Biological Trace Element Research (2019) 187:273–280
https://doi.org/10.1007/s12011-018-1361-7

# Selenium Ameliorates $AFB_1$_Induced Excess Apoptosis in Chicken Splenocytes Through Death Receptor and Endoplasmic Reticulum Pathways

Jing Fang[1,2] · Panpan Zhu[1,2] · Zhuangzhi Yang[3] · Xi Peng[4] · Zhicai Zuo[1,2] · Hengmin Cui[1,2] · Ping Ouyang[1] · Gang Shu[1] · Zhengli Chen[1] · Chao Huang[1] · Wentao Liu[1]

Received: 21 February 2018 / Accepted: 23 April 2018 / Published online: 16 May 2018


## Abstract

Aflatoxin $B_1$ ($AFB_1$) can cause hepatotoxicity, genotoxicity, and immunosuppressive effects for a variety of organisms. Selenium (Se), as an essential nutrient element, plays important protective effects against cell apoptosis induced by $AFB_1$. This research aimed to reveal the ameliorative effects of selenium on $AFB_1$-induced excess apoptosis in chicken splenocytes through death receptor and endoplasmic reticulum pathways in vivo. Two hundred sixteen neonatal chickens, randomized into four treatments, were fed with basal diet (control treatment), 0.4 mg/kg Se supplement (+Se treatment), 0.6 mg/kg $AFB_1$ ($AFB_1$ treatment), and 0.6 mg/kg $AFB_1$ + 0.4 mg/kg Se ($AFB_1$ + Se treatment) during 21 days of experiment, respectively. Compared with the $AFB_1$ treatment, the levels of splenocyte apoptosis in the $AFB_1$ + Se treatment were obviously dropped by flow cytometry and TUNEL assays although they were still significantly higher than those in the control or + Se treatments. Furthermore, the mRNA expressions of CASP-3, CASP-8 and CASP-10, GRP78, GRP94, TNF-α, TNF-R1, FAS, and FASL of splenocytes in the $AFB_1$ + Se treatment by qRT-PCR assay were significantly decreased compared with the $AFB_1$ treatment. These results indicate that Se could partially ameliorate the $AFB_1$-caused excessive apoptosis of chicken splenocytes through downregulation of endoplasmic reticulum and death receptor pathway molecules. This research may rich the knowledge of the detoxification mechanism of Se on $AFB_1$-induced apoptosis.

**Keywords** Sodium selenite · $AFB_1$ · Splenocyte apoptosis · Endoplasmic reticulum molecules · Death receptor molecules · Chicken

## Introduction

Aflatoxins are secondary toxic metabolites produced by the genus *Aspergillus*-coumarin derivatives containing a dihydrofurofuran moiety [1]. Aflatoxin $B_1$ ($AFB_1$), the most toxic form of aflatoxins, is frequently encountered in cereal crops and peanut meal [2, 3]. This toxin can disturb protein synthesis, and impede metabolic systems, resulting in hepatotoxic, mutagenic, and immunosuppressive effects on animals and humans [4–8].

The spleen, the most important periphery lymphoid organ, contains a large number of lymphocytes and macrophages, playing a crucial role in the cellular and humoral immunity [9]. Documents have revealed that $AFB_1$ could impede the development of spleen [10, 11], inhibit the expression of cytokines [12], as well as led oxidative stress [13] and excessive apoptosis of splenocyte [14].

Jing Fang and Panpan Zhu contributed equally to this work.

✉ Xi Peng
pengxi197313@163.com

✉ Zhicai Zuo
zzcjl@126.com

[1] College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, Sichuan, People's Republic of China

[2] Key Laboratory of Animal Diseases and Environmental Hazards of Sichuan Province, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, Sichuan, People's Republic of China

[3] Chengdu Academy of Agriculture and Forestry Sciences, Chengdu 611130, Sichuan, People's Republic of China

[4] College of Life Sciences, China West Normal University, Nanchong 637002, Sichuan, People's Republic of China

Selenium (Se), an essential element for the animal body, participates in the normal function of immune system [15–17]. Se could amend the disease process [18] and ameliorate the $AFB_1$-induced negative effects [19, 20] including the hindered development and injured histological structure of immune organs, lowered T cell subset proportion, as well as declined antibody levels [17, 21–23].

However, no information on the ameliorative aspects of Se on $AFB_1$-caused excess apoptosis in chicken splenocytes through endoplasmic reticulum and death receptor and pathways in vivo was available. This research aimed to explore these unknown areas by flow cytometry and TUNEL assays as well as qRT-PCR method. The outcomes from the present study may rich the knowledge of the detoxification mechanism of Se.

## Materials and Methods

### Animals and Diets

Two hundred sixteen healthy neonatal Cobb chickens (male) were purchased from a commercial rearing farm (Wenjiang poultry farm, Sichuan province, China) and were randomized into four dietary treatments: control (0 mg/kg $AFB_1$), +Se (0.4 mg/kg supplement Se), $AFB_1$ (0.6 mg/kg $AFB_1$), and $AFB_1$ + Se (0.6 mg/kg $AFB_1$ + 0.4 mg/kg supplement Se), respectively. There were three replicates/treatment and 24 birds/replicate. The control diet was the corn-soybean basal diet and provided by the Animal Nutrition Institute of Sichuan Agricultural University (Chengdu, Sichuan, China). Nutritional requirements were sufficient in accordance with National Research Council (1994) (National Research Council, 1994) [24] and Chinese Feeding Standard of Chicken (NY/T33–2004) (Table 1). The 0.6 mg/kg $AFB_1$ (Sigma-Aldrich, USA, A6636)-contaminated diets were produced based on the Kaoud's report [25]. Furthermore, 1% sodium selenite (feed-grade) was mingled into the control diet by a stepwise dilution method to make +Se and $AFB_1$ + Se diets with 0.4 mg/kg Se supplement. The Se content of control treatment diet was 0.332 mg/kg by the test of hydride-generation atomic absorption spectroscopy. The doses of $AFB_1$ and Se supplement were determined on the basis of our early studies [26, 27]. Chickens in cages with electric heating installation were given water and the above described diets ad libitum throughout 21 days of experiment. Sichuan Agricultural University Animal Care and Use Committee approved this operation in this research involving animals.

### Apoptosis Analysis by Flow Cytometry

The spleen tissues from six chickens in each treatment were obtained for determination of the apoptotic percentages by flow cytometry (BD FACSCalibur) on day 7, 14, and 21 of the experiment. Immediately, the sample splenocyte suspensions that could pass through the nylon screen with a 300-mesh were made by scissors. After being rinsed with ice-cold PBS, the suspensions were adjusted to be $1 \times 10^6$ cells/mL. One hundred microliters of cell suspensions with 5 μL Annexin V-Fluorescein isothiocyanate (V-FITC) and 5 μL propidium iodide (PI) were incubated for 15 min at 25 °C in the dark. After 400 μL 1 × Annexin-binding buffer was put into the mixture; the apoptotic percentages were immediately tested by flow cytometer. The annexin V-FITC Kit was obtained from BD Pharmingen (USA, 556547).

### TUNEL Assay

On day 7, 14, and 21, six spleen tissues from each treatment were obtained, immediately fixed in 4% paraformaldehyde (PFA), and processed for the routine paraffin embedded section with 5 μm for TUNEL assay as reported by Zheng [28]. The numbers of TUNEL-positive cells in spleens were conducted by a digital microscope camera system (Nikon DS-Ri1, Japan) and Image-Pro Plus5.1 (USA) image analysis software. Five fields at × 400 magnification (corresponding to 0.064 $mm^2$ per field) were randomly chosen in each section, and all TUNEL-positive cells showing yellow or brown nuclear were counted in each field of vision. The TUNEL cell apoptosis detection kit was obtained from QIA33, Merck, Germany.

### Quantitative Real-Time PCR

On day 7, 14, and 21 of the experiment, six spleens from four treatments were taken and stored in liquid nitrogen. Total RNA of spleen was extracted by RNAiso Plus (9108/9109, Takara, Otsu, Japan) based on the manufacturer's guide. The mRNA was reversely transcribed, and cDNA was formed by Prim-Script™ RT reagent Kit (RR047A, Takara, Japan). The cDNA as a template was used for PCR analysis. The β- actin gene expression value was chosen as calibration of gene expression tool in the control group. The results were analyzed using the $2^{-\Delta\Delta CT}$ method [29]. Sangon Biotech (Shanghai, China) provided the genes primers designed with Primer 5 (Table 2).

### Statistical Analysis

The statistical analyses were done by one-way analysis of variance and Dunnett T3 in SPSS 20.0 software (IBM Corp, Armonk, NY, USA) for windows. The data of this experiment were shown as mean ± standard deviation ($X \pm$ SD). Statistical significant differences were considered at

**Table 1** Composition of the basal diet

| Composition | Content (%) | Nutrient | Content (%) |
|---|---|---|---|
| Corn | 51.95 | Crude protein (CP) | 21.5 |
| Soybean | 39.5 | Methionine (Met) | 0.5 |
| Rapeseed oil | 4.1 | Calcium (Ca) | 1 |
| DL-Metionine | 0.2 | All phosphorus (P) | 0.7 |
| Calcium hydrogen phosphate | 1.85 | Methionine+Cystine (Met+Cys) | 0.84 |
| Calcium carbonate | 1.3 | Lysine (Lys) | 1.15 |
| Sodium chloride | 0.4 | Threonie (Thr) | 0.83 |
| Trace element premix[a] | 0.5 | Metabolizable energy (ME) (MJ/kg) | 12.52 |
| Choline | 0.17 | | |
| Multivitamins[b] | 0.03 | | |
| Total | 100 | | |

[a] Trace element premix (mg/kg): $FeSO_4 \cdot 7H_2O$, 530; $CuSO_4 \cdot 5H_2O$, 30; $MnSO_4 \cdot H_2O$, 400; $ZnSO_4 \cdot 7H_2O$, 470; KI, 18; $NaSeO_3$, 0.3

[b] Multivitamins: Vitamin A, 13,500 IU/kg; Vitamin D, 3000 IU/kg; Vitamin E, 24 IU/kg; Vitamin $K_3$, 3 mg/kg; Pantothenic acid, 15 mg/kg; Folic acid, 1.05 mg/kg; Nicotinamide, 30 mg/kg; Biotin, 0.14 mg/kg

$p < 0.05$ and markedly significant differences were considered at $p < 0.01$.

## Results

### Apoptosis Percentage of Splenocytes by Flow Cytometry

The apoptotic percentages of splenocytes were demonstrated in Fig. 1. The percentages of apoptosis in the $AFB_1$ treatment were evidently raised during the experiment compared with the control treatment ($p < 0.01$). In comparison to the $AFB_1$ treatment, the apoptotic percentages in the $AFB_1$ + Se treatment were significantly dropped ($p < 0.05$) although they still kept obviously higher level compared with +Se or control treatments ($p < 0.05$ or 0.01). There was on evident difference in these parameters between the control and + Se treatments ($p > 0.05$).

### The Apoptosis of Splenocytes by TUNEL Assay

The apoptosis of splenocytes using TUNEL test was presented in Fig. 2. TUNEL-positive cells which showed yellow or brown nuclear were mainly distributed in the white pulp. The TUNEL-positive cell numbers in the $AFB_1$ and $AFB_1$ + Se treatments were significantly increased ($p < 0.01$) compared with the +Se and control treatments. However, in comparison to the $AFB_1$ treatment, the TUNEL-positive cell numbers in the $AFB_1$ + Se treatment were significantly declined ($p < 0.05$). These parameters had no obvious difference between the +Se and control treatments ($p > 0.05$).

### The mRNA Expression of Apoptosis Regulatory Molecules by RT-qPCR

The mRNA expressions of CASP-3, CASP-8, CASP-10, TNF-α, TNF-R1, FAS, and FASL in spleen involved in death receptor pathway were exhibited in Fig. 3. When compared

**Table 2** Primer sequence for apoptotic genes

| Gene symbol | RefSeq mRNA mRNA number | Forward primers | Reverse primers |
|---|---|---|---|
| CASPASE −3 | NM_204725 | TGGCCCTCTTGAACTGAAAG | TCCACTGTCTGCTTCAATACC |
| CASPASE −8 | NM_204592 | GTCTCCGTTCAGGTATCTGCT | TCTCAATGAAAACGTCCGGC |
| CASPASE-10 | XM_421936 | CTGGGGGCTCCAAAAGTCC | AAAGGGGGACAAAGCCAACA |
| FAS | NM_001199487 | TCCACCTGCTCCTCGTCATT | GTGCAGTGTGTGTGGGAACT |
| FASL | NM_001031559 | GGCATTCAGTACCGTGACCA | CCGGAAGAGCACATTGGAGT |
| GRP78 | NM_205491 | GGTGTTGCTTGATGTGTGTCC | GCTGATTGTCAGAAGCTGTGG |
| GRP94 | NM_204289 | TGACCTGGATGCAAAGGTGGA | TTAAACCCCACACCATCCCTCAAC |
| TNF-α | AY765397 | TGTGTATGTGCAGCAACCCGTAGT | GGCATTGCAATTTGGACAGAAGT |
| TNF-R1 | NM_001030779 | CCTGTCTGTCTTCCCTGTCC | GGTGCATGGGGTCTTTTCTA |
| β-actin | L08165 | TGCTGTGTTCCCATCTATCG | TTGGTGACAATACCGTGTTCA |

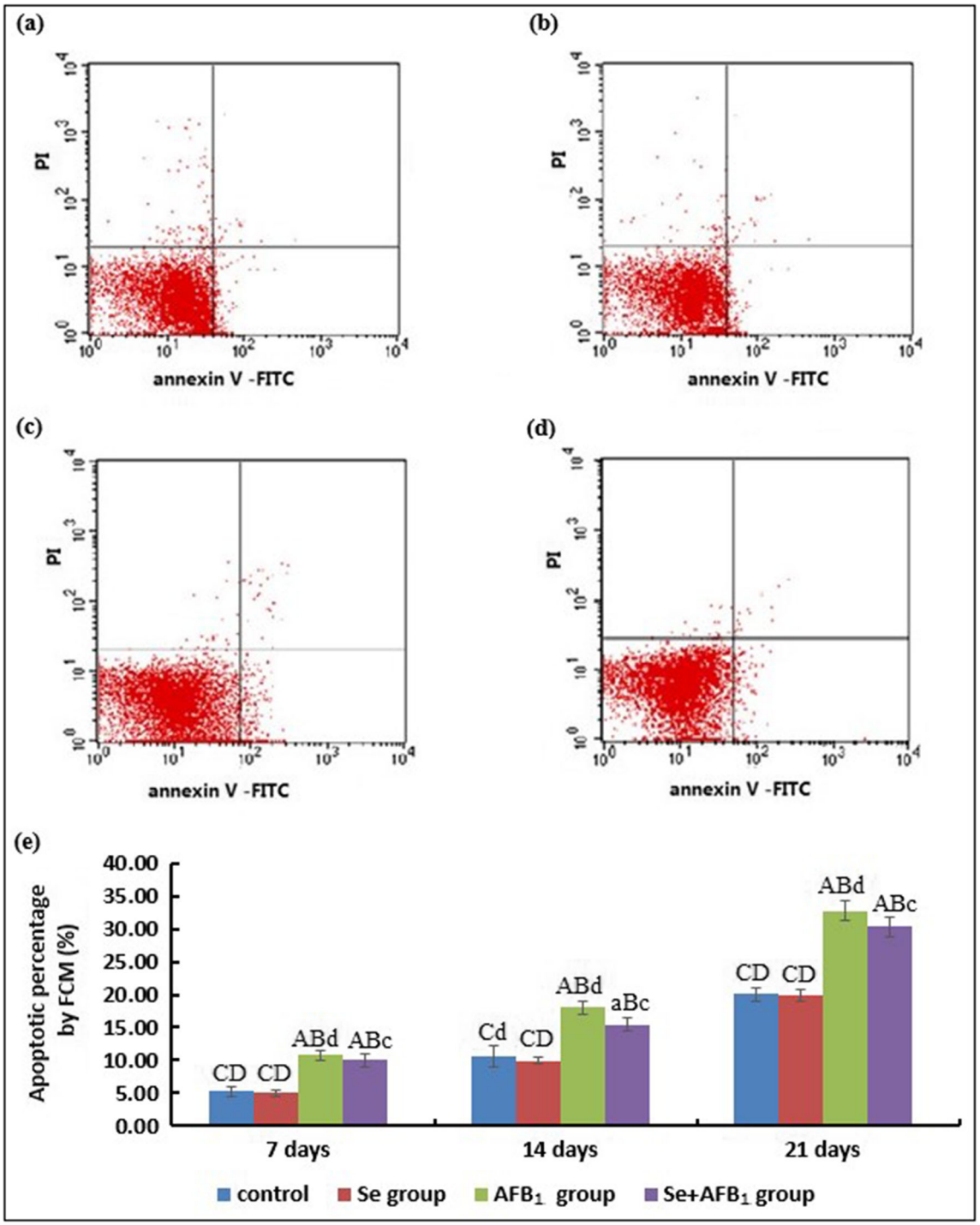


**Fig. 1** The splenic cell apoptosis by flow cytometry analysis. **a**–**d** Representative scattergram of apoptotic splenocytes by flow cytometry assay in the control (**a**), +Se (**b**), $AFB_1$ (**c**), and $AFB_1$ + Se (**d**) treatments on 21 days of the experiment. **e** Apoptotic percentages of splenocytes by flow cytometry assay. Uppercase letters A, B, C, and D represent the significant difference ($p < 0.01$) between the treatment and control, +Se, $AFB_1$, and $AFB_1$ + Se treatments, respectively. Lowercase letters a, b, c, and d represent difference ($p < 0.05$) between the treatment and control, +Se, $AFB_1$, and $AFB_1$ + Se treatments, respectively.

with the control and + Se treatments, the mRNA expressions of CASP-3, CASP-8, CASP-10, TNF-α, TNF-R1, FAS, and FASL in the $AFB_1$ treatment were obviously increased during the experiment ($p < 0.05$ or $p < 0.01$). In comparison to the $AFB_1$ treatment, these parameters in the $AFB_1$ + Se treatment were obviously decreased ($p < 0.05$ or $p < 0.01$). No evident differences were observed in these gene expressions between the +Se and control treatments ($p > 0.05$).

The mRNA expression levels of GRP78 and GRP94 related to endoplasmic reticulum pathway were demonstrated in

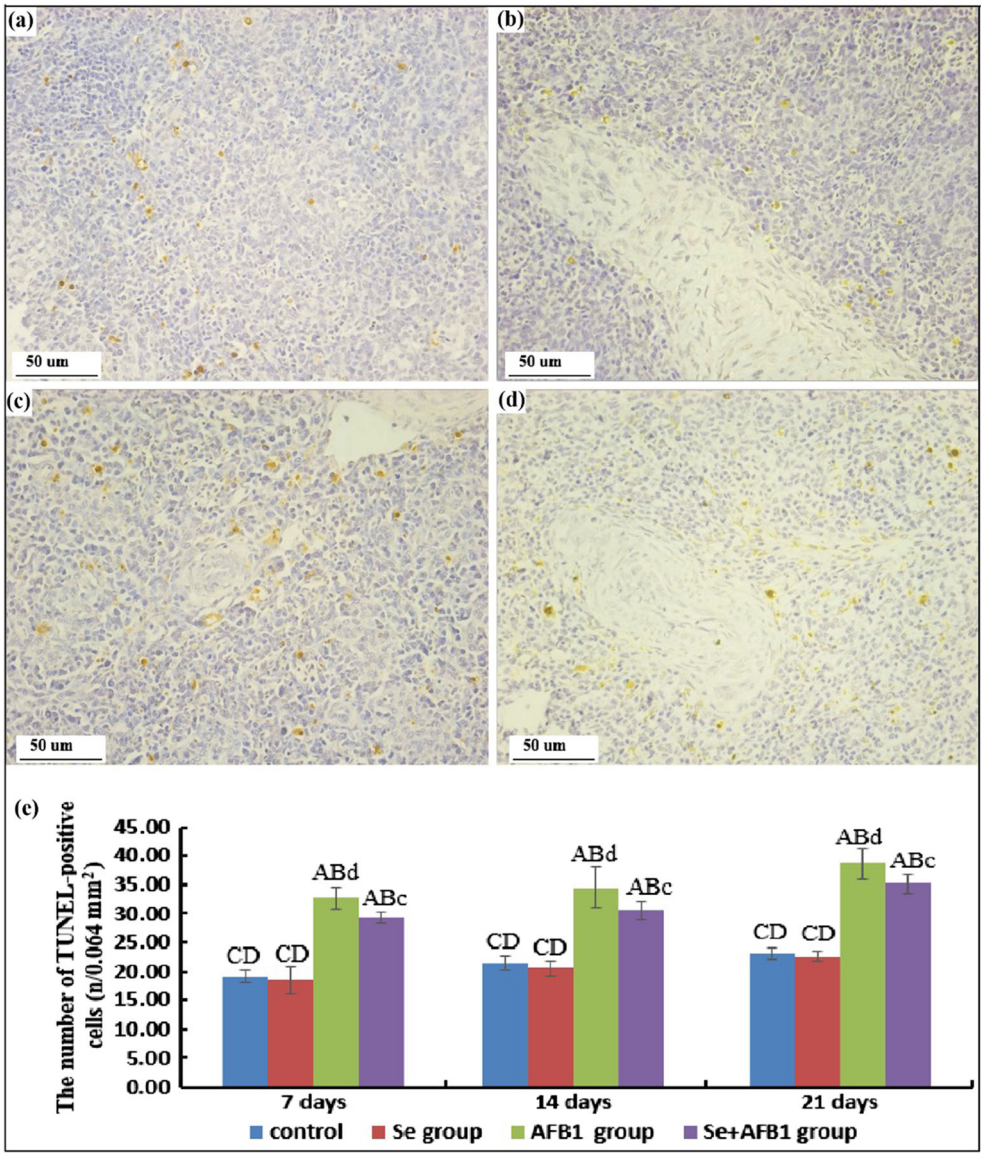


**Fig. 2** The apoptosis splenocytes by TUNEL assay. **a**–**d** TUNEL-positive cells (TUNEL assay, Scale bar 50 μm) in the control (**a**), +Se (**b**), $AFB_1$(**c**), and $AFB_1$ + Se treatments (**d**) on day 21. **e** The numbers of TUNEL-positive cells (microscopic quantitative analysis). Uppercase letters A, B, C, and D represent the significant difference ($p < 0.01$) between the treatment and control, +Se, $AFB_1$, and $AFB_1$ + Se treatments, respectively. Lowercase letters a, b, c, and d represent difference ($p < 0.05$) between the treatment and control, +Se, $AFB_1$, and $AFB_1$ + Se treatments, respectively.

Fig. 4. Compared with the control treatment, the gene expression levels of GRP78 and GRP94 in the $AFB_1$ and $AFB_1$+ Se treatments were evidently raised during the experiment ($p < 0.05$ or $p < 0.01$). However, the parameters in the $AFB_1$ + Se treatment were evidently lower than those in the $AFB_1$ treatment ($p < 0.05$ or $p < 0.01$). Furthermore, these parameters exhibited no obvious differences between the +Se and control treatments ($p > 0.05$).

## Discussions

As a crucial component in several vital metabolic pathways, Se displays an antioxidant effective oxygen-free radical scavenging, protects the organs and tissues from oxidative damage, and improves the immune function [30, 31]. Generally, Se is absorbed for animals by food as the main source. Increasing reports showed that Se can defend animal and humans from different toxic substances [30, 32].

Se can alleviate $AFB_1$-induced damages in broilers' immune system, including the growth retardation of immune organs [23], the declined percentages of T cell subsets in spleen and thymus [17, 21], the lowered contents of immunoglobulins in the small intestine [22], and the G2/M phase arrest of jejunum [26], along with the excess apoptosis in immune organs [17, 23, 33]. In this study, the flow cytometry results showed that apoptotic levels in the $AFB_1$ treatment were obviously raised compared with the +Se and control treatments. And, these parameters in the $AFB_1$+ Se treatment were significantly declined compared with the $AFB_1$ treatment, although they were still evidently higher than those of the +Se and control treatments. In addition, the changing pattern of the apoptotic numbers using TUNEL staining was similar to that by flow cytometry assay. The above results

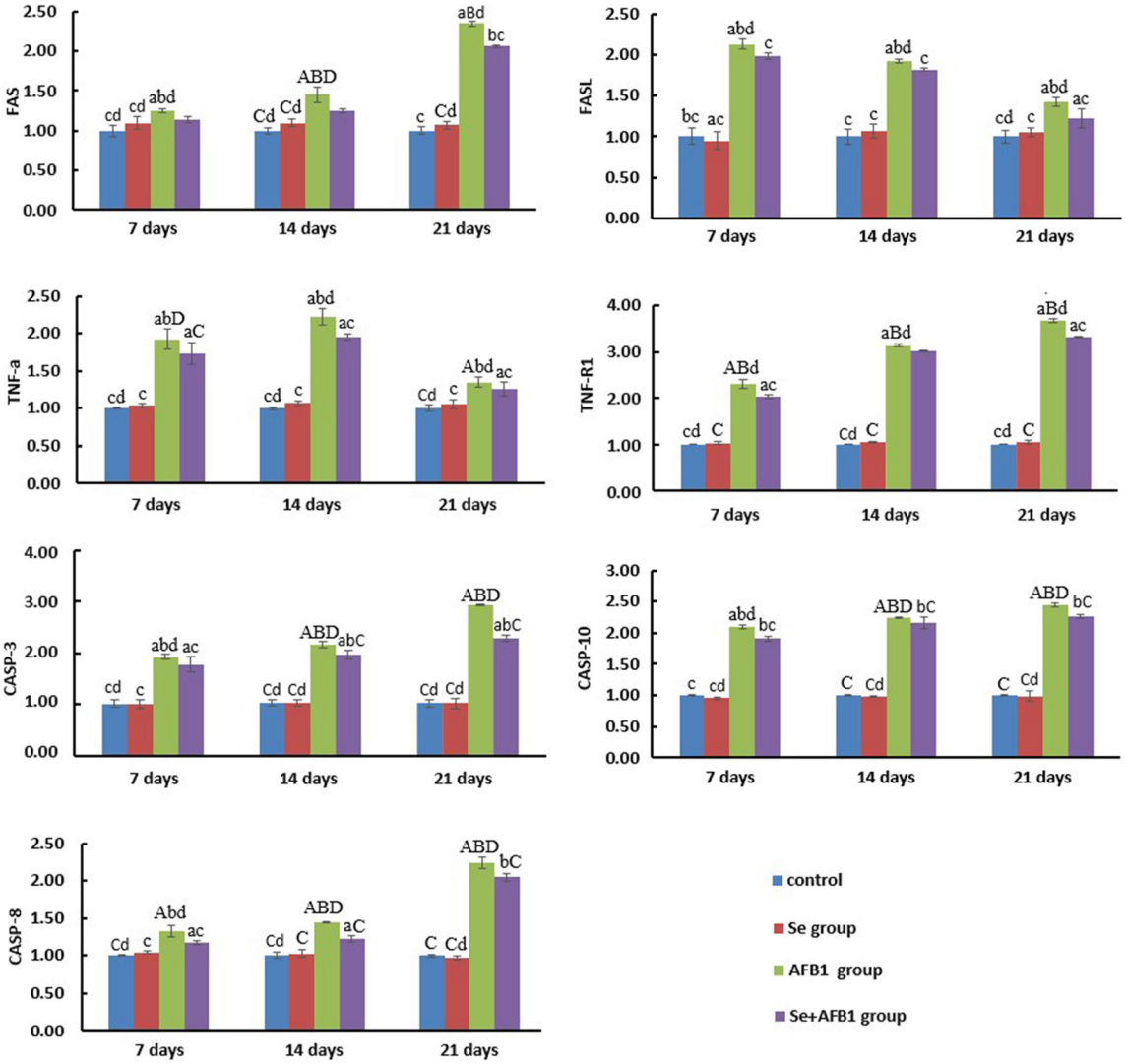


**Fig. 3** The mRNA expression levels of FAS, FASL, TNF-a, TNF-R1, CASP-3, CASP-10, and CASP-8 of splenocytes (fold of control). Uppercase letters A, B, C, and D represent the significant difference ($p < 0.01$) between the treatment and control, +Se, $AFB_1$, and $AFB_1$ + Se treatments, respectively. Lowercase letters a, b, c, and d represent difference ($p < 0.05$) between the treatment and control, +Se, $AFB_1$, and $AFB_1$ + Se treatments, respectively.

indicated that dietary 0.4 mg/kg Se supplement partially alleviated excess apoptosis caused by 0.6 mg/kg $AFB_1$, which was in accordance with previous researches in the chicken's central immune organs [17, 23]. However, the alliterative mechanism of Se on the $AFB_1$-induced excess apoptosis of splenocytes was required to be elucidated by further investigations. Meanwhile, the apoptosis rates in four groups by flow cytometry test showed an increasing trend with the age, while

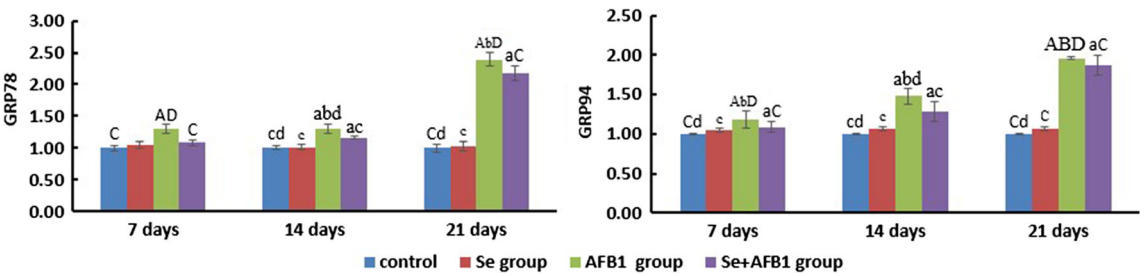


**Fig. 4** The mRNA expression levels of GRP78 and GRP94 of splenocytes (fold of control). Uppercase letters A, B, C, and D represent the significant difference ($p < 0.01$) between the treatment and control, +Se, $AFB_1$, and $AFB_1$ + Se treatments, respectively. Lowercase letters a, b, c, and d represent difference ($p < 0.05$) between the treatment and control, +Se, $AFB_1$, and $AFB_1$ + Se treatments, respectively.

the TUNEL-positive cells' numbers did not show such a tendency by age, which may be due to the fact that TUNEL staining mainly reveals the late apoptotic cells, whereas the flow cytometry detects both the early and late apoptotic cells.

Apoptosis is a modulated physiological process resulting in cell death in order to keep homeostasis. It is also an active course that contains a variety of actions such as activation, expression, and regulation of genes so as to accommodate to the living surroundings [34]. CASP-8, -9, -10, and -12 belong to pro-apoptotic molecules, while, CASP-3,-6, and -7 function as effector apoptotic molecules [35]. FAS and TNF-α binding with its ligand FASL and TNF-R1 respectively activate CASP- 8 and -10 and CASP-3, resulting in cell apoptosis [36, 37]. In this research, compared with the +Se and control treatments, the mRNA expressions of CASP-3, CASP-8, CASP-10, TNF-α, TNF-R1, FAS, and FASL in the $AFB_1$ treatment were obviously increased. And, these gene expressions in the $AFB_1$ + Se treatment were significantly decreased compared with the $AFB_1$ treatment, although they were still evidently higher than those of the control treatment. Moreover, no obvious differences in these gene expressions between the +Se and control treatments were observed. These results implicated that supplement dietary Se with 0.4 mg/Kg could down regulate the gene expression of death receptor pathway molecules induced by $AFB_1$.

The endoplasmic reticulum (ER) has many functions such as lipid biosynthesis and glycosylation, as well as protein folding and assembly [38]. Unfolded or misfolded proteins are accumulated in the ER lumen when the protein-folding capacity in the ER is overwhelmed, resulting in ER stress [39] and then the activation of CASP-12 mediated apoptosis [40]. These reactions will induce the expression of glucose 2-regulated protein 78kD (GRP78), GRP94, and other endoplasmic reticulum molecular chaperones which have protective roles and also lead to apoptosis independently [41–43]. In the present research, compared with the control treatment, the gene expressions of molecular chaperone GRP78 and GRP94 in the $AFB_1$ treatment were evidently raised. However, these values in the $AFB_1$ + Se treatment were significantly decreased in comparison to the $AFB_1$ treatment. These results demonstrated that supplement dietary Se with 0.4 mg/kg could partially downregulate the gene expressions of GRP78 and GRP94 caused by $AFB_1$.

In summary, supplement dietary Se with 0.4 mg/kg could partially alleviate $AFB_1$-induced excess apoptosis in chicken splenocytes via downregulation of the gene expressions of endoplasmic reticulum and death receptor pathway molecules. The outcomes from the present study may enrich the knowledge of the detoxification mechanism of Se on $AFB_1$-induced apoptosis.

**Funding information** This work was supported by the program for Changjiang scholars, the University Innovative Research Team (IRT 0848), the Education Department of Sichuan Province (2012FZ0066) and (2013FZ0072) and Huimin project of Chengdu science and technology (2016-HM01-00337-SF).

## Compliance with Ethical Standards

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

## References

1. Trandinh N (2013) Mycotoxins and food. Microbiol Aust 34(2): 70–72
2. Gowda NKS, Malathi V, Suganthi RU (2003) Screening for aflatoxin and effect of moisture, duration of storage and form of feed on fungal growth and toxin production in livestock feeds. Anim Nutr Feed Techn 3(1):45–51
3. Yunus AW, Razzazi-Fazeli E, Bohm J (2011) Aflatoxin $B_1$ in affecting broiler's performance, immunity, and gastrointestinal tract: a review of history and contemporary issues. Toxins 3(6):566–590
4. Mohamed AM, Metwally NS (2009) Antiaflatoxigenic activities of some plant aqueous extracts against aflatoxin-$B_1$ induced renal and cardiac damage. J Pharmacol Toxicol 4(1):1–16
5. Wangikar PB, Dwivedi P, Sinha N, Sharma AK, Telang AG (2005) Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin $B_1$ with special reference to microscopic effects. Toxicology 215(1–2):37–47
6. Lakkawar AW, Rajiv Gandhi College of Veterinary an Animal Sciences Chattopadhyay S and Johri TS (2004) Experimental aflatoxin B1 toxicosis in young rabbits—a clinical and pathoanatomical study. Anatol Stud 36(12):467–472
7. Dai Y, Huang K, Zhang B, Zhu L, Xu W (2017) Aflatoxin $B_1$-induced epigenetic alterations: an overview. Food Chem Toxicol 109(Pt 1):683–689
8. Purchase IFH (1967) Acute toxicity of $AFB_1$ and $M_1$ in one-day-old ducklings. Food Cosmet Toxicol 5(3):339–342
9. Mebius RE, Kraal G (2005) Structure and function of the spleen. Nat Rev Immunol 5(8):606–616
10. Guo SN, Liao SQ, Su RS, Lin RQ, Chen YZ, Tang ZX, Wu H, Shi DY (2012) Influence of longdan xiegan decoction on body weights and immune organ indexes in ducklings intoxicated with aflatoxin $B_1$. J Anim Vet Adv 11(8):1162–1165
11. Peng X, Zhang K, Bai S, Ding X, Zeng Q, Yang J, Fang J, Chen K (2014) Histological lesions, cell cycle arrest, apoptosis and T cell subsets changes of spleen in chicken fed aflatoxin-contaminated corn. Inter J Env Res Pub Heal 11(8):8567–8580
12. Qian G, Tang L, Guo X, Wang F, Massey ME, Su J, Guo TL, Williams JH, Phillips TD, Wang JS (2014) Aflatoxin $B_1$ modulates the expression of phenotypic markers and cytokines by splenic lymphocytes of male F344 rats. J Appl Toxicol 34(3):241–249
13. Chen J, Chen K, Yuan S, Peng X, Fang J, Wang F, Cui H, Chen Z, Yuan J, Geng Y (2016) Effects of aflatoxin $B_1$ on oxidative stress markers and apoptosis of spleens in broilers. Toxicol Ind Health 32(2):278–284
14. Zhu PP, Zuo ZC, Zheng ZX, Wang FY, Peng X, Fang J, Cui HM, Gao CX, Song HT, Zhou Y (2017) Aflatoxin $B_1$ affects apoptosis and expression of death receptor and endoplasmic reticulum molecules in chicken spleen. Oncotarget 8(59):99531–99540
15. McKenzie, Roderick C, Rafferty S, Teresa and Geoffrey J (1998) Selenium: an essential element for immune function. Immunol Today 19(8):342–345
16. Hoffmann PR, Berry MJ (2010) The influence of selenium on immune responses. Mol Nutr Food Res 52(11):1273–1280

17. Chen K, Shu G, Peng X, Fang J, Cui H, Chen J, Wang F, Chen Z, Zuo Z, Deng J (2013) Protective role of sodium selenite on histopathological lesions, decreased T-cell subsets and increased apoptosis of thymus in broilers intoxicated with aflatoxin $B_1$. Food Chem Toxicol 59(3):446–454
18. Jakhar KK, Sadana JR (2004) Sequential pathology of experimental aflatoxicosis in quail and the effect of selenium supplementation in modifying the disease process. Mycopathologia 157(1):99–109
19. Shi D, Guo S, Liao S, Su R, Pan J, Lin Y, Tang Z (2012) Influence of selenium on hepatic mitochondrial antioxidant capacity in ducklings intoxicated with aflatoxin $B_1$. Biol Trace Elem Res 145(3): 325–329
20. Guo S, Shi D, Liao S, Su R, Lin Y, Pan J, Tang Z (2012) Influence of selenium on body weights and immune organ indexes in ducklings intoxicated with aflatoxin $B_1$. Biol Trace Elem Res 146(2): 167–170
21. Chen K, Peng X, Fang J, Cui H, Zuo Z, Deng J, Chen Z, Geng Y, Lai W, Tang L (2014) Effects of dietary selenium on histopathological changes and T cells of spleen in broilers exposed to aflatoxin $B_1$. Inter J Env Res Pub Heal 11(2):1904–1913
22. He Y, Fang J, Peng X, Cui H, Zuo Z, Deng J, Chen Z, Geng Y, Lai W, Shu G (2014) Effects of sodium selenite on aflatoxin $B_1$-induced decrease of ileal IgA+ cell numbers and immunoglobulin contents in broilers. Biol Trace Elem Res 160(1):49–55
23. Chen K, Jing F, Xi P, Cui H, Jin C, Wang F, Chen Z, Zuo Z, Deng J, Lai W (2014) Effect of selenium supplementation on aflatoxin $B_1$ - induced histopathological lesions and apoptosis in bursa of Fabricius in broilers. Food Chem Toxicol 74(74):91–97
24. Dale N (1994) National research council nutrient requirements of poultry–ninth revised edition. J Appl Poult Res 3(1):101–101
25. Kaoud H (2015) Innovative methods for the amelioration of aflatoxin ($AFB_1$) effect in broiler chicks. Sjar Net 1(2):19–24
26. Fang J, Yin H, Zheng Z, Zhu P, Peng X, Zuo Z, Cui H, Zhou Y, Ouyang P, Geng Y (2018) The molecular mechanisms of protective role of Se on the G2/M phase arrest of jejunum caused by $AFB_1$. Biol Trace Elem Res 181(1):142–153
27. Xi P, Yu Z, Na L, Chi X, Li X, Min J, Jing F, Cui H, Lai W, Yi Z (2016) The mitochondrial and death receptor pathways involved in the thymocytes apoptosis induced by aflatoxin $B_1$. Oncotarget 7(11):12222–12234
28. Zheng Z, Zuo Z, Zhu P, Wang F, Yin H, Peng X, Fang J, Cui H, Gao C, Song H (2017) A study on the expression of apoptotic molecules related to death receptor and endoplasmic reticulum pathways in the jejunum of $AFB_1$-intoxicated chickens. Oncotarget 8(52):89655–89664
29. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4):402–408
30. Liu CY, Zuo ZC, Zhu PP, Zheng ZX, Xi P, Jing F, Cui HM, Zhou Y, Ping O, Yi G (2017) Sodium selenite prevents suppression of mucosal humoral response by $AFB_1$ in broiler's cecal tonsil. Oncotarget 8(33):54215–54226
31. Harsharn G, Glen W (2008) Selenium, immune function and resistance to viral infections. Nutr Diet 65(s3):S41–S47
32. Dorado RD, Eduardo APMD, Aquino TM (1985) Effects of dietary selenium on hepatic and renal tumorigenesis induced in rats by diethylnitrosamine. Hepatology 5(6):1201–1208
33. Wang F, Gang S, Xi P, Jing F, Chen K, Cui H, Chen Z, Zuo Z, Deng J, Yi G (2013) Protective effects of sodium selenite against aflatoxin B1-induced oxidative stress and apoptosis in broiler spleen. Inter J Env Res Pub Heal 10(7):2834–2844
34. Degterev A, Yuan J (2008) Expansion and evolution of cell death programs. Nat Rev Mol Cell Bio 9(5):378–390
35. Cowan CM, Thai J, Krajewski S, Reed JC, Nicholson DW, Kaufmann SH, Roskams AJ (2001) Caspases 3 and 9 send a pro-apoptotic signal from synapse to cell body in olfactory receptor neurons. J Neurosci 21(18):7099–7109
36. Miao K, Zhang L, Yang S, Qian W, Zhang Z (2013) Intervention of selenium on apoptosis and Fas/FasL expressions in the liver of fluoride-exposed rats. Environ Toxicol Pharmacol 36(3):913–920
37. Merger M, Viney JL, Borojevic R, Steele-Norwood D, Zhou P, Clark DA, Riddell R, Maric R, Podack ER, Croitoru K (2002) Defining the roles of perforin, Fas/FasL, and tumour necrosis factor alpha in T cell induced mucosal damage in the mouse intestine. Gut 51(2):155–163
38. English AR, Voeltz GK (2013) Endoplasmic reticulum structure and interconnections with other organelles. CSH Perspect Biol 5(4):a013227
39. Cybulsky AV (2017) Endoplasmic reticulum stress, the unfolded protein response and autophagy in kidney diseases. Nat Rev Nephrol 13(11):681–696
40. Bustany S, Cahu J, Guardiola P, Sola B (2015) Cyclin D1 sensitizes myeloma cells to endoplasmic reticulum stress-mediated apoptosis by activating the unfolded protein response pathway. BMC Cancer 15(11):262
41. Kamil M, Haque E, Irfan S, Sheikh S, Hasan A, Nazir A, Lohani M, Mir SS (2017) ER chaperone GRP78 regulates autophagy by modulation of p53 localization. Front Biosci 1(9):54–66
42. Wu BX, Hong F, Zhang Y, Ansaaddo E, Li Z (2016) GRP94/gp96 in cancer: biology, structure, immunology, and drug development. Adv Cancer Res 129:165–190
43. Little E, Ramakrishnan M, Roy B, Gazit G, Lee AS (1994) The glucose-regulated proteins (GRP78 and GRP94): functions, gene regulation, and applications. Crit Rev Eukar Gene 4(1):1–18