D (2016) Dietary exposure to cadmium and risk of breast cancer in postmenopausal women: a systematic review and meta-analysis. Environ Int 86:1–13
2. Sun H, Wang D, Zhou Z, Ding Z, Chen X, Xu Y, Huang L, Tang D (2016) Association of cadmium in urine and blood with age in a general population with low environmental exposure. Chemosphere 156:392–397
3. Gu X, Chen R, Hu G, Zhuang Y, Luo J, Zhang C, Guo X, Huang A, Cao H (2015) Cell apoptosis of caprine spleen induced by toxicity of cadmium with different levels of molybdenum. Environ Toxicol Pharmacol 40(1):49–56
4. Pollock B, Machin KL (2009) Corticosterone in relation to tissue cadmium, mercury and selenium concentrations and social status of male lesser scaup (*Aythya affinis*). Ecotoxicology 18(1):5–14
5. Liu S, FP X, Yang ZJ, Li M, Min YH, Li S (2014) Cadmium-induced injury and the ameliorative effects of selenium on chicken splenic lymphocytes: mechanisms of oxidative stress and apoptosis. Biol Trace Elem Res 160(3):340–351
6. Banos-Lara Mdel R, Ghosh A, Guerrero-Plata A (2013) Critical role of MDA5 in the interferon response induced by human

metapneumovirus infection in dendritic cells and in vivo. J Virol 87(2):1242–1251
7. Poeck H, Ruland J (2012) From virus to inflammation: mechanisms of RIG-I-induced IL-1beta production. Eur J Cell Biol 91(1):59–64
8. Rietdijk ST, Burwell T, Bertin J, Coyle AJ (2008) Sensing intracellular pathogens—NOD-like receptors. Curr Opin Pharmacol 8(3): 261–266
9. Dostert C, Meylan E, Tschopp J (2008) Intracellular pattern-recognition receptors. Adv Drug Deliv Rev 60(7):830–840
10. Quicke KM, Diamond MS, Suthar MS (2017) Negative regulators of the RIG-I-like receptor signaling pathway. Eur J Immunol 47(4): 615–628
11. Han J, Sun Y, Song W, Xu T (2017) microRNA-145 regulates the RLR signaling pathway in miiuy croaker after poly(I:C) stimulation via targeting MDA5. Dev Comp Immunol 68:79–86
12. Hartwig A (2010) Mechanisms in cadmium-induced carcinogenicity: recent insights. Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine 23(5):951–960
13. Emmanouil C, Sheehan TM, Chipman JK (2007) Macromolecule oxidation and DNA repair in mussel (*Mytilus edulis* L.) gill following exposure to Cd and Cr(VI). Aquat Toxicol 82(1):27–35
14. Hu X, Zhang R, Xie Y, Wang H, Ge M (2017) The protective effects of polysaccharides from *Agaricus blazei* Murill against cadmium-induced oxidant stress and inflammatory damage in chicken livers. Biol Trace Elem Res 178(1):117–126
15. Fanhani JC, Murakami AE, Guerra AFQG, GRd N, Pedroso RB, Alves MCF (2016) Effect of *Agaricus blazei* in the diet of broiler chickens on immunity, serum parameters and antioxidant activity. Semina: Ciências Agrárias 37(4):2235
16. Mizuno T (1995) Bioactive biomolecules of mushrooms: food function and medicinal effect of mushroom fungi. Food Reviews International 11(1):5–21
17. Cui L, Sun Y, Xu H, Xu H, Cong H, Liu J (2013) A polysaccharide isolated from *Agaricus blazei* Murill (ABP-AW1) as a potential Th1 immunity-stimulating adjuvant. Oncol Lett 6(4):1039–1044
18. Liu Y, Zhang L, Zhu X, Wang Y, Liu W, Gong W (2015) Polysaccharide *Agaricus blazei* Murill stimulates myeloid derived suppressor cell differentiation from M2 to M1 type, which mediates inhibition of tumour immune-evasion via the Toll-like receptor 2 pathway. Immunology 146(3):379–391
19. Gonzaga ML, Bezerra DP, Alves AP, de Alencar NM, Mesquita Rde O, Lima MW, Soares Sde A, Pessoa C, de Moraes MO, Costa-Lotufo LV (2009) In vivo growth-inhibition of Sarcoma 180 by an alpha-(1–>4)-glucan-beta-(1–>6)-glucan-protein complex polysaccharide obtained from *Agaricus blazei* Murill. J Nat Med 63(1):32–40
20. Sui Z, Yang R, Liu B, Gu T, Zhao Z, Shi D, Chang D (2010) Chemical analysis of *Agaricus blazei* polysaccharides and effect of the polysaccharides on IL-1beta mRNA expression in skin of burn wound-treated rats. Int J Biol Macromol 47(2):155–157
21. Wu S, Li F, Jia S, Ren H, Gong G, Wang Y, Lv Z, Liu Y (2014) Drying effects on the antioxidant properties of polysaccharides obtained from *Agaricus blazei* Murrill. Carbohydr Polym 103:414–417
22. Lv A, Ge M, Hu X, Liu W, Li G, Zhang R (2017) Effects of *Agaricus blazei* Murill polysaccharide on cadmium poisoning on the MDA5 signaling pathway and antioxidant function of chicken peripheral blood lymphocytes. Biological trace element research
23. Guo Y, Zhao P, Guo G, Hu Z, Tian L, Zhang K, Sun Y, Zhang X, Zhang W, Xing M (2016) Effects of arsenic trioxide exposure on heat shock protein response in the immune organs of chickens. Biol Trace Elem Res 169(1):134–141
24. Gill LW, Ring P, Casey B, Higgins NMP, Johnston PM (2017) Long term heavy metal removal by a constructed wetland treating rainfall runoff from a motorway. Sci Total Environ 601-602:32–44
25. Jin X, Xu Z, Zhao X, Chen M, Xu S (2017) The antagonistic effect of selenium on lead-induced apoptosis via mitochondrial dynamics pathway in the chicken kidney. Chemosphere 180:259–266
26. Li X, Xing M, Chen M, Zhao J, Fan R, Zhao X, Cao C, Yang J, Zhang Z, Xu S (2017) Effects of selenium-lead interaction on the gene expression of inflammatory factors and selenoproteins in chicken neutrophils. Ecotoxicol Environ Saf 139:447–453
27. Liu Q, Ji X, Ge Z, Diao H, Chang X, Wang L, Wu Q (2017) Role of connexin 43 in cadmium-induced proliferation of human prostate epithelial cells. Journal of applied toxicology : JAT 37(8):933–942
28. Manquian-Cerda K, Escudey M, Zuniga G, Arancibia-Miranda N, Molina M, Cruces E (2016) Effect of cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (*Vaccinium corymbosum* L.) plantlets grown in vitro. Ecotoxicol Environ Saf 133:316–326
29. Liu W, Zhao H, Wang Y, Jiang C, Xia P, Gu J, Liu X, Bian J, Yuan Y, Liu Z (2014) Calcium-calmodulin signaling elicits mitochondrial dysfunction and the release of cytochrome c during cadmium-induced apoptosis in primary osteoblasts. Toxicol Lett 224(1):1–6
30. Zou H, Zhuo L, Han T, Hu D, Yang X, Wang Y, Yuan Y, Gu J, Bian J, Liu X, Liu Z (2015) Autophagy and gap junctional intercellular communication inhibition are involved in cadmium-induced apoptosis in rat liver cells. Biochem Biophys Res Commun 459(4): 713–719
31. Al-Dbass AM, Al-Daihan SK, Bhat RS (2012) *Agaricus blazei* Murill as an efficient hepatoprotective and antioxidant agent against CCl4-induced liver injury in rats. Saudi journal of biological sciences 19(3):303–309
32. de Jesus Pereira NC, Regis WC, Costa LE, de Oliveira JS, da Silva AG, Martins VT, Duarte MC, de Souza JR, Lage PS, Schneider MS, Melo MN, Soto M, Soares SA, Tavares CA, Chavez-Fumagalli MA, Coelho EA (2015) Evaluation of adjuvant activity of fractions derived from *Agaricus blazei*, when in association with the recombinant LiHyp1 protein, to protect against visceral leishmaniasis. Exp Parasitol 153:180–190
33. Bernardshaw S, Hetland G, Ellertsen LK, Tryggestad AM, Johnson E (2005) An extract of the medicinal mushroom *Agaricus blazei* Murill differentially stimulates production of pro-inflammatory cytokines in human monocytes and human vein endothelial cells in vitro. Inflammation 29(4–6):147–153
34. Liu W, Ge M, Hu X, Lv A, Ma D, Huang X, Zhang R (2017) The effects of *Agaricus blazei* Murill polysaccharides on cadmium-induced apoptosis and the TLR4 signaling pathway of peripheral blood lymphocytes in chicken. Biological trace element research
35. Pathak N, Khandelwal S (2006) Influence of cadmium on murine thymocytes: potentiation of apoptosis and oxidative stress. Toxicol Lett 165(2):121–132
36. Alvarez-Olmedo DG, Biaggio VS, Koumbadinga GA, Gomez NN, Shi C, Ciocca DR, Batulan Z, Fanelli MA, O'Brien ER (2017) Recombinant heat shock protein 27 (HSP27/HSPB1) protects against cadmium-induced oxidative stress and toxicity in human cervical cancer cells. Cell Stress Chaperones 22(3):357–369
37. Nazir A, Saxena DK, Kar Chowdhuri D (2003) Induction of hsp70 in transgenic Drosophila: biomarker of exposure against phthalimide group of chemicals. Biochim Biophys Acta Gen Subj 1621(2):218–225
38. Wang Z, Wu Z, Jian J, Lu Y (2009) Cloning and expression of heat shock protein 70 gene in the haemocytes of pearl oyster (*Pinctada fucata*, Gould 1850) responding to bacterial challenge. Fish & shellfish immunology 26(4):639–645
39. Zhao P, Guo Y, Zhang W, Chai H, Xing H, Xing M (2017) Neurotoxicity induced by arsenic in Gallus Gallus: regulation of oxidative stress and heat shock protein response. Chemosphere 166:238–245

40. Truettner JS, Hu K, Liu CL, Dietrich WD, Hu B (2009) Subcellular stress response and induction of molecular chaperones and folding proteins after transient global ischemia in rats. Brain Res 1249:9–18
41. Gao Q, Song L, Ni D, Wu L, Zhang H, Chang Y (2007) cDNA cloning and mRNA expression of heat shock protein 90 gene in the haemocytes of Zhikong scallop *Chlamys farreri*. Comparative biochemistry and physiology Part B, Biochemistry & molecular biology 147(4):704–715
42. Nemmiche S, Chabane-Sari D, Kadri M, Guiraud P (2012) Cadmium-induced apoptosis in the BJAB human B cell line: involvement of PKC/ERK1/2/JNK signaling pathways in HO-1 expression. Toxicology 300(3):103–111
43. Nemmiche S, Guiraud P (2016) Cadmium-induced oxidative damages in the human BJAB cells correlate with changes in intracellular trace elements levels and zinc transporters expression. Toxicology in vitro : an international journal published in association with BIBRA 37:169–177
44. Oliveira RJ, Matuo R, da Silva AF, Matiazi HJ, Mantovani MS, Ribeiro LR (2007) Protective effect of beta-glucan extracted from *Saccharomyces cerevisiae*, against DNA damage and cytotoxicity in wild-type (k1) and repair-deficient (xrs5) CHO cells. Toxicology in vitro : an international journal published in association with BIBRA 21(1):41–52
45. Oliveira RJ, Ribeiro LR, da Silva AF, Matuo R, Mantovani MS (2006) Evaluation of antimutagenic activity and mechanisms of action of beta-glucan from barley, in CHO-k1 and HTC cell lines using the micronucleus test. Toxicology in vitro : an international journal published in association with BIBRA 20(7):1225–1233
46. Tohamy AA, El-Ghor AA, El-Nahas SM, Noshy MM (2003) β-Glucan inhibits the genotoxicity of cyclophosphamide, adriamycin and cisplatin. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 541(1–2):45–53
47. Sahin E, Gumuslu S (2004) Cold-stress-induced modulation of antioxidant defence: role of stressed conditions in tissue injury followed by protein oxidation and lipid peroxidation. Int J Biometeorol 48(4):165–171
48. Dwivedi VK, Bhatanagar A, Chaudhary M (2012) Protective role of ceftriaxone plus sulbactam with VRP1034 on oxidative stress, hematological and enzymatic parameters in cadmium toxicity induced rat model. Interdiscip Toxicol 5(4):192–200
49. Falfushinska HI, Romanchuk LD, Stolyar OB (2008) Different responses of biochemical markers in frogs (*Rana ridibunda*) from urban and rural wetlands to the effect of carbamate fungicide. Comparative biochemistry and physiology Toxicology & pharmacology : CBP 148(3):223–229
50. Wang Y, Fang J, Leonard SS, Rao KM (2004) Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radic Biol Med 36(11):1434–1443
51. Li JL, Jiang CY, Li S, SW X (2013) Cadmium induced hepatotoxicity in chickens (*Gallus domesticus*) and ameliorative effect by selenium. Ecotoxicol Environ Saf 96:103–109
52. Kudr J, Nguyen HV, Gumulec J, Nejdl L, Blazkova I, Ruttkay-Nedecky B, Hynek D, Kynicky J, Adam V, Kizek R (2014) Simultaneous automatic electrochemical detection of zinc, cadmium, copper and lead ions in environmental samples using a thin-film mercury electrode and an artificial neural network. Sensors 15(1):592–610
53. Lee JC, Son YO, Pratheeshkumar P, Shi X (2012) Oxidative stress and metal carcinogenesis. Free Radic Biol Med 53(4):742–757
54. Yuan SS, Lv ZM, Zhu AY, Zheng JL, CW W (2017) Negative effect of chronic cadmium exposure on growth, histology, ultrastructure, antioxidant and innate immune responses in the liver of zebrafish: preventive role of blue light emitting diodes. Ecotoxicol Environ Saf 139:18–26
55. Nemmiche S (2017) Oxidative signaling response to cadmium exposure. Toxicological sciences : an official journal of the Society of Toxicology 156(1):4–10
56. Black PH (2002) Stress and the inflammatory response: a review of neurogenic inflammation. Brain Behav Immun 16(6):622–653
57. Xu F, Liu S, Li S (2015) Effects of selenium and cadmium on changes in the gene expression of immune cytokines in chicken splenic lymphocytes. Biol Trace Elem Res 165(2):214–221
58. Li JL, Li S, Tang ZX, Xu SW (2010) Oxidative stress-mediated cytotoxicity of cadmium in chicken splenic lymphocytes. Toxicol Lett 196:S122
59. Zheng JL, Yuan SS, CW W, Li WY (2016) Chronic waterborne zinc and cadmium exposures induced different responses towards oxidative stress in the liver of zebrafish. Aquat Toxicol 177:261–268
60. Zheng JL, Yuan SS, CW W, Lv ZM (2016) Acute exposure to waterborne cadmium induced oxidative stress and immunotoxicity in the brain, ovary and liver of zebrafish (*Danio rerio*). Aquat Toxicol 180:36–44
61. Ding Z, An K, Xie L, Wu W, Zhang R, Wang D, Fang Y, Chen H, Xiao S, Fang L (2017) Transmissible gastroenteritis virus infection induces NF-kappaB activation through RLR-mediated signaling. Virology 507:170–178
62. Lan W, Petznick A, Heryati S, Rifada M, Tong L (2012) Nuclear factor-kappaB: central regulator in ocular surface inflammation and diseases. The ocular surface 10(3):137–148
63. Bustos-Arriaga J, Garcia-Machorro J, Leon-Juarez M, Garcia-Cordero J, Santos-Argumedo L, Flores-Romo L, Mendez-Cruz AR, Juarez-Delgado FJ, Cedillo-Barron L (2011) Activation of the innate immune response against DENV in normal non-transformed human fibroblasts. PLoS Negl Trop Dis 5(12):e1420
64. Decembre E, Assil S, Hillaire ML, Dejnirattisai W, Mongkolsapaya J, Screaton GR, Davidson AD, Dreux M (2014) Sensing of immature particles produced by dengue virus infected cells induces an antiviral response by plasmacytoid dendritic cells. PLoS Pathog 10(10):e1004434
65. Cheng F, Yan X, Zhang M, Chang M, Yun S, Meng J, Liu J, Feng CP (2017) Regulation of RAW 264.7 cell-mediated immunity by polysaccharides from *Agaricus blazei* Murill via the MAPK signal transduction pathway. Food Funct 8(4):1475–1480