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The Protective Role of Selenium Against AFB_1-Induced Liver Apoptosis by Death Receptor Pathway in Broilers

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Abstract

Aflatoxin B1 (AFB_1) is the most toxic among the mycotoxins and causes detrimental health effects on the liver of human and animals. Selenium (Se) plays an important role in protection of various animal species against numerous notorious toxic agents. The present study is designed to explore the protective effects of Se against AFB_1-induced liver pathogenesis by the methods of histopathology, flow cytometry, quantitative real-time polymerase chain reaction (qRT-PCR), and biochemical analysis. A total of 312, 1-day-old healthy Cobb-500 broilers were randomly divided into four groups and fed with basal diet (control group), 0.6 mg/kg AFB_1 (AFB_1 group), 0.4 mg/kg Se (+ Se group), and 0.6 mg/kg AFB_1 + 0.4 mg/kg Se (AFB_1 + Se group) for 21 days, respectively. Our results showed that 0.4 mg/kg Se supplement in broiler's diets could alleviate the AFB_1-induced histological lesions in the liver. The apoptosis analysis by flow cytometry showed that 0.4 mg/kg Se ameliorated the AFB_1-induced apoptosis in the liver. Moreover, the mRNA expression levels of Fas, TNF-α, FAS-associated death domain, TNF receptor-associated death domain, TNF receptor-associated factor 2, caspase 10, caspase 8, B cell lymphoma 2, IκB kinase, X-linked inhibitor of apoptosis protein, caspase 9, and caspase 3 analyzed by qRT-PCR demonstrated that 0.4 mg/kg Se could relieve the impact caused by AFB_1 to these parameters. The biochemical analyses of activities of CAT, GSH-Px and SOD, hydroxyl ion scavenging and contents of MDA and GSH in liver cells also indicated that 0.4 mg/kg Se has positive effect on AFB_1-induced oxidative stress in the liver. In conclusion, Se could relieve AFB_1-induced apoptosis by the molecular regulation of death receptors pathway in the liver of broilers. The outcomes from the present study may lead to a better understanding of the nature of selenium's essentiality and its protective roles against AFB_1.

Keywords Sodium selenite · AFB_1 · Apoptosis · Death receptors · Liver · Broiler

Introduction

Aflatoxins belong to group of approximately 20 related fungal metabolites mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxins can affect more than one organ system simultaneously, thereby producing a multitude of responses in the affected organisms [1]. This malicious nature of aflatoxins made them comprehensively researched mycotoxin. The most prevalent aflatoxins are B1, B2, G1, G2, M1, and M2, which are naturally found in many food and dairy products [2]. Among these, aflatoxin B1 (AFB_1) is the widely known aflatoxin [3] due to its proven carcinogenicity in human, lab animal, pets, and domestic animals [4–6]. AFB_1 has been reported to provoke hepatotoxic, genotoxic, and immunotoxic effects in several animal species [7]. The toxicity of AFB_1 depends on its dose, duration of intake, and affected animal species [8]. Poultry birds are considered highly susceptible to AFB_1 due to their high metabolic rate and AFB_1 prevalence in poultry feed, along with other toxins. Since AFB_1 is naturally present in poultry feed, it poses hepatotoxic, immune toxic, and other high detrimental threats to the poultry production which can range from lower egg/meat production to mortality [7]. The liver is considered most crucial organ in

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human and animals performing many vital functions, along with serving as the incinerator for toxins and harmful substances. It is also the main affected organ following the ingestion of the AFB_1 [9]. Studies have proved that aflatoxins react negatively with different cell proteins, leading to inhibition of carbohydrate and lipid metabolism and protein synthesis, and ultimately induce apoptosis [10]. Our previous work has also revealed that AFB_1 negatively affect the liver, spleen, thymus, jejunum, and ileum, when fed to broiler [11, 12].

Selenium (Se) is an essential nutrient for normal physiological processes [13]. It has also been shown to protect various animal species against numerous notorious toxic agents [14]. For instance, Se protected cadmium-induced liver toxicity in rat by improving oxidative stress status [15] and ameliorated DNA damage caused by mercury [16]. Se and alpha-tocopherol amended the lead-induced toxicity by improving serum biochemical parameters, lipid peroxidation, and antioxidant enzyme activities in fish [17]. Another recent study in chicken has shown to improve the lead-induced oxidative stress and immune damage, when selenium was fed to the lead intoxicated birds [18] .

Our previous study has revealed that AFB_1 negatively affects broiler's liver, and we also elucidated the mechanisms by which AFB_1 induces apoptosis at cellular and molecular level [19]. Looking at our previous results, it would be enthralling to find out how Se can amend the toxic effects of AFB_1 on the broiler liver as Se has been shown to provide protective effects against several toxic agents. Therefore, the present study is designed to explore the protective effects of Se against AFB_1-induced liver pathogenesis by analyzing histological, ultra-structural, biochemical, flow cytometry, and relative gene expression changes in chicken hepatocytes.

Materials and Methods

Birds and Diet

A total of 312, 1-day-old healthy Cobb-500 broilers were purchased from Chia Tai Group of Wenjiang, Sichuan, China. The broilers were weighed and randomly divided into four groups which are the control group (0 mgAFB_1/kg of basal diet), AFB_1 group (0.6 mg AFB_1/kg of basal diet), Se group (0.4 mg Se/kg of basal diet), and AFB_1 + Se group (0.6 mg AFB_1/kg + 0.4 mg Se/kg of basal diet). Each group was in three replicates, each with 26 birds. Chicks were provided the corresponding diets and water ad libitum throughout the 21 days of experimentation. Room lights were set on a 24-h continuous schedule, temperature was initially maintained at 33 °C and gradually lowered by 2 °C each week, and relative humidity was maintained between 65 and 67%. All procedures performed in studies involving animals were in accordance with the international guidelines, and the laws and the ethical standards of China West Normal University Animal Care and Use Committee.

The control diet (basal diet) was formulated according to National Research Council (NRC, 1994) [20] and Chinese Feeding Standard of Chicken (NY/T33-2004) recommendations.

AFB_1-contaminated diet was made by following method: 27 mg AFB_1 (A6636, Sigma-Aldrich, USA) was dissolved into 30 ml methanol, and then this 30 ml mixture was mixed into 45 kg corn–soybean basal diet to formulate AFB_1-contaminated diet. The equivalent methanol was mixed into 45 kg corn–soybean basal diet to produce the control diet. The methanol of diets was evaporated at 98 °F (37 °C). After preparing the diet, the control and the AFB_1-contaminated diets were analyzed by high-performance liquid chromatography (HPLC) and fluorescence detection (Waters Model 2475) to ensure the AFB_1 concentration in the experimental diets. AFB_1 content was 0.601 mg/kg in the contaminated diet and less than 0.001 mg/kg in the control diet. Basal diet composition is mentioned in Table 1.

Gross Observation of the Liver

At days 7, 14, and 21, six chickens in each group were randomly selected and weighed. After euthanization, gross pathological condition of each organ was observed with a special emphasis on livers. After the liver from each chicken was dissected, gross pathological changes were observed, and weight was detected and liver index was calculated using following formula.

$$\text{Organ index} = \text{Organ weight}/\text{Body weight} \times 100\%(\text{g/kg})$$

Histopathological and Ultrastructural Examination of the Liver

At the age of 7, 14, and 21 days, six chickens in each group were euthanized and the livers were fixed in 4% paraformaldehyde (PFA) and routinely processed in paraffin. Thin sections (5 μm) of tissue were sliced, mounted on glass slides, and stained with hematoxylin and eosin Y. The histological organization of the tissues was contemplated and snapped with a digital camera (Nikon, eclipse 50i, Japan).

One chick per replicate in each group was euthanized and then immediately necropsied at the end of the trial. Small pieces of liver tissues were immediately fixed with 2.5% glutaraldehyde and post-fixed in 2% Veronal acetate-buffered OsO_4. The tissues were embedded in Araldite after dehydrating in alcohol gradient. The blocks were sectioned in 65–75-nm-thick sections in a microtome with a glass knife

Table 1 Composition of the experimental diets

Diet formulation	Contents (%)	Nutritional values	Contents (%)
Corn	51.95	Crude protein (CP %)	21.5
Soybean meal	39.50	ME metabolic energy (MJ/kg), Kcal	2.99
Rapeseed oil	4.10	Calcium (Ca %)	1.00
DL-Met	0.20	Total phosphorus (P %)	0.70
Calcium hydrogen	1.85	Lysine (Lys, %)	1.15
Calcium carbonate	1.30	Methionine (Met, %)	0.50
Salt	0.40	Methionine + cysteine (Met+ Cys, %)	0.84
Multidimensional	0.03	Threonine (Thr, %)	0.83
Choline	0.17		
Trace element premix	0.50		
Total	100		

$FeSO_4 \cdot 7H_2O = 0.53$ g, $CuSO_4 \cdot 5H_2O = 0.03$ g, $MnSO_4 \cdot H_2O = 0.4$ g, $ZnSO_4 \cdot 7H_2O = 0.47$ g, KI = 0.018 g, $NaSeO_3 = 0.03$ g, vitamin A = 13,500 IU, vitamin D = 3000 IU, vitamin E = 24 IU, vitaminK3 = 3 mg, pantothenic acid = 15 mg, folic acid = 1.05 mg, nick amides = 30 mg, biotin = 0.14 mg was also used in feed

and placed in uncoated copper grids. The sections were stained with uranyl acetate and post-stained with 0.2% lead citrate. The subcellular architecture of liver was examined with a Hitachi H-600 transmission electron microscope (Japan).

Annexin-V Apoptosis Detection of the Liver by Flow Cytometry

At 7, 14, and 21 days of the experiment, six chickens in each group were euthanized, and the livers were sampled from each chick to determine the percentage of apoptotic cells by flow cytometer, using the method by Del Bino (2010) [21]. Briefly, the dissected livers were thereupon homogenized to form a cell suspension. After filtered and washed, these cells were resuspended in phosphate buffer at a concentration of 1×10^6 cells/mL. Five microliters of Annexin-V-Fluorescein isothiocyanate (V-FITC) and 5 μL propidium iodide (PI) were added into 100 μL cell suspension, and then the mixture was incubated at 25 °C for 15 min in the dark. Four hundred microliters of 1× Annexin binding buffer was added to the mixture, and then the apoptotic cells were assayed by flow cytometer (BD FACSCalibur) within 1 h. The Annexin-V-FITC Kit was obtained from BD Pharmingen (USA, 556547).

Detection of Death Receptors of the Liver by qRT-PCR

The livers from six chickens in each group were removed at 7, 14, and 21 days of age and instantly stored in liquid nitrogen. The liver samples were homogenized in liquid nitrogen, by crushing with a mortar and pestle and the powdered tissues were collected into eppendorf tubes and stored at − 80 °C. Total RNA was extracted using TriPure Isolation Reagent (Cat No. 11667165001, Roche Applied Science, Germany) following manufacturer's protocol. The quality and quantity of total RNA were measured spectrophotometrically. Extracted RNA was forthwith reverse-transcribed into cDNA using Transcriptor First Strand cDNA Synthesis Kit (Cat No: 04897030001, Roche Applied Science, Germany). qRT-PCR reactions were performed in a total volume of 20 μL using FastStart Universal SYBR Green Master mix (Cat No: 04913914001, Roche Applied Science, Germany), at the following thermocycler program: initial denaturation at 95 °C for 10 min, followed by 44 cycles of "10 s at 95 °C and 30 s at melting temperature (T_m) of a specific primer pair", and melt curve analysis by 10 s at 95 °C, and 72 °C for 10 s, using Thermal Cycler (Step One Plus, Applied BioSystems, USA). β-Actin was used as an internal control [22, 23]. Primer information is provided in Table 2. The qRT-PCR data were analyzed, and fold changes in expressions were calculated using $2^{-\Delta\Delta Ct}$ calculation method described by reference [24]. The forward and reverse primers are listed at Table 2.

Biochemical Analysis of the Liver

Six chickens in each group were euthanized and immediately dissected at 7, 14, and 21 days of age. Then, the livers were immediately taken out and chilled to 0 °C in 0.85% NaCl solution and then dried, weighed, and homogenized in nine times volume of ice cold 0.85% NaCl in a chilled homogenizer and centrifuged at 3500×*g* at 4 °C for 10 min.

Table 2 List of oligonucleotides used as primers in qRT-PCR analysis

Gene symbol	RefSeq mRNA number	Forward primer	Reverse primer	Amplicon length (bp)
FAS	NM_001199487	TCCACCTGCTCCTCGTCATT	GTGCAGTGTGTGTGGGAACT	78
TNF-R1	NM_001030779	CCTGTCTGTCTTCCCTGTCC	GGTGCATGGGGTCTTTTCTA	120
TRADD	XM_414067	CTAGAGCCCAAAGGAAGTCGAT	TGGCTGCTTCTCTGTGACAT	100
FADD	XM_421073	GGGGTAAAGAGGCTGAACTCTTA	TGAGTCCTATTGCACTGCTGTC	163
TRAF2	XM_015279623	CGTGGTGATGAAAGGACCCA	AATGATGTGCTCCCGGTTGT	100
Casp-10	XM_421936	CTGGGGGCTCCAAAAGTCC	AAAGGGGGACAAAGCCAACA	204
Casp-9	AY057940	CCAACCTGAGAGTGAGCGATT	GTACACCAGTCTGTGGGTCGG	87
Casp-8	NM_204592	GTCTCCGTTCAGGTATCTGCT	TCTCAATGAAAACGTCCGGC	143
Casp-3	NM_204725	TGGCCCTCTTGAACTGAAAG	TCCACTGTCTGCTTCAATACC	139
IKK (IKBIP)	XM_001232182	GGCTTGGTTTTGGCAGTGAG	CGGCTTTGACGTTTGCTGAA	144
XIAP	NM_204588	GCAGAATATGAGAGGCGGATAC	TCCTTCCACTCTTGCAATCC	149
BCL-2	NM_205339	TGTTTCTCAAACCAGACACCAA	CAGTAGGCACCTGTGAGATCG	205
β-Actin	L08165	TGCTGTGTTCCCATCTATCG	TTGGTGACAATACCGTGTTCA	178

Total protein was measured by the method of Bradford. The commercial kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and used to detect total protein (Total protein quantification kit No.A045), activities of SOD (superoxide dismutase detection kit no. A001-1), CAT (catalase detection kit no. A007), GSH-Px (glutathione peroxidase detection kit no. A005) and contents of GSH (glutathione detection kit no. A006) and MDA (malonaldehyde detection kit no. A003-1), and $^{-}$OH radical (hydroxyl free radical detection kit no. A018) in the supernatant, according to the manufacturer's instructions.

Statistical Analysis

The results were expressed as mean ± standard deviation (X ± SD). Statistical analyses were performed using one-way analysis of variance and Tukey was employed for multiple comparisons. Letters A, B, C, and D represent the significant difference ($p < 0.01$) between the control group, AFB_1 group, Se group, and AFB_1 + Se group respectively, and letters a, b, c, and d represent the difference ($p < 0.05$) between the control group, AFB_1 group, Se group, and AFB_1 + Se group respectively.

Results

Body Weight and Clinical Observation

At day 7, no significant differences ($p < 0.05$ or $p < 0.01$) among the control and treatment groups were found. There was no significant reduction in the body weight of chickens at day 7, whereas at days 14 and 21, a marked reduction in the body weight of AFB_1 group was seen ($p < 0.05$ or $p < 0.01$). Furthermore, an increase in the body weight was seen in the Se group in comparison to the control group ($p < 0.05$). What is more, the body weight of the AFB_1 + Se group was comparable to the control group ($p > 0.05$). The results are shown in Fig. 1a.

Grossly, no major differences were observed in the liver in all four groups at day 7. A mild yellowish discoloration and enlargement of the liver was seen at days 14 and 21 in AFB_1 exposed chickens as compared to the control, whereas in the Se group, there were no such gross pathological changes observed when compared to the control group. In the AFB_1 + Se group, these gross characteristics were found to be comparable to the control.

There was no significant change ($p > 0.05$) in the relative weight of livers at day 7 in all four groups, whereas at days 14 and 21, the relative weight of livers were seen increasing ($p < 0.05$ or $p < 0.01$) in the AFB_1 group as compare to other groups. Furthermore, in the AFB_1 + Se group, the relative weight of the liver was equivalent to the control group as shown in Fig. 1b.

Histological and Ultrastructural Changes of the Liver

Compared to the control group, microscopically degenerative reversible lesions were observed in a varying manner in AFB_1 group (Fig. 2a), such as congestion, slight to moderate vacuolar degeneration, and proliferation of bile duct (Fig. 2b), while no such histological differences were seen in the control, Se group and AFB_1 + Se group except slight hydropic degeneration (insignificant) in the AFB_1 + Se group (Fig. 2a, c, d).

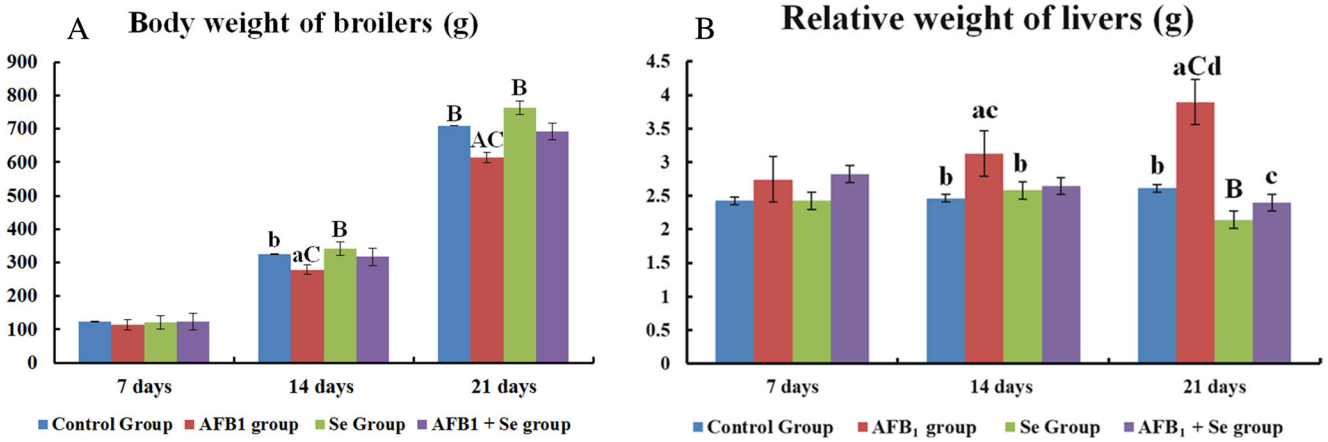

Fig. 1 Body weights of experimental chickens (**a**) and relative liver weight of experimental chicken (**b**). Letters A, B, C, and D represent the significant difference ($p < 0.01$) between the control group, AFB_1 group, Se group, and AFB_1 + Se group, respectively, and letters a, b, c, and d represent difference ($p < 0.05$) between the control group, AFB_1 group, Se group, and AFB_1+Se group, respectively, the same as followings

Results of ultrastructural observation revealed irregular, fragmented, and condensed nucleus, swollen mitochondria with reduced number of cristae, and swollen endoplasmic reticulum in the AFB_1 group (Fig. 2f) when compared to the control (Fig. 2e). Slightly swollen mitochondria and fatty degeneration were also seen in the AFB_1 + Se group (Fig. 2h) in comparison to the control group, while no obvious ultrastructural changes were observed in the Se (Fig. 2g) and control group.

Apoptotic Percentage by Flow Cytometer

Annexin-V FITC was used to quantitatively determine the percentage of apoptotic cells. Apoptotic cell counts were determined by examining the total percentage of early (Annexin-V positive and PI negative) and late (both Annexin-V and PI positive) apoptotic cells. Figure 3a–d shows scattered analysis of apoptosis at 21 days in the control, AFB_1 group, Se group, and AFB_1 + Se group respectively. At days 7, 14, and 21, a significant increase ($p < 0.01$) in apoptotic cell percentage was observed in the AFB_1 group, while a significant decrease ($p < 0.01$) in apoptotic cell percentage was seen in the AFB_1 + Se group (Fig. 3e) when compared with the AFB_1 group.

qRT-PCR Analysis of Relative Expression of Genes Involved in Death Receptor–Induced Apoptosis

The death receptor genes FAS (fatty acid synthesize receptor) and TNF-R1 (tumor necrosis factor receptor 1) and the genes involved in DISC (death-inducing signaling complex) formation i–e FADD (FAS-associated death domain), TRADD (TNF receptor-associated death domain), and TRAF2 (TNF receptor-associated factor 2) were seen significantly downregulated ($p < 0.05$ or $p < 0.01$) at 7, 14, and 21 days with an exception of TRAF2 which was not significantly downregulated ($p > 0.05$) at day 7 and 14 in the AFB_1 + Se group when compared with the AFB_1 group. The mRNA levels of CASPASE (cysteine-aspartic protease) family genes, i.e., caspase 3, caspase 8, caspase 9, and caspase 10 were seen significantly decreased at 7, 14, and 21 days ($p < 0.05$ or $p < 0.01$) in AFB_1 + Se group compared to the AFB_1 group. BCL-2 (B cell lymphoma 2), XIAP (X-linked inhibitor of apoptosis protein) and IKK (IκB kinase) genes displayed notable upregulation ($p < 0.05$ or $p < 0.01$) at days 7, 14, and 21 in the AFB_1 + Se group when compared with the AFB_1 group (Fig. 4).

A time bound significant increase ($p < 0.05$ or $p < 0.01$) was observed in the expression of TNF-R, FAS, FADD, TRADD, caspase 8, caspase 9, and caspase 3 at days 7, 14, and 21 in the AFB_1 group when compared to the control group, whereas no significant difference ($p > 0.05$) was observed in the AFB_1 group at days 7 and 14. However, the mRNA expressions of TRAF2 and caspase 10 displayed maximum expression at day 14 in the AFB_1 group. Also, BCL-2 and XIAP displayed the time bound decrease in the expression at days 7, 14, and 21 in the AFB_1 group when compared to the control group. Se group exhibited the similar expression of the genes involved in death receptor–induced apoptosis, when compared to the control group at all the time points.

Biochemical Analysis

Compared to the control group, the activities of CAT (Catalase) and GSH-Px (glutathione peroxidase) in the AFB_1 + Se group were observed significantly upregulated ($p < 0.05$ or $p < 0.01$) throughout the experiment at different

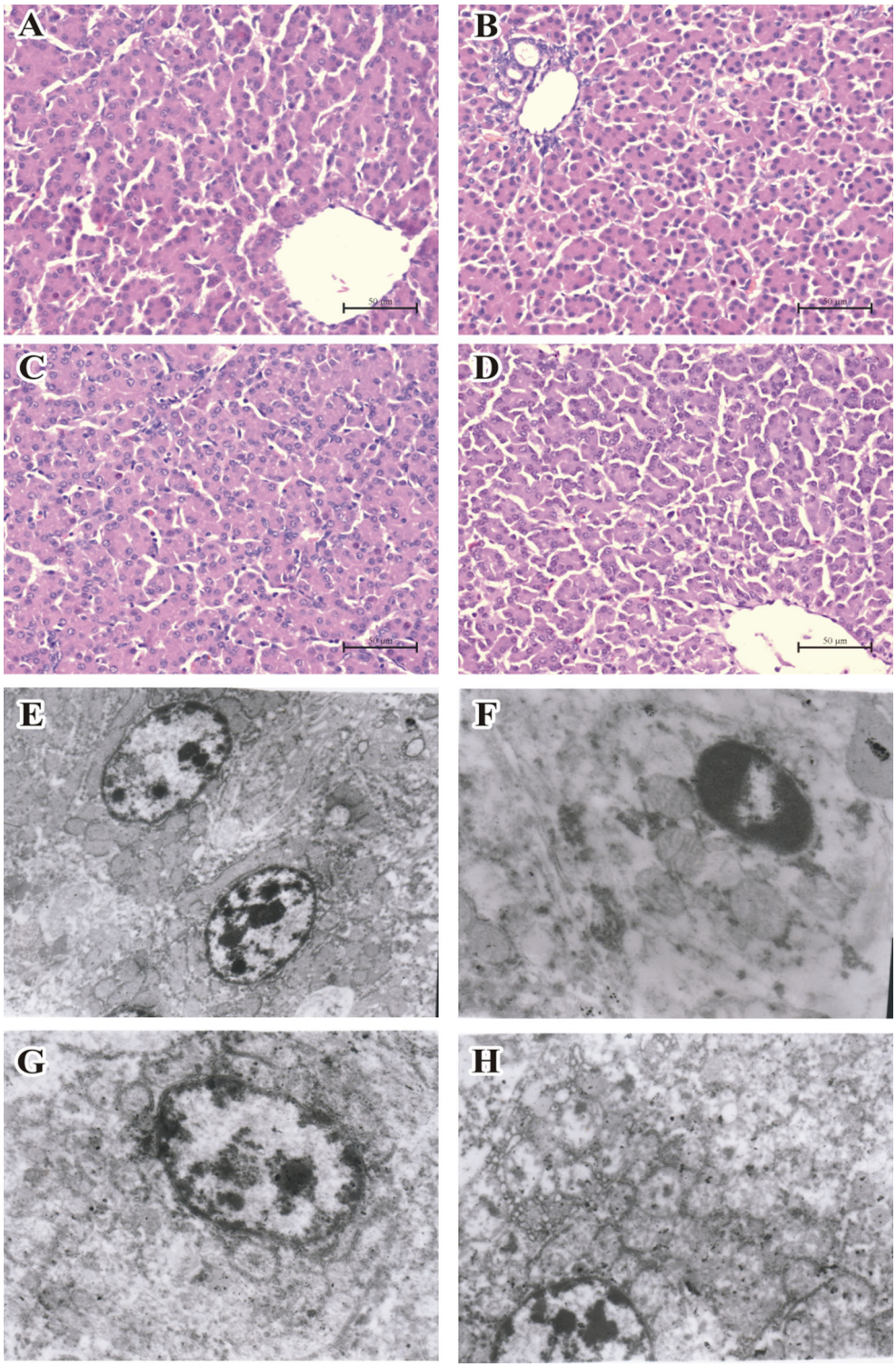

Fig. 2 Histopathological and ultrastructural changes displayed by liver cells exposed to AFB_1. **a**–**d** The histological examination of H & E stained liver tissues of control and treatment groups from 21 days old chickens (Bar = 50 μm). **e**–**h** The ultrastructural examination of uranyl acetate and lead citrate stained liver tissue from the control group and treatment groups (control group, AFB_1 group, Se group, AFB_1 + Se group)

time points when compared to the AFB_1 group. Activity of SOD (superoxide dismutase) and hydroxyl free radical scavenging were also seen remarkably increase ($p < 0.05$ or $p < 0.01$) in the AFB_1+ Se group compared to the AFB_1 group at 7, 14, and 21 days. A significantly downregulated contents of MDA (Malondialdehyde) were observed at 7, 14, and 21 days ($p < 0.01$), while GSH (glutathione) contents were seen increased ($p < 0.05$ or $p < 0.01$) at 7, 14, and 21 days in the AFB_1 + Se group when compared to the AFB_1 group as represented in Fig. 5. The activities and contents of the same enzymes were not seen significantly different among control, Se group, and AFB_1 + Se groups.

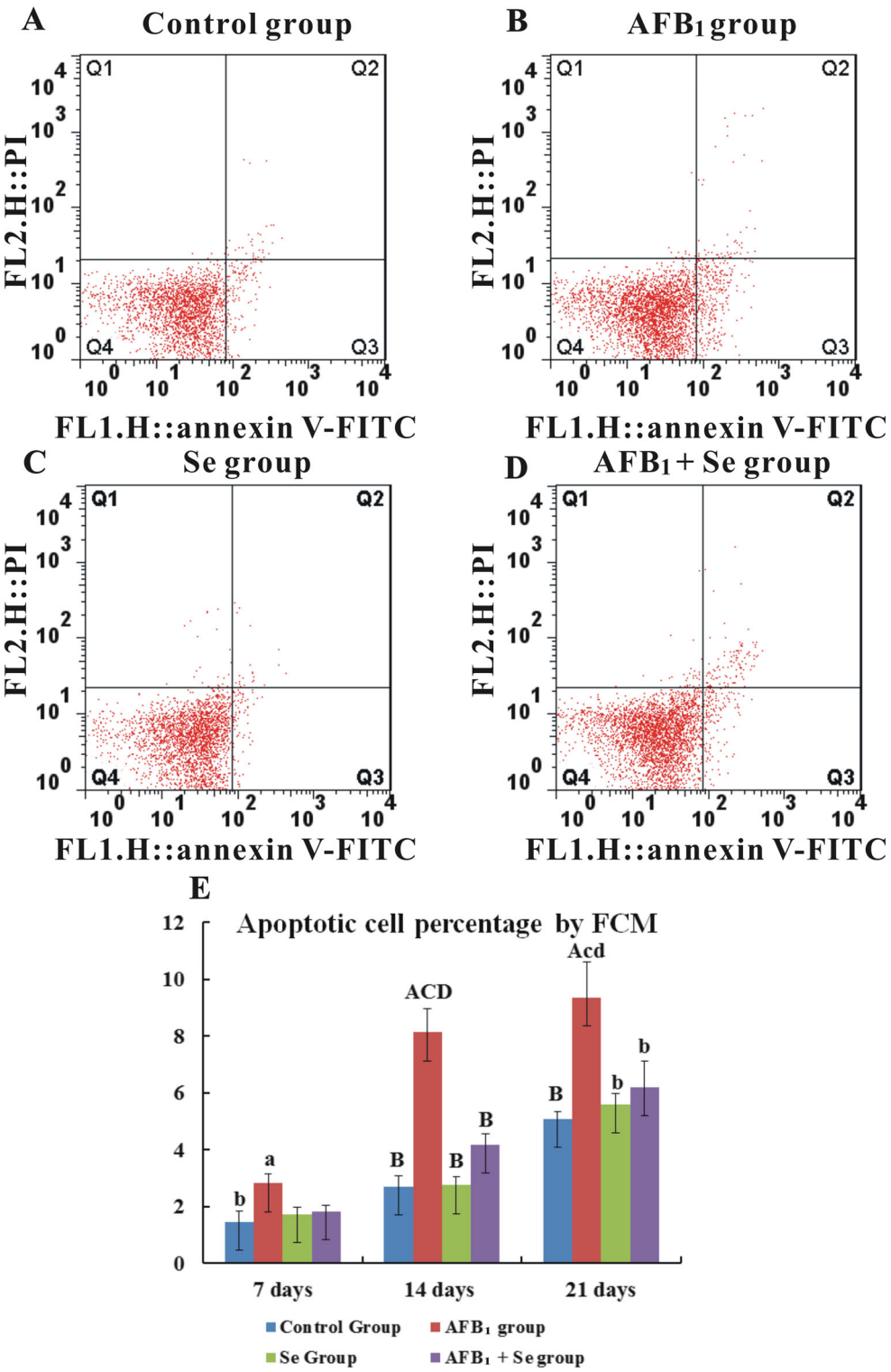

Fig. 3 Apoptotic percentage and scattered analysis of early and late apoptosis in chicken hepatocytes between control, AFB_1, Se group, and AFB_1 + Se. Figure **a**, **b**, **c**, and **d** represent scattered analysis of early (Annexin-V positive and PI negative) and late (Annexin-V and PI positive) hepatocytes apoptosis in the control, AFB_1, Se group and AFB_1 + Se at 21 days respectively, while **e** is a bar graph showing apoptotic percentage rate in the same groups at days 7, 14, and 21, measured by flow cytometer when compared to the control

Discussion

Many studies have documented that AFB_1 has hepatotoxic effects in human beings and animals [25–27]. Se, as an essential micronutrient, plays a key role in antioxidant and detoxification functions [28–30]. Previous studies showed that Se could ameliorate AFB_1-induced hepatotoxicity [31, 32], and our studies have proven that diet with supplementation of 0.4 mg/kg Se has negative effects on immune organs [12, 33, 34]. In this study, we also observed AFB_1-induced hepatotoxicity, including the cellular degeneration, and subcellular lesions including swollen mitochondria with reduced number of cristae and swollen endoplasmic

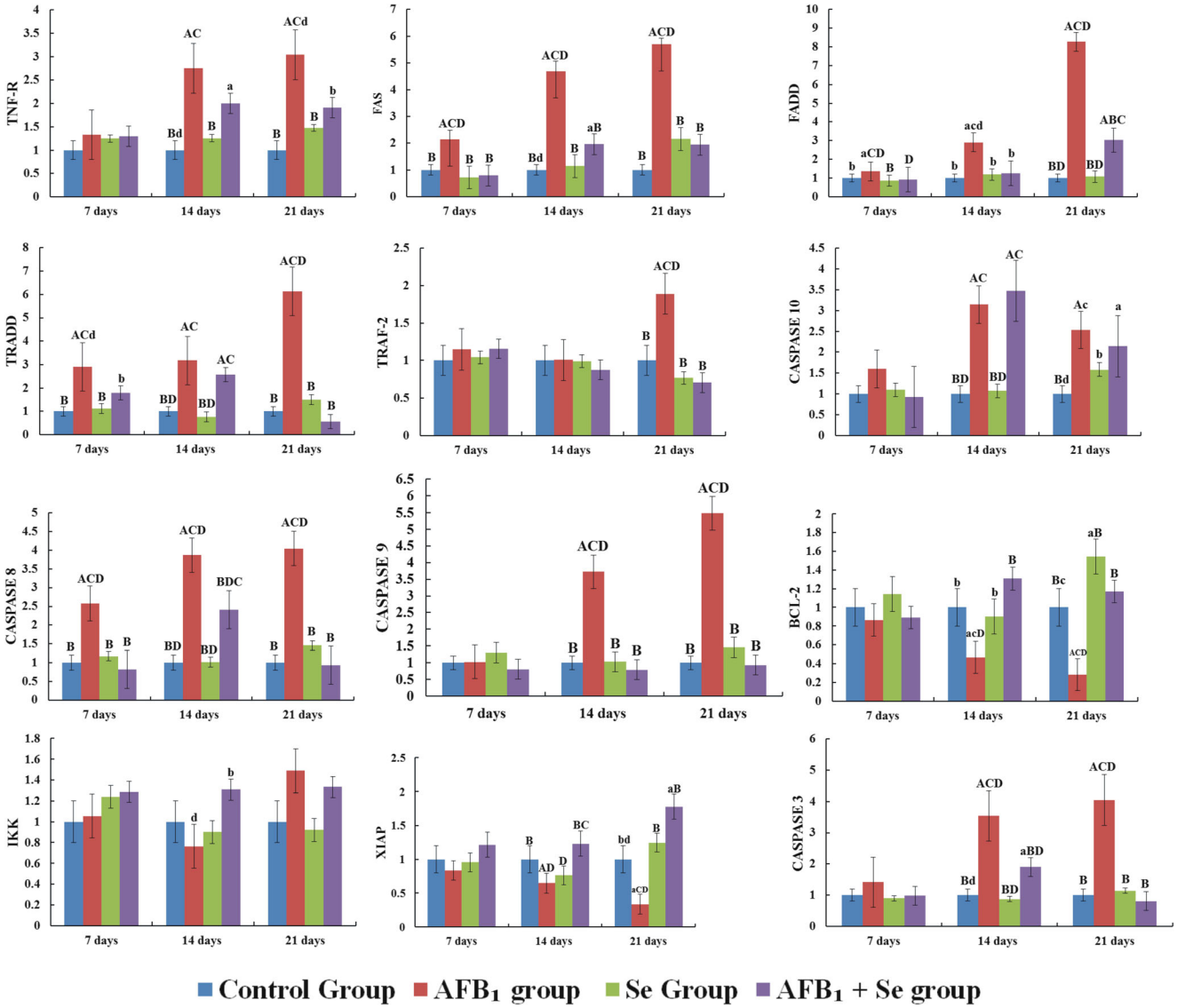

Fig. 4 Relative expression of genes involved in death receptor–induced apoptosis in the liver of chickens exposed to AFB_1. Figure represents the mRNA levels of Fas, TNF-α, FADD, TRADD, TRAF2, caspase 10, caspase 8, BCL-2, IKK, XIAP, caspase 9, and caspase 3 in the liver of the AFB_1-fed chickens and expressed as fold change relative to the control group ($n = 6$)

reticulum. However, no obvious lesions were found in the AFB_1 + Se group, + Se group, and control group. Looking at our previous results, it would be enthralling to find out how Se can amend the toxic effects of AFB_1 on the broiler liver as Se has been shown to provide protective effects against several toxic agents.

Biochemical analysis suggested that Se supplementation could protect hepatocytes from AFB_1-induced oxidative damage by upregulation of GSH content, SOD, CAT, GSH-Px activities, and downregulation of MDA level in the AFB_1 + Se groups. It has been reported that excessive production of ROS or deactivation of antioxidant enzymes will cause oxidative stress [35] and then induce apoptosis. In addition, these enzymatic antioxidants have been recognized to play an important role in the anti-oxidant mechanism of the body [36]. Therefore, we speculated that supplemented 0.4 mg/kg Se could effectively protect the chicken's liver against the AFB_1-induced histopathological lesions by reducing oxidative stress.

It is reported that excessive apoptosis is actively involved in liver injury in various circumstances [37, 38]. In our study, a significant increase in apoptotic cell percentage was observed in AFB_1 group by flow cytometry assay, but it was alleviated in the AFB_1 + Se group. Why can Se alleviate AFB_1-induced cell apoptosis? In this study, we detected the relative expression of genes involved in death receptors pathway, including FAS, TNF-R1, TRADD, FADD, TRAF2, Casp-10, Casp-9, Casp-8, Casp-3, IKK (IKBIP), XIAP, and BCL-2. The results showed that when compared with those of the AFB_1 group, the death receptor genes FAS and TNF R1 and the genes involved in death-inducing signaling complex formation such as FADD, TRADD, and TRAF2 were significantly downregulated in Se + AFB_1 group. The

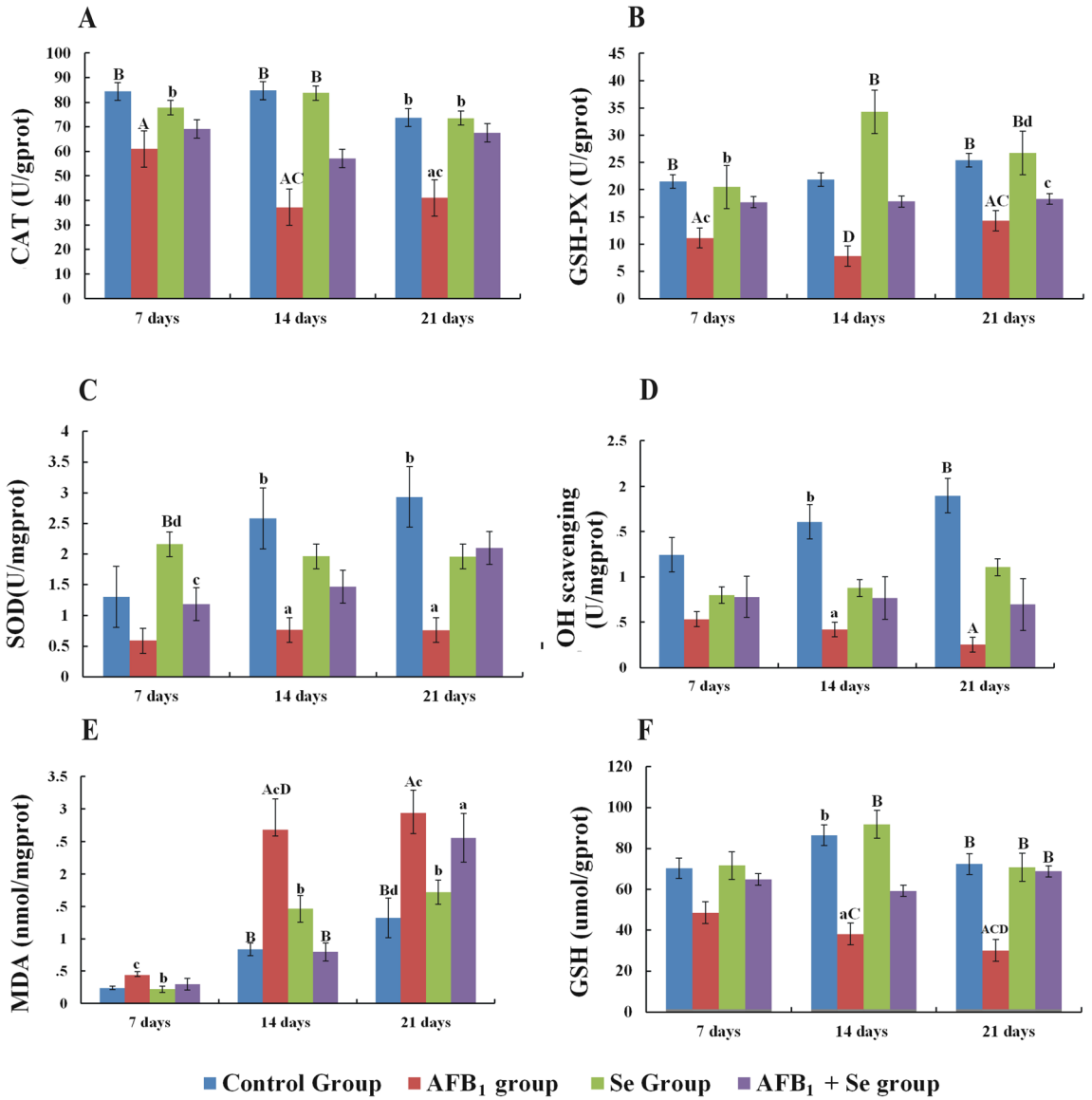

Fig. 5 Biochemical analyses of activities of CAT, GSH-Px, SOD, hydroxyl ion scavenging, and contents of MDA and GSH in liver cells in different groups. **a**–**c** Activities of CAT, GSH-Px, and SOD among groups respectively. Bar graph **d** shows hydroxyl free radical scavenging while **e** and **f** bar graphs display contents of MDA and GSH in chicken hepatocytes when compared to the control

mRNA levels of caspase family genes, caspase 3, caspase 8, and caspase 9 were seen significantly decreased, while BCL-2 and XIAP genes were increased in Se + AFB_1 group.

TNF is a major mediator of apoptosis, the initial step in TNF signaling involves in the binding of the TNF trimer to the extracellular domain of TNF-R1 and the release of the inhibitory protein silencer of death domains (SODD) from TNF-R1's intracellular domain (ICD) [39]. The resulting aggregated TNF-R1 ICD is recognized by the adaptor protein TRADD, which recruits additional adaptor proteins receptor-interacting protein (RIP), TRAF2, and FADD. FADD is a pivotal component of death receptor-mediated extrinsic apoptosis and necroptosis [40]. Fas plays an initial role for extrinsic pathway of apoptosis and it acts as a death-receptor for triggering apoptosis [41]. The Fas death inducing signaling complex (DISC) also contains the adaptor protein FADD and caspases 8 and 10, which can initiate the process of apoptosis [42]. Fas-mediated apoptosis is controlled by a plethora of regulators of the mitochondrial pathway of cell death, for example, by BCL-2 family members [43]. In this study, the above-mentioned factors were decreased in the AFB_1 + Se group, which indicated that the AFB_1-induced apoptosis is inhibited by appropriate dietary Se.

It is well known that XIAP is the prominent member of the inhibitor of apoptosis proteins (IAPs) [44]. IκB kinase (IKK) is essential for nuclear factor κB activation and prevention of apoptosis [45]; IKK inhibits TNFα-induced apoptosis [46]. IKK appears to directly interfere with BCL-2 activity through phosphorylation in HNE-mediated apoptosis independent of NFκB

signaling [47]. BCL-2 is known to target the protein kinase Raf-1 to mitochondria, allowing the kinase to phosphorylate and thereby inactivate proapoptotic Bad [48], and then causes caspase activation by forming a complex with Apaf-1 and procaspase-9, leading to activation of caspase-9, which in turn activates procaspase-3 [49, 50] and then induced apoptosis. It has been reported that selenium can lead to significant reduction in caspase-3 level [32], which consists with our present findings. It is suggested that Se could inhibit the activation of death receptors pathway in the liver caused by AFB_1.

In conclusion, 0.4 mg/kg Se supplied in diet could protect the liver from AFB_1-induced histopathological lesions by diminishing oxidative damage and relieve AFB_1-induced apoptosis by the molecular regulation of death receptors pathway in the liver of broilers. The outcomes from the present study may lead to a better understanding of the nature of selenium's essentiality and its protective roles against AFB_1.

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Compliance with Ethical Standards

All procedures performed in studies involving animals were in accordance with the international guidelines, and the laws and the ethical standards of China West Normal University Animal Care and Use Committee.

Conflict of Interest The authors declare that there is no competing interest.

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