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Ameliorated effect of *Lactobacillus plantarum* SCS2 on the oxidative stress in HepG2 cells induced by AFB1

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Abstract

Aflatoxin B1 (AFB1), a widespread mycotoxin found in food, poses a significant threat to the food industry and production. The primary target of AFB1 is the liver. Probiotic-mediated antitoxicity has been proposed to overcome aflatoxin toxicity. In the present study, to investigate the protective effects and molecular mechanisms of *Lactobacillus plantarum* SCS2 (*L. plantarum* SCS2) against HepG2 cells oxidative stress induced by AFB1. HepG2 cells were cultured and treated with different concentrations of AFB1 (0-10 μmol/L) to induce oxidative stress and *L. plantarum* SCS2 (total protein, 0.4 mg/mL) was pretreated HepG2 for 2 h and then supplemented with AFB1 (10 μmol/L) for 22 h to observe cellular oxidative stress. The cytotoxicity, reactive oxygen species, malondialdehyde, activities and mRNA expressions of antioxidant enzymes and activation of the Nrf2 signaling pathway were measured. The results showed that AFB1 exposure caused oxidative stress in HepG2. Supplementation of *L. plantarum* SCS2 prevented AFB1-induced HepG2 cells activity decreased and activated antioxidant enzymes activity by inhibiting Nrf2 over expression. The results of in vitro experiments revealed that *L. plantarum* SCS2 evidently protected against AFB1-induced oxidative stress.

Keywords: *Lactobacillus plantarum* SCS2, aflatoxin B1, oxidative stress.

Practical Application: The results of this study showed that *L. plantarum* SCS2 could effectively improve oxidative stress by increasing the activity of antioxidant enzymes. In the future, people could promote the application of lactic acid bacteria (LAB) which is found in traditional foods with the ability of improving oxidative damage in food nutrition and related fields, so as to guide residents to form good dietary habits. Meanwhile, it also can enhance the edible value of traditional foods.

1 Introdution

As a class of toxic fungal secondary metabolites, mycotoxins produce mainly from *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. and *Alternaria* spp. (Bennett & Klich, 2003). The contamination of food and feed by mycotoxins is considered one of the worst food safety problems in the world because these fungal metabolites may have teratogenic, mutagenic, oncogenic and immunosuppressive effects and may cause serious damage to animal and human health (Luo et al., 2021; Ostry et al., 2017). Aflatoxins (AFs) are the most common among mycotoxins, producing mainly by *Aspergillus* flavus and *Aspergillus* parasiticu (Campagnollo et al., 2016). According to the Food and Agriculture Organization of the United Nations (FAO) (Eskola et al., 2020), about 25% of all crops worldwide are mycotoxin-contaminated every year with AFs polluting the most seriously. Currently, more than 20 kinds of AFs have been identified, including AFB1, B2, G1 and G2, of which AFB1 is the most toxic and contaminated (Luo et al., 2015) in crop growth, agricultural harvest, transportation and storage, which seriously endangers human health, and is listed as grade I carcinogen (International Agency for Research on Cancer, 2012) by the International Agency for Research on Cancer (IARC). AFs contamination generally refers to AFB1 contamination. AFB1 causes significant damage and is gaining increasing attention because of its toxicity, carcinogenicity, and universality (Chaytor et al., 2011; Mohammadi et al., 2014). Jakšić et al. (2021) and Pimpitak et al. (2020) found an accurate and reliable determination of AFs in food. However, as AFs accumulation is harmful to human health and environmental safety, it is of more economic and social significance to explore effective methods and mechanisms to prevent AFs contamination.

AFB1 affects the internal organs of human and animals, especially the liver, and consuming a certain amount of AFB1 can lead to acute poisoning, including acute hepatitis, hemorrhagic necrosis, steatosis, and bile duct proliferation (Bishayee, 2014). Epoxy chloropropane Kelch sample related protein-1-nuclear factor erythroid related factor-2/antioxidant response element (Keap1-Nrf2/ARE) signal pathway is critical in cellular antioxidant response. Nrf2-regulated downstream target proteins have been identified as antioxidant proteases (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx, heme oxygenase-1, HO-1), phase II metabolic enzymes, proteasome/molecular chaperones, anti-inflammatory factors, and phase III metabolic enzymes (Hu et al., 2016). AFB1 induces a large amount of reactive

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oxygen species (ROS) in cells, causing a series of changes in the cellular antioxidant mechanism, such as increased expression of Nrf2 gene, and decreased expression of SOD gene (Wang et al., 2017; Yuan et al., 2016). Chen et al. (2016) reported that the addition of 0.15, 0.30 and 0.60 mg/kg AFB1 to the broiler diet caused decreased activity of GPx, glutathione reductase (GR), and catalase (CAT), decreased glutathione (GSH) content and increased malondialdehyde (MDA) in the broiler spleen. The reduced expression of antioxidant enzymes promote oxidative damage in liver cells, it suggested that activation of antioxidant enzyme expression may be a new avenue to improve the prevention and treatment of AFB1 poisoning.

Our previous study showed that *Lactobacillus plantarum* SCS2 (*L. plantarum* SCS2) showed good antioxidant effects in the treatment of diabetic mice and reducing the degree of oxidative damage in mouse pancreas (Wu et al., 2021). However, whether lactic acid bacteria have the same effects on the oxidative stress damage caused by AFB1, and the mechanism by which lactic acid bacteria regulate antioxidant enzymes to mitigate AFB1 poisoning remains to be further investigated.

2 Materials and methods

2.1 Cell Culture

Human liver cancer cell line HepG2 was purchased by Wuhan Procell Technology Company (Wuhan, China) cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY, USA), supplemented with 10% (v/v) fetal bovine serum (Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 100 U/mL streptomycin (HyClone, Utah, Logan, USA) at 37 °C under an atmosphere of 5% CO_2 in humidified air. Cell number was determined by blood counting chamber.

2.2 Preparation of bacteria subjects

L. plantarum SCS2 kept in the Laboratory Center of Public Health Institute of Chengdu University of Traditional Chinese Medicine was isolated from Chinese sausage. 50 μL *L. plantarum* SCS2 was inoculated into 100 mL MRS (Biosharp Beijing, China) and cultured at 37 °C for 24 hours. The strain was washed and resuspended with 0.1mol/L sterile phosphate-buffered saline (PBS) (Solarbio, Beijing, China) for 3 times after centrifugalizing (8000rpm for 10 min at 4 °C) and kept at -80 °C.

2.3 HepG2 cells viability assay

The Cell Counting Kit-8 (CCK-8) (Biosharp, Beijing, China) was used to evaluate the viability of HepG2 cells. And HepG2 cells were seeded at the density of 5×10^4/mL in 96-well plates. For the control group, HepG2 cells were incubated with serum-free DMEM medium for 24 h. For the AFB1 groups, HepG2 cells were cultured with different concentrations of AFB1 (1.25, 2.5, 5, 10, 20 μmol/L). For *L. plantarum* SCS2 intervention dose selection experiments, the HepG2 cells were treated with 10^8, 10^7, 10^6, 10^5 CFU *L. plantarum* SCS2, 10^8, 10^7, 10^6, 10^5 CFU (counted by live bacteria) heat-killed (95 °C for 2 h) bacterial suspension and 0.05, 0.1, 0.2, 0.4mg/mL (calculated by total protein) cell-free extractions of *L. plantarum* SCS2 (The bacterial was added to the zirconia beads for grinding) for 24 h. After that, 0.1% DMSO, 10^6 CFU *L. plantarum* SCS2 and 0.05mg/mL cell-free extractions of *L. plantarum* SCS2 were added in serum-free DMEM medium to pretreated HepG2 cells for 2 h and 10 μmol/L AFB1 treated for another 22 h after preculture.

2.4 Determination of ROS release

HepG2 cells were cultured in 96-well plates with the density of 8×10^4/mL and treated with different concentrations of AFB1 (0, 2.5, 5, 10 μmol/L). After 24h, the cells were washed three times with PBS, and then fluorescent probe (2',7'-dichlorofluorescin diacetate, DCFH-DA, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was added to the serum-free DMEM medium. HepG2 cells with 1 μmol/L DCFH-DA continued to be cultured at 37°C for 30 min. At the end of the culture, HepG2 cells were washed three times with pre-cooling PBS buffer solution again. The fluorescence intensity of ROS was detected by multi-function microplate reader (MD iD5, USA).

2.5 Determination of MDA

HepG2 cells were cultured in 6-well plates with the density of 15×10^4/mL were treated with different concentrations of AFB1 (0, 2.5, 5, 10 μmol/L) for 24 h. After that, HepG2 cells were washed with pre-cooling PBS and lysed in RIPA lysis buffer on ice for 15min, followed by centrifugation at 12000 rpm for 15 min to obtain HepG2 cells lysate. According to the manufacturer's guidelines, the concentration of MDA in HepG2 cells were determined by ELISA kit (Shanghai Enzyme Union Biotechnology, Shanghai, China).

2.6 Determination of Nrf2

After AFB1 treatment, nuclei were extracted according to the manufacturer's guidelines (Solarbio, Beijing, China). The concentration of Nrf2 in the nucleus of HepG2 was detected using ELISA kit (Shanghai Enzyme Union Biotechnology, Shanghai, China).

2.7 Activities of SOD, GPx1, CAT and HO-1

The enzymatic activities of GPx1 and SOD in HepG2 cells were detected using ELISA kit (Shanghai Enzyme Union Biotechnology, Shanghai, China). And the HepG2 cells was operated according to the previous experimental methods.

2.8 Real-time quantitative PCR

HepG2 cells were treated with different concentrations of AFB1 (0, 2.5, 5, 10 μmol/L) for 24 h. Then, total RNA was extracted from HepG2 cells using Cell Total RNA Isolation Kit (Chengdu Foregene Biological Technology Co., Ltd., Chengdu, China). One microgram RNA was reverse-transcribed to complementary DNA (cDNA) via RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA). Real-time quantitative PCR (RT-qPCR) was used with 2 × SYBR green master mix (Chengdu Foregene Biological Technology Co., Ltd., Chengdu, China) by Two-Step Real-Time System (Jena qTOWER 2.0, DE). The primers are displayed in Table 1. And the $2^{-\Delta\Delta CT}$ method

Table 1. Sequence of primers used for Real-time quantitative PCR.

Gene	Gene ID	Primer sequences (5'-3')	Product Size (bp)
β-actin	4780	F: 5'-CCTTCCTGGGCATGGAGTC-3'	189
		R: 5'-TGATCTTCATTGTGCTGGGTG-3'	
Nrf2	6647	F: 5'-GGAGACACACTACTTGGCCT-3'	136
		R: 5'-CCTGAGATGGTGACAAGGGT-3'	
SOD1	6648	F: 5'-AGCGAGTTATGGCGACGAAG-3'	244
		R: 5'-TCTTCATCCTTTGGCCCACC-3'	
SOD2	847	F: 5'-CTCCGCCATGTTCGCCTTCT-3'	137
		R: 5'-CCAGATACCCCAAAACCGGAG-3'	
CAT	2876	F: 5'-TCTCACCAAGGTTTGGCCTC-3'	196
		R: 5'-GCGGTGAGTGTCAGGATAGG-3'	
GPx1	3162	F: 5'-GCCTCCCCTTACAGTGCTTG-3'	184
		R: 5'-TCTTGGCGTTCTCCTTGCC-3'	
HO-1	60	F: 5'-ATGCCCCAGGATTTGTCAGA-3'	198
		R: 5'-GAAGACTGGGCTCTCCTTGT-3'	

and normalized to the housekeeping gene (β-actin) were used to analyze the relative changes in the target gene expression.

2.9 Intervention of L. plantarum SCS2

HepG2 cells were treated with 0.1% DMSO and 10 μmol/L AFB1 for 24 h. HepG2 cells were incubated with 0.4 mg/mL cell-free extractions of *L. plantarum* SCS2 for 2 h. After being cultured with it, HepG2 cells were treated with 10 μmol/L AFB1 for another 22 h. The content of ROS, MDA and Nrf2, the activities of antioxidant enzymes and their relative RNA expression were measured to examine the antioxidant capacity of *L. plantarum* SCS2.

2.10 Statistical analysis

The data were analyzed with SPSS 17.0, and are presented as mean ± standard deviation. Statistical calculations were performed with GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, USA). Statistical significance was evaluated by one-way analysis of variance (ANOVA) with LSD test for post hoc analysis. Differences with $p < 0.05$ and $p < 0.01$ were considered statistically significant and highly significant, respectively.

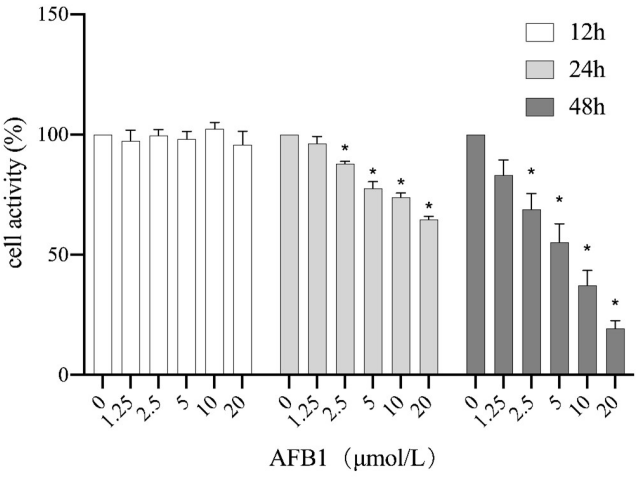

Figure 1. Effects of AFB1 on HepG2 cells viability. The cells viability of AFB1-treated HepG2 cells was determined using CCK-8 method. Control group was treated with 0.1% DMSO and AFB1 groups were treated with 1.25-20 μmol/L AFB1. The values are presented as means ± of SD. Significant differences with control group were designated as *P < 0.05.

3 Results

3.1 Suitable concentration of AFB1 and L. plantarum SCS2 for HepG2 cells

According to previous studies, 20 μmol/L AFB1 was selected as the maximum concentration. The CCK-8 method was used to investigate the effects of AFB1 on HepG2 cells viability. Except the treatment of 12 h experiments, the toxic effects of 0-20 μmol/L AFB1 on the viability (Figure 1) of HepG2 cells were concentration-dependent. Treatment with 2.5-20 μmol/L AFB1 significantly decreased cell via-bility when compared with the control group ($P < 0.05$). Therefore, 2.5-10 μmol/L AFB1 and experiment time 24 h was used for subsequent experiments.

The toxic effects of 105-108 CFU *L. plantarum* SCS2, heat-killed *L. plantarum* SCS2 and 0.1-0.4 mg/mL cell-free extractions of *L. plantarum* SCS2 on the viability (Figure 2a-c) of HepG2 cells were significantly decreased cell viability when compared with the control group ($P < 0.05$). 10^6 CFU *L. plantarum* SCS2 and 0.05 mg/mL cell-free extractions of *L. plantarum* SCS2 were the cellular non-toxic doses. The toxic effects of 0.05 mg/mL cell-free extractions of *L. plantarum* SCS2 (81.19% ± 2.93) significantly de-creased when compared with the AFB1 group (70.66% ± 5.08) (Figure 2d, $P < 0.05$). Therefore, 0.05 mg/mL extractions of *L. plantarum* SCS2 was used for the subsequent experiments.

3.2 AFB1 induced oxidative stress

The release of ROS and content of MDA are important indicators for detecting oxidative stress damage. The results of ROS fluorescence intensity showed that AFB1 memorably increased the release of ROS, reaching the max at 10 μmol/L AFB1 (163.42 ± 7.16) (Figure 3a, $P < 0.05$). Similarly, the

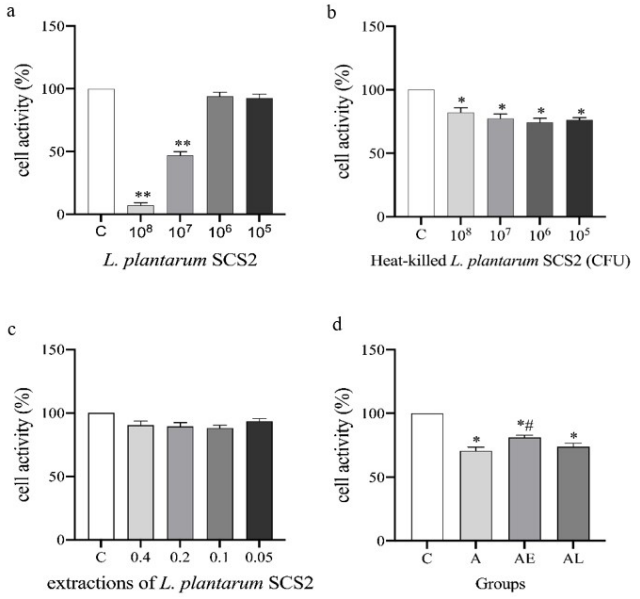

Figure 2. Effects of various concentrations of (a) *L. plantarum* SCS2, (b) heat-killed *L. plantarum* SCS2 and (c) cell-free extractions of *L. plantarum* SCS2 on the viability of HepG2 cells. (d) A: 10 μmol/L AFB1 treated for 24 h, AE: 0.05 mg/mL total protein extracted from *L. plantarum* SCS2 pretreated for 2 h, 10 μmol/L AFB1 treated for 22 h and AL: 10^6 CFU *L. plantarum* SCS2 pretreated for 2 h, 10 μmol/L AFB1 treated for 22 h. Each value represents the mean ± S.D. * and ** denoted significant differences ($p < 0.05$ and $p < 0.01$, respectively) between the control and other groups. # denoted significant differences ($p < 0.05$) between the AFB1 group and other groups.

content of MDA was significantly increased at 10 μmol/L AFB1 (1.56 ± 0.13 nmol/mL) (Figure 3b, $P < 0.01$). The results showed that the balance of the oxidative response in HepG2 cells has been broken, and that the cells undergo an oxidative stress under exposure to AFB1.

3.3 AFB1 inhibited antioxidant enzymes and activated Nrf2 mRNA expressions.

The Nrf2 is recognized as a key transcription factor for oxidative cellular damage. In this study, mRNA levels of Nrf2 and downstream antioxidant enzymes (SOD1, SOD2, CAT, GPx1, HO-1) were detected using the RT-qPCR method. The results showed that relative mRNA expressions of antioxidant enzymes were dose-dependent decrease, while the Nrf2 expression levels were dose-dependent increase. And the most significant differences (Figure 4, $P < 0.01$) compared with control group were at AFB1 concentration of 10 μmol/L (Nrf2, 1.38 ± 0.1, SOD1, 0.59 ± 0.04, SOD2, 0.60 ± 0.05, CAT, 0.61 ± 0.04, GPx1, 0.50 ± 0.07, HO-1, 0.58 ± 0.07).

3.4 AFB1 decreased the activities of antioxidant enzymes and stimulated Nrf2 transferring to the nucleus

The concentration of Nrf2 in the nucleus of HepG2 cells was significantly in-creased when HepG2 cells were treated with 5 μmol/L AFB1 (Figure 5a, $P < 0.01$). The current study indicated that 10 μmol/L AFB1 induced redox imbalance in

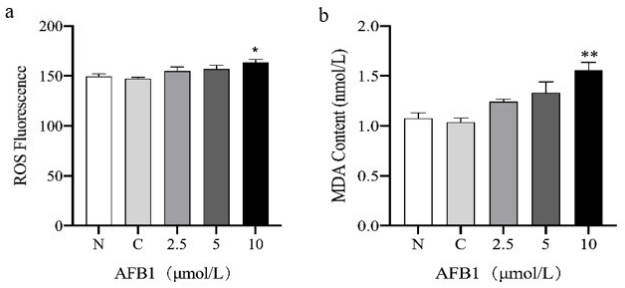

Figure 3. Effects of AFB1 on oxidative stress damage in HepG2 cells. (a) Effects of AFB1 on release of ROS in HepG2 cells. (b) Effects of AFB1 on content of MDA in HepG2 cells. The values are presented as means ± of SD. Significant differences with control group were designated as *$P < 0.05$ and **$P < 0.01$.

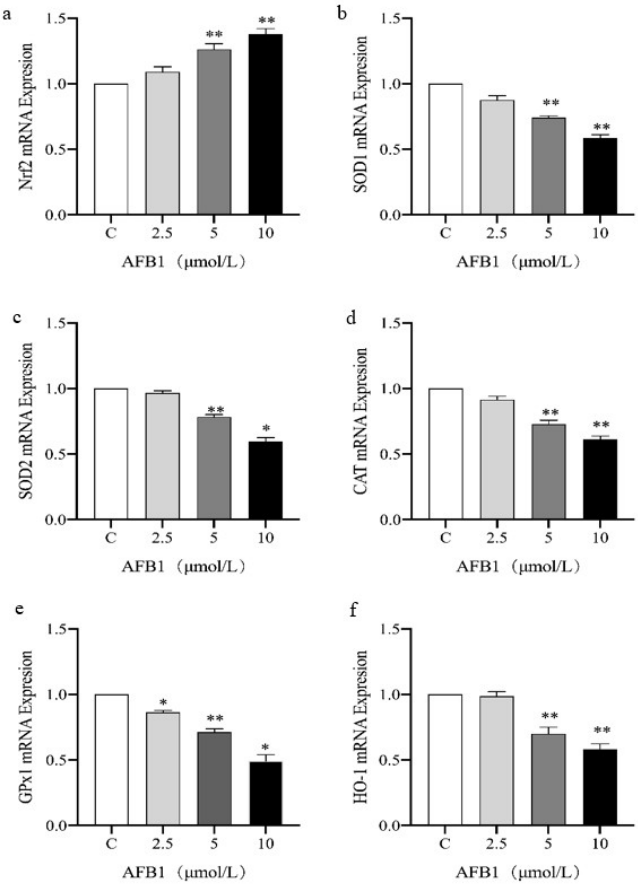

Figure 4. Effects of AFB1 on mRNA expressions of Nrf2 and antioxidant enzymes in HepG2 cells. The mRNA expression of (a) Nrf2, (b) SOD1, (c) SOD2, (d) CAT, (e) GPx1 and (f) HO-1 were detected by RT-qPCR, normalized by β-actin and expressed as $2^{-\Delta\Delta CT}$. HepG2 cells treated for 24 h with AFB1 (0, 2.5, 5, 10 μmol/L). The values are presented as means ± of SD. Significant differences with control group were designated as *$P < 0.05$ and **$P < 0.01$.

HepG2 cells. Meanwhile, AFB1 consumed a large amount of antioxidant enzymes, so the activities of SOD (204.60 ± 2.0 U/L), CAT (258.91 ± 13.58 U/L), GPx1 (84.55 ± 5.88 U/L) and

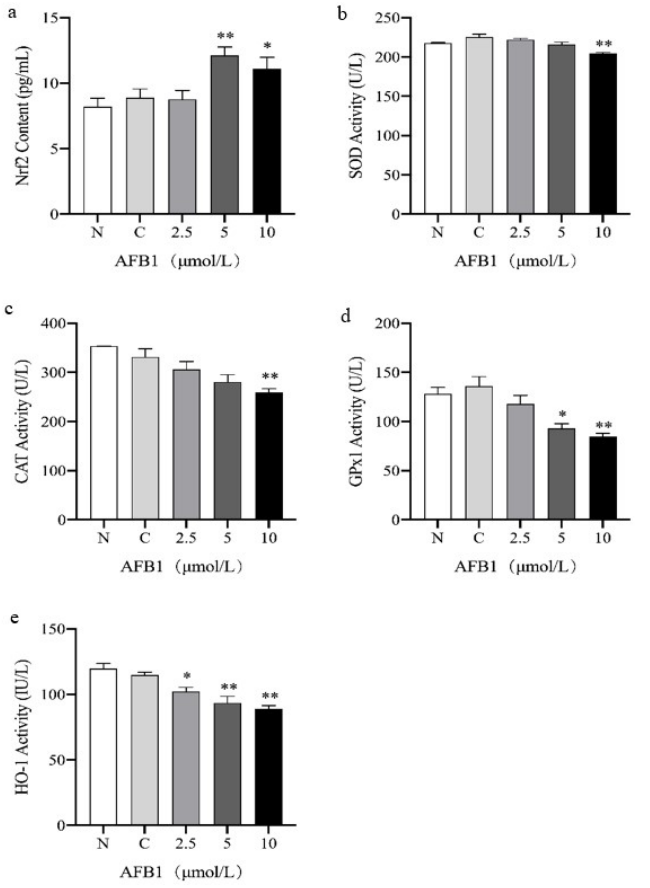

Figure 5. (a) Effects of AFB1 on content of Nrf2 in the nucleus of HepG2 cells. Effects of AFB1 on antioxidant enzymes in HepG2 cells. (b) SOD, (c) CAT, (d) GPx1, (e) HO-1. The values are presented as means ± of SD. Significant differences with control group were designated as *P < 0.05 and **P < 0.01.

HO-1 (88.99 ± 4.23 IU/L) (Figure 5b-e, P < 0.01) substantially decreased compared with control group in HepG2 cells.

3.5 The improvement of pretreatment with L. plantarum SCS2 on the oxidative stress in HepG2

The oxidative stress induced by 10 μmol/L AFB1 in HepG2 cells was improved after pretreatment with 0.05mg/mL cell-free extractions of *L. plantarum* SCS2. The re-sults of ROS fluorescence intensity showed that the increased release of ROS activated by AFB1 had significant decreased in AE group (165.31 ± 4.24) (P < 0.01, Figure 6a), nearly reaching the normal levels (152.33 ± 13.55). The change trend in MDA was also con-sistent. This study showed that the extractions of *L. plantarum* SCS2 ameliorated for redox imbalance in HepG2 cells. The results (Figure 7) showed that the mRNA expression of Nrf2 in HepG2 cells pretreated with *L. plantarum* SCS2 significantly decreased and the mRNA expression of antioxidant enzymes significantly increased compared to the AFB1 treated group (p < 0.01). The concentration of Nrf2 in the nucleus of HepG2 cells was significantly decreased when HepG2 cells were pretreated with extractions of *L. plantarum* SCS2 (P < 0.01, Figure 8a). Meanwhile, AFB1 consumed a large

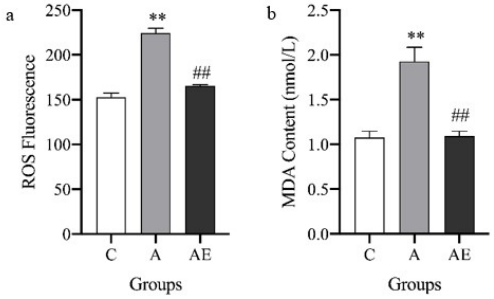

Figure 6. Effects of oxidative stress damage in HepG2 cells. (a) Effects of AFB1 on release of ROS in HepG2 cells. (b) Effects of AFB1 on content of MDA in HepG2 cells. A: 10 μmol/L AFB1 treated for 24 h, AE: 0.05 mg/mL total protein extracted from *L. plantarum* SCS2 pretreated for 2 h, 10μmol/L AFB1 treated for 22 h. The values are presented as means ± of SD. Significant differences with control group were designated as **P < 0.01 and significant differences with AFB1 group were designated as ## P < 0.0.

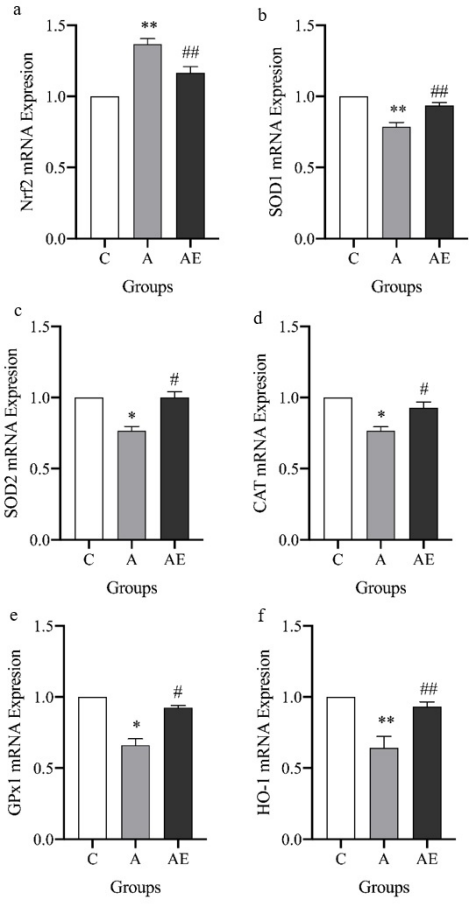

Figure 7. Effects of mRNA expressions of Nrf2 and antioxidant enzymes in HepG2 cells. The mRNA expression of (a) Nrf2, (b) SOD1, (c) SOD2, (d) CAT, (e) GPx1 and (f) HO-1 were detected by RT-qPCR, normalized by β-actin and expressed as $2^{-\Delta\Delta CT}$. A: 10 μmol/L AFB1 treated for 24 h, AE: 0.05 mg/mL total protein extracted from *L. plantarum* SCS2 pretreated for 2 h, 10μmol/L AFB1 treated for 22 h. The values are presented as means ± of SD. Significant differences with control group were designated as *P < 0.05 or **P < 0.01 and significant differences with AFB1 group were designated as #P < 0.05 or ## P < 0.01.

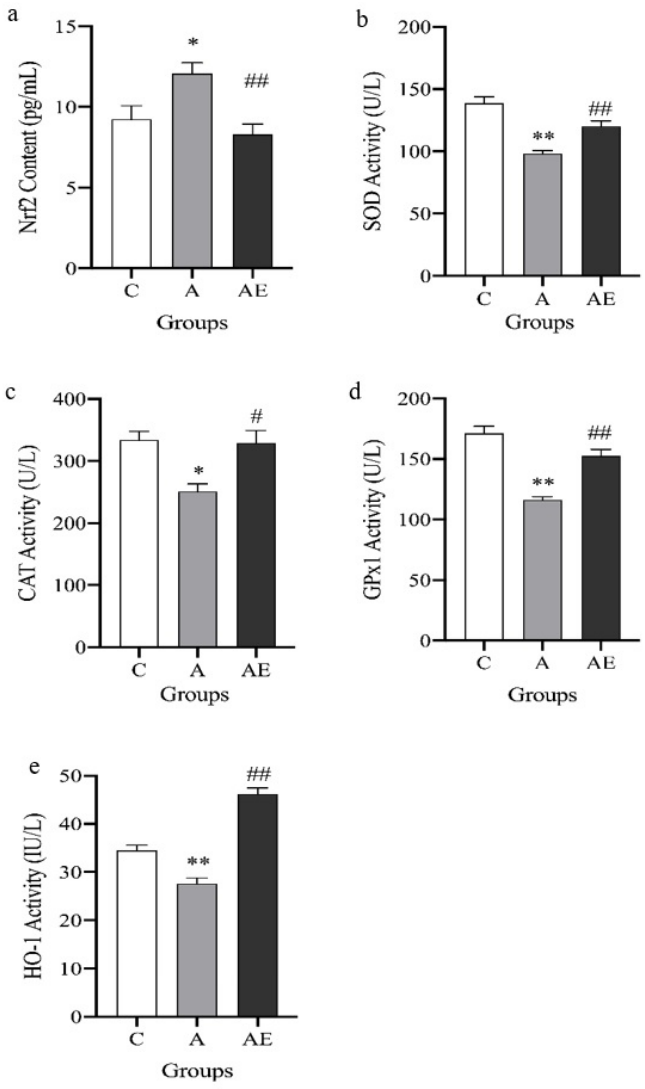

Figure 8. (a) Effects of AFB1 on content of Nrf2 in the nucleus of HepG2 cells. Effects of antioxidant enzymes in HepG2 cells. (b) SOD, (c) CAT, (d) GPx1, (e) HO-1. A: 10 μmol/L AFB1 treated for 24 h, AE: 0.05 mg/mL total protein extracted from *L. plantarum* SCS2 pretreated for 2 h, 10 μmol/L AFB1 treated for 22 h. The values are presented as means ± of SD. Significant differences with control group were designated as *P < 0.05 or **P < 0.01 and significant differences with AFB1 group were designated as ## P < 0.01.

amount of antioxidant enzymes, but the activities of SOD, CAT, GPx1 and HO-1 substantially in-creased in AE group (P < 0.01, Figure 8b-e). The changes in the antioxidant enzymes activities were consistent with the changes in the mRNA expressions. These results suggested that the intervention in *L. plantarum* SCS2 did ameliorate the oxidative stress in HepG2 cells induced by AFB1.

4 Discussion

HepG2 cells are characterized by rapid proliferation, immortalization, and contain most of the hepatocyte enzymes, such as phase I and phase II detoxification enzymes, and are often used as models for exploring chemotoxicity and cytoprotection (Baeza et al., 2016; Yan et al., 2016). Recent studies have shown that certain probiotics have the ability to effectively protect from liver injury (Abbès et al., 2016; Peltonen et al., 2001; Wang et al., 2013). Our previous study showed that *L. plantarum* SCS2 showed good antioxidant effects, whereas the role of *L. plantarum* SCS2 is poorly understood. Therefore, we selected HepG2 cells to explore how AFB1 triggered oxidative stress response and the improvement of *L. plantarum* SCS2.

AFB1 is a common pollutant in the grain crops and exhibits complex toxicity mechanism. The present study is to explore if AFB1 induces the release of ROS, which is mediated by oxidative stress response to activate the Nrf2 signaling pathway in HepG2 cells. The nrf2 belongs to the family of Cap-n-Colla (CNC) regulatory proteins, is a transcription factor with a basic leucine zipper structure, widely found in various organs of the body, and is the master regulator of the cellular redox response (Sykiotis et al., 2011). Under normal physiological conditions, Nrf2 mainly binds mainly to its inhibitor Keapl, present in the cytosol in its inactive state and is rapidly degraded by the ubiquitin proteasome pathway to maintain the low transcriptional activity of Nrf2 in the physiological state (Liu et al., 2013). When cells are stimulated with ROS or other nucleophilic, Nrf2 is uncoupled to Keapl, activated Nrf2 transports into the nucleus, binds to are after binding to Maf protein into a heterodimer in a manner, activates target gene expression and regulates the transcriptional activity of phase metabolic enzymes, antioxidant enzymes or drug transporters, thus exerting antioxidant damage effects (Bellezza et al., 2018; de Haan, 2011; McMahon et al., 2010). SOD, also known as liver protein, is considered one of the most important enzymes in living organisms, which is the leading killer of oxygen free radicals in the body, and is closely related to the human physiopathology and the occurrence and development of various diseases (Piao et al., 2010). The SOD transforms the superoxygen radical into H2O2 and H2O by a disambiguation reaction, after which GPx and CAT rets H2O2 to H2O, thereby protecting cells from oxidative stress damage (Rubiolo et al., 2008)). As a stress protein, HO-1 plays a role in hemoglobin metabolism, inflammation, and antioxidant processes, and is also protective against the cardiovascular and nervous system (de Freitas Silva et al., 2018; Zhang et al., 2014).

Consistent with our speculation, stimulation of AFB1 triggered oxidative stress damage in HepG2 cells and intervention by *L. plantarum* SCS2 effectively improved the damage sustained by the cells. Activation of Nrf2 signaling initiates the expression of multiple downstream target proteins. Antioxidases are a class of proteins with important functions in regulating redox balance in the body, including SOD, CAT, GPx, HO-1, etc. Extensive release of ROS as well as the elevated MDA content suggested that HepG2 cells indeed undergo oxidative stress induced by AFB1. And with the mRNA expressions and activities of antioxidases in HepG2 cells significantly decreased (P < 0.05), while the mRNA expressions and content of Nrf2 in the nucleus significantly increased (P < 0.05). Hassan et al. (2015), also found that levels of MDA were increased in the spleen by AFB1 or fumonisin B1 (FB1) were in accordance with earlier findings of the effects of these mycotoxins on induced oxidative stress and lipid peroxidation. The results of Mary et al. (2012) were also in agreement with. They reported elevated levels of AFB1 and FB1,

along with reduced protective antioxidant-promoting enzyme activity. Interestingly, on the one hand, levels of oxidative stress and antioxidant enzyme activity were correspondingly controlled and restored to almost normal levels of cells by supplementing *L. plantarum* SCS2 intervention and, on the other hand, significantly increased cell activity of HepG2 cells ($P < 0.05$), which suggested that the presence of some substance in the cell extractions of *L. plantarum* SCS2 reversed the cellular oxidative damage caused by AFB1. Abbès et al. (2013) and Abdellatef & Khalil (2016) also found that probiotics may induce its protective role via increasing the antioxidant capacity and inhibition of lipid peroxidation in liver and kidney of experimental animals tested.

Many micro-organisms, e.g. bacteria, yeasts, molds, actinomycetes, are able to remove or degrade small amounts of mycotoxins in food/ feed (Styriak et al., 2001). Strains *L. rhamnosus* GG and LC-705 seemed to be effective in such detoxifications (Lahtinen et al., 2004). However, the binding mechanism still remains not fully understood. It has been suggested that carbohydrate-rich mannoproteins or glucans might be involved in the binding, the complex stucture maybe be responsible for strain- and toxin-specific binding (Shetty & Jespersen, 2006). Raju & Devegowda (2000) attributed the binding of aflatoxins by yeast cell walls to mannan oligosaccharides, while Haskard et al. (2001) explained the toxin binding was attributed to carbohydrate and protein components. Therefore, in partly, the protective effects of lactic acid bacteria may be explained by the ability of the bacterium to bind AFs and reduce toxin bioavailability. Studies showed that *L. plantarum*, *L. bulgaricus* or *L. rhamnosus* prevented AFB1-induced secretion of pro-inflammatory cytokines by modulating NF-κB pathways (Chen et al., 2019; Huang et al., 2019). Li et al. (2021) demonstrated the anti-oxidant effect of *L. plantarum* KSFY06 in molecular biology, histology and at the gene level, but they have not examined these effects at the protein level. Currently, the protective mechanisms of extractions from *L. plantarum* SCS2 have been explained in terms of mRNA and antioxidant enzyme activity. Obviously, systematic studies are still needed to understand the precise anti-oxidative mechanisms. We have not yet clarified the key material for *L. plantarum* SCS2 and its mechanism of action in ameliorating oxidative stress induced by AFB1 in HepG2 cells, but we plan to do so in future experiments.

5 Conclusion

The results of in vitro experiments revealed that *L. plantarum* SCS2 evidently protected against AFB1-induced oxidative stress. *L. plantarum* SCS2 is a high-quality lactic acid bacterium with antioxidant effects and may be used for the development of related probiotic products.

Conflict of interest

The authors declare no conflict of interest.

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