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Evaluation of Hepatic Detoxification Effects of Enteromorpha prolifera Polysaccharides against Aflatoxin B₁ in Broiler Chickens

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Abstract: Aflatoxin B₁ (AFB₁) is a major risk factor in animal feed. Seaweed (Enteromorpha prolifera)-derived polysaccharides (SDP) are natural antioxidants with multiple biological functions, which may have an in vivo detoxification effect on AFB₁. The current study aimed to evaluate whether SDP could mitigate AFB₁-induced hepatotoxicity in broilers. A total of 216 chickens (male, 5 weeks old) were randomly allocated to three groups with differing feeding patterns, lasting 4 weeks: (1) control group (CON, fed a basal diet); (2) AFB₁ group (fed a basal diet mixed with 0.1 mg/kg AFB₁); and (3) AFB₁ + SDP group (AFB₁ group + 0.25% SDP). The results showed that dietary SDP improved the liver function-related biochemical indicators in serum, and reversed the increase in relative liver weight, hepatic apoptosis and histological damage of broilers exposed to AFB₁. SDP treatment also reduced the activity and mRNA expression of phase I detoxification enzymes, while increasing the activity and mRNA expression of phase II detoxification enzymes in the livers of AFB₁-exposed broilers, which was involved in the activation of p38 mitogen-activated protein kinase (p38MAPK)/nuclear factor erythroid 2-related factor 2 (Nrf2) signaling. In conclusion, dietary SDP alleviated AFB₁-induced liver injury of broilers through inhibiting phase I detoxification enzymes and upregulating p38MAPK/Nrf2-mediated phase II detoxification enzymes pathway.

Keywords: aflatoxin B₁; hepatotoxicity; detoxification; seaweed polysaccharides; Enteromorpha prolifera; natural antioxidants

1. Introduction

At present, chicken is a major meat consumer product worldwide, and the food safety of chicken is critical to public health [1]. Feed toxins are easily transmitted to humans through the food chain during chicken production [2]. The contamination of mycotoxins in feed is a significant risk factor for broiler production and food safety [3]. Grains and other feed materials breed mold and produce mycotoxins during production procedures, transportation and storage, and more than 300 kinds of mycotoxins have been found [4]. Among these mycotoxins, aflatoxins are highly toxic secondary metabolites produced by Aspergillus flavus and A. parasiticus [5]. The diverse derivatives of aflatoxins have been identified, including aflatoxin B₁, B₂, G₁, G₂, M₁ and M₂, etc., and the aflatoxin B₁ (AFB₁) is classified as a class I carcinogen because it has the strongest toxicity and the widest distribution [6]. The AFB₁ accumulation in broiler muscle was found to be mediated by dietary intake, which leads to chicken meat contamination and endangers human health [7].

The liver is the AFB₁ detoxification organ, and it is also the main target organ for the toxic effects of AFB₁ in chickens [8]. Broiler chickens are highly susceptible to AFB₁.
According to Chinese feed hygiene standards, the maximum limit of AFB₁ is set at 10 µg/kg for juvenile chickens and 20 µg/kg for adult chickens; exceeding the maximum limit will damage the health status of broilers [9]. However, China is a large-scale chicken producing and consuming country, and among 175 feed ingredients collected from different regions, the detection rate of AFB₁ was found to reach 94.9%, and the AFB₁ content of some samples reached 50–100 µg/kg [10]. Numerous direct pieces of evidence demonstrated that broiler chickens exposed to greater than 50 µg/kg AFB₁ exhibited reduced performance, morphological damage and physiological dysfunction of the liver [11–15].

The bioactivation of AFB₁ is mainly dependent on the phase I detoxification enzyme system (cytochrome P450, CYP450) [6]. AFB₁ is metabolized to AFB₁-8,9-epoxide (AFBO)-DNA by phase I detoxification enzymes, which triggers hepatotoxicity and oxidative damage to liver [16]. On the contrary, phase II detoxification enzymes (e.g., glutathione S-transferase, GST; glutathione peroxidase, GSH-Px) promote the metabolism and excretion of AFBO-DNA and alleviate oxidative stress, thereby attenuating AFB₁-induced hepatotoxicity [17].

There are a rising number of studies indicating that natural antioxidants can prevent AFB₁ hepatotoxicity by targeting phase I and/or phase II detoxification enzymes in broilers [18]. For instance, curcumin has been reported to alleviate AFB₁-induced liver injury of chickens; this beneficial effect was achieved via inhibition of CYP450 isozymes, and activation of nuclear factor erythroid 2-related factor-2 (Nrf2) involved GSTs pathway [19–23]. Lycopene and proanthocyanidins also exerted protective effects against AFB₁ hepatotoxicity and showed similar mechanisms of action to curcumin [24–27]. Therefore, the phytoproducts’ combatting of AFB₁ toxicity may be mainly owed to their antioxidant function [18].

Enteromorpha prolifera is a green seaweed with abundant sources, which is widely distributed in the East Asian Ocean Belt [28]. E. prolifera has long been used as a traditional medicinal plant in China. The polysaccharide is the main bioactive component, and has health benefits for humans [29]. It has been proven that seaweed-derived polysaccharides (SDP) from E. prolifera exhibit various biological activities, such as antioxidant, hepatoprotective and anti-inflammatory activities [29–33]. Previous reports suggested that SDP could improve the antioxidant capacity and upregulate phase II detoxification enzyme gene expression through modulating the Nrf2 signaling pathway in broilers [34,35]. A more recent study demonstrated that SDP mitigated immune organ injury caused by AFB₁ exposure by regulating the p38MAPK/Nrf2-mediated phase II detoxification enzyme signaling pathway [36]. These findings indicate that SDP has the potential to act as a decontamination agent of AFB₁ in vivo. However, to date, no report has defined the protective effect of SDP against hepatotoxicity in broiler chickens fed an AFB₁-contaminated diet. Therefore, this study was conducted to investigate the effects of SDP in attenuating AFB₁-induced liver injury of chickens, and to uncover the underlying mechanism.

2. Materials and Methods

2.1. Animals and Experimental Design

A total of two hundred and sixteen yellow-feathered broilers (five weeks old, male) were obtained from the local producers and used in this study. The chickens were randomly split into three treatment groups: (1) control group (CON, provided basal diet); (2) AFB₁ group (basal diet mixed with 0.1 mg/kg AFB₁); (3) AFB₁ + SDP group (AFB₁ group + 0.25% SDP). The duration of this animal study was four weeks. The AFB₁ was obtained from a commercial reagents company (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA). Each treatment group was subjected to six replicate cages (12 broilers/replicate cage). The SDP products were extracted from the seaweed E. prolifera, the details of SDP such as purity, chemical composition, structure and molecular weight, were analyzed in our previous report [31] and the chemical and monosaccharide composition of SDP was described in Table S1. The environmental conditions and basal diet composition (including the AFB₁...
content in basal diet) were also referred to in our earlier study [36]; the formulation and nutrient levels of the basal diet were presented in Table S2.

2.2. Growth Performance and Sampling

During the feeding trial, feed intake was monitored daily on a per-cage basis for the detection of average daily feed intake (ADFI). At the end of the animal experiment, the body weights of broilers were measured per cage, and then the average daily gain (ADG) and the ratio of feed/gain (F/G) were calculated.

One bird was selected randomly from each replicate cage at the end of the animal study for sampling (n = 6 per group). These birds were weighed individually, and the blood samples were collected into vacuum tubes without anticoagulants via subwing veins. The relative liver weight was determined as previously reported [19]. The coagulated bloods were centrifuged to collect serum samples for a biochemical parameters assay (3000 r/min, 5 min). Then, the chickens were slaughtered by neck bleeding, and the livers were separated, rinsed with saline and weighed. Subsequently, the liver tissues were fixed with 4% paraformaldehyde for histological, apoptosis and immunohistochemical analysis. Other liver samples were stored at –80 °C until further analysis of enzyme activity, mRNA and protein expression.

2.3. Serum Biochemical Analysis

The activity of alanine transaminase (ALT) and aspartate transaminase (AST), and the total protein levels in serum, was determined using the Chemray 800 Automatic Biochemical Analyzer (Redu Life Technology Co., Ltd., Shenzhen, China).

2.4. Hepatic Histopathology and Apoptosis Analysis

The preparation and observation of histological sections and histopathological analysis were based on our previous report [36]. The TdT-mediated dUTP Nick-End Labeling (TUNEL) method was used to detect hepatic apoptosis using the paraffinized sections; the details of the TUNEL method are as we previously reported [33]. Green indicates apoptosis-positive cells in the figures.

2.5. Determination of Enzymatic Activity and AFBO-DNA Content of Liver

The activity of total-superoxide dismutase (T-SOD), GSH-Px, catalase (CAT) and GST and the content of malondialdehyde (MDA) in the liver were analyzed using commercial kits from the Jiancheng Institute (Nanjing, China) following the manufacturer’s instructions. The activity of heme oxygenase-1 (HO-1) and CYP450, and the content of AFBO-DNA, were analyzed using the commercial kits (Jiangsu Enzyme Immunology Co., Ltd., Suzhou, China), according to the manufacturer’s instructions.

2.6. Gene Expression Analysis of Liver

The reagents and protocols of RNA extraction, cDNA transcription and quantitative real-time polymerase chain reaction (qPCR) reaction were described in our previous report [33]. The internal control gene was the β-actin. In addition, the information of used primers was based on the former studies [19,33]. The method of $2^{-\Delta\Delta Ct}$ was used for data processing [37], and the relative mRNA expression compared with the CON group was used to express the gene expression results.

2.7. Immunohistochemical Analysis and Western Blotting

Immunohistochemistry was performed to determine the Nrf2 protein expression in the liver samples, in accordance with the earlier report [24]. The protein expression of p38MAPK, HO-1, Nrf2, total-Nrf2 and nuclear-Nrf2 was determined using Western blotting; detailed methods and information on antibodies have been described previously [33,36]. The immunohistochemical results were expressed as areal density, and the Western blotting results were expressed as relative protein expression (target protein/β-actin).
2.8. Statistical Analysis

The general linear model procedure in SAS 9.4 (SAS, 2013. SAS Institute Inc., Cary, NC, USA) was used to process and analysis the data in this study. The analysis of variance was used for significance testing, and then the significant differences among the three treatment groups were determined using Tukey’s test. \( p < 0.05 \) was considered statistically significant.

3. Results

3.1. Growth Performance, Serum Biochemical Indicators and Relative Liver Weight

As presented in Table 1, AFB\(_1\) exposure and SDP treatment had no significant impacts on ADG, ADFI and F/G of chickens \( (p > 0.05) \). The results of serum biochemical indicators and relative liver weight are shown in Table 2. Compared with the CON group, chickens fed an AFB\(_1\)-contaminated diet showed increased serum ALT and AST activity \( (p < 0.05) \), and an elevated relative liver weight \( (p < 0.05) \). Dietary SDP could reduce the serum ALT and AST activity \( (p < 0.05) \) and decrease the relative liver weight \( (p < 0.05) \) in broilers after being subjected to AFB\(_1\) exposure.

Table 1. The impacts of polysaccharides from seaweed (\textit{Enteromorpha prolifera}) on growth performance of broiler chickens exposed to AFB\(_1\).

| Items   | CON   | AFB\(_1\) | AFB\(_1\) + SDP | SEM  | \( p\)-Value |
|---------|-------|-----------|----------------|------|-------------|
| ADG, g  | 21.34 | 20.77     | 21.06          | 0.41 | 0.629       |
| ADFI, g | 75.05 | 75.42     | 73.96          | 1.31 | 0.732       |
| F/G     | 3.51  | 3.63      | 3.54           | 0.04 | 0.152       |

ADG: average daily gain; ADFI: average daily feed intake; F/G: the ratio of feed/gain; SDP: seaweed-derived polysaccharides; SEM: standard error of the mean; ANOVA: analysis of variance. CON: control group; AFB\(_1\): fed 0.1 mg/kg AFB\(_1\); AFB\(_1\) + SDP: fed AFB\(_1\) + 0.25% SDP.

Table 2. The impacts of polysaccharides from seaweed (\textit{Enteromorpha prolifera}) on serum liver function-related indexes and relative liver weight of broiler chickens exposed to AFB\(_1\).

| Items                | CON       | AFB\(_1\)  | AFB\(_1\) + SDP | SEM  | \( p\)-Value |
|----------------------|-----------|------------|----------------|------|-------------|
| ALT activity, U/L    | 6.68      | 8.05       | 6.82           | 0.35 | 0.038       |
| AST activity, U/L    | 232.72    | 272.59     | 249.48         | 6.60 | 0.005       |
| TP content, g/L      | 40.55     | 44.28      | 43.01          | 1.49 | 0.243       |
| Relative liver weight| 2.06      | 2.35       | 2.12           | 0.07 | 0.049       |

ALT: alanine transaminase; AST: aspartate transaminase; TP: total protein; SDP: seaweed-derived polysaccharides; SEM: standard error of the mean; ANOVA: analysis of variance. CON: control group; AFB\(_1\): fed 0.1 mg/kg AFB\(_1\); AFB\(_1\) + SDP: fed AFB\(_1\) + 0.25% SDP.

3.2. Histopathology and Apoptosis of Liver

The hepatic histopathology is illustrated in Figure 1. There were no histopathological alterations of liver in the CON group. Vacuolization, inflammatory cell infiltration and hepatocyte necrosis were observed in the AFB\(_1\) group. Dietary SDP restored the AFB\(_1\)-induced histopathological changes in the liver. As shown in Figure 2, compared with the CON group, the AFB\(_1\) group had a higher apoptosis rate in hepatocytes \( (p < 0.01) \), while SDP intervention reduced the apoptosis rate in hepatocytes \( (p < 0.05) \).
hepatocyte necrosis were observed in the AFB1 group. Dietary SDP restored the AFB1-induced histopathological changes in the liver. As shown in Figure 2, compared with the CON group, the AFB1 group had a higher apoptosis rate in hepatocytes ($p < 0.01$), while SDP intervention reduced the apoptosis rate in hepatocytes ($p < 0.05$).

**Figure 1.** The protective effects of dietary seaweed (*Enteromorpha prolifera*)-derived polysaccharides on histopathological changes in liver in broiler chickens. Scale bars for 200× are 50 μm and 400× are 25 μm. Arrows indicate the vacuolization, inflammatory cell infiltration and hepatocyte necrosis.

**Figure 2.** The protective effects of dietary seaweed (*Enteromorpha prolifera*) derived polysaccharides on apoptosis of hepatocytes in broiler chickens. The scale bar is 100 μm. * $p < 0.05$, ** $p < 0.01$, no superscript marks indicated that $p > 0.10$. CON, control group; AFB1 group (fed diet with 0.1 mg/kg AFB1); AFB1 + SDP group (AFB1 group + 0.25% SDP).

### 3.3. Enzymatic Activity and AFBO-DNA level of Liver

The results of enzyme activity and AFBO-DNA content in the liver are shown in Figure 3. Chickens exposed to AFB1 had lower activity of GSH-Px, GST and HO-1 ($p < 0.05$), while showing higher activity of CYP450 and MDA and AFBO-DNA content in the liver ($p < 0.05$). Dietary SDP intervention promoted the activity of GSH-Px, GST and HO-1 ($p < 0.05$) and reduced the activity of CYP450 and the content of MDA and AFBO-DNA in the liver of broilers after being fed an AFB1-contaminated diet ($p < 0.05$).
Dietary SDP intervention promoted the activity of GSH-Px, GST and HO-1 \((p < 0.05)\) and reduced the activity of CYP450 and the content of MDA and AFBO-DNA in the liver of broilers after being fed an AFB1-contaminated diet \((p < 0.05)\).

**Figure 3.** The protective effects of dietary seaweed \((Enteromorpha prolifera)\)-derived polysaccharides on enzymatic activity and AFBO-DNA level in liver of broiler chickens. \(* p < 0.05, ** p < 0.01, \) no superscript marks indicated that \(p > 0.10\). CON: control group; AFB1 group (fed diet with 0.1 mg/kg AFB1); AFB1 + SDP group (AFB1 group + 0.25% SDP).

### 3.4. Hepatic mRNA Expression of Phase I Detoxification Enzyme-Related Genes

As depicted in Figure 4, compared with the CON group, AFB1 exposure upregulated the relative mRNA expression of CYP1A1, CYP1A2 and CYP3A4 in the liver \((p < 0.05)\). Conversely, dietary SDP downregulated the relative mRNA expression of CYP1A1, CYP1A2 and CYP3A4 in the liver of broilers exposed to AFB1 \((p < 0.05)\). When compared to the CON group, the AFB1 + SDP group showed a higher relative mRNA expression of CYP1A1 and CYP1A2 in the liver \((p < 0.05)\).

**Figure 4.** The protective effects of dietary seaweed \((Enteromorpha prolifera)\)-derived polysaccharides on hepatic mRNA expression of phase I detoxification enzyme-related genes in broiler chickens. \(* p < 0.05, ** p < 0.01, \) no superscript marks indicated that \(p > 0.10\). CON: control group; AFB1 group: (fed diet with 0.1 mg/kg AFB1); AFB1 + SDP group (AFB1 group + 0.25% SDP).
3.5. Hepatic mRNA Expression of Antioxidant and Phase II Detoxification Enzyme Related Genes

As shown in Figure 5, compared with the CON group, AFB\textsubscript{1} exposure downregulated the relative mRNA expression of hepatic GPx1, GSTT1, GSTO1, GSTA3, GSTM2, GSTP1, HO-1 and Nrf2 (p < 0.05). Compared to the AFB\textsubscript{1} group, the relative mRNA expression of hepatic GPx1, GSTT1, GSTO1, GSTM2, GSTP1, HO-1 and Nrf2 was elevated in the AFB\textsubscript{1} + SDP group (p < 0.05). Even then, broilers in the AFB\textsubscript{1} + SDP group had a lower relative mRNA expression of hepatic GSTT1, GSTO1 and GSTM2 than those in the CON group (p < 0.05).

![Figure 5. The protective effects of dietary seaweed (Enteromorpha prolifera)-derived polysaccharides on hepatic mRNA expression of antioxidant phase II detoxification enzyme-related genes in broiler chickens. * p < 0.05, ** p < 0.01, no superscript marks indicated that p > 0.10. CON: control group; AFB\textsubscript{1} group: (fed diet with 0.1 mg/kg AFB\textsubscript{1}); AFB\textsubscript{1} + SDP group (AFB\textsubscript{1} group + 0.25% SDP).](image)

3.6. Protein Expression of p38MAPK/Nrf2 Signaling Pathway in the Liver

The results of Nrf2 immunohistochemistry are shown in Figure 6. Compared to the CON group, broilers in the AFB\textsubscript{1} and AFB\textsubscript{1} + SDP groups had lower protein expression levels of Nrf2 in the liver (p < 0.01). Compared to the AFB\textsubscript{1} group, dietary SDP supplementation improved the protein expression level of Nrf2 in the liver (p < 0.05).
4. Discussion

Prolonged consumption of an AFB1-contaminated diet is not only detrimental to broiler health and production, but also causes residue in chicken meat, threatening human health [9]. In production practice, there is typically 20–100 μg/kg AFB1 contamination in feed [10]. Accordingly, this study defined 0.1 mg/kg as the toxic dose of AFB1 in broilers. The results showed that 0.1 mg/kg AFB1 and 0.25% SDP treatment had no significant impacts on the growth performance of broilers, which is similar to a previous study [38].

The Western blotting results are presented in Figure 7. AFB1 exposure reduced the protein expression level of p38MAPK, total Nrf2 and nuclear Nrf2 in the liver (p < 0.05). Inclusion of SDP in the diet could increase the protein expression level of p38MAPK, total Nrf2 and nuclear Nrf2 in the liver (p < 0.05).

![Figure 6. The protective effects of dietary seaweed (Enteromorpha prolifera)-derived polysaccharides on hepatic Nrf2 protein expression in broiler chickens based on immunohistochemical analysis. * p < 0.05, ** p < 0.01, no superscript marks indicated that p > 0.10. CON: control group; AFB1 group (fed diet with 0.1 mg/kg AFB1); AFB1 + SDP group (AFB1 group + 0.25% SDP).](image)

![Figure 7. The protective effects of dietary seaweed (Enteromorpha prolifera)-derived polysaccharides on hepatic protein expression of p38MAPK, HO-1, total Nrf2, and nuclear Nrf2 in broiler chickens. * p < 0.05, no superscript marks indicated that p > 0.10. CON: control group; AFB1 group (fed diet with 0.1 mg/kg AFB1); AFB1 + SDP group (AFB1 group + 0.25% SDP).](image)
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Interestingly, dietary SDP supplementation improved the impairment of these liver parameters in AFB$_1$-exposed broilers. Similarly, Zhang et al. [44] found that dietary natural polysaccharides play a role in hepatoprotection by improving histomorphology and reducing apoptosis of the liver, as well as decreasing the activity of ALT and AST in serum of mice exposed to toxins. Solis-Cruz et al. [41] demonstrated that supplementation of cellulosic polymers mitigated AFB$_1$-induced histological disruption and elevation of serum ALT and AST activity in broiler chickens. Feed including other phytoproducts, such as curcumin and lycopene, also showed protective effects on hepatic histopathology and serum ALT and AST, thus preventing the liver damage from AFB$_1$ exposure in chickens [19,20,23,24,26]. It is worth mentioning that these effective protection agents are natural antioxidants. Therefore, it is reasonable to speculate that SDP alleviates AFB$_1$-induced liver injury, likely due to their antioxidant properties. The specific mechanisms of action are yet to be elucidated.

AFB$_1$ bioactivation is depends on the phase I detoxification enzymes (CYP450 isozymes); CYP450 catalyzes the formation of toxic AFBO from AFB$_1$ in the liver, which further forms the toxic adduct AFBO-DNA, thereby impairing liver function [6]. Additionally, CYP450 isozymes (namely, CYP1A1, CYP1A2, CYP2A6 and CYP3A4) are responsible for AFB$_1$ bioactivation in avian species [9]. Therefore, reducing the activity of CYP450 isozymes can inhibit the bioactivation of AFB$_1$ and attenuate the hepatotoxicity of AFB$_1$. In this study, broilers in AFB$_1$ group had a higher hepatic AFBO-DNA level, CYP450 activity and mRNA expression of CYP1A1, CYP1A2 and CYP3A4. Consistently, extensive research has confirmed that AFB$_1$ exposure increases the content of AFBO-DNA and the activity of CYP450 isozymes in chickens’ livers, indicating the bioactivation of AFB$_1$ [13–15]. It is worth mentioning that our study observed that SDP supplementation decreased the AFBO-DNA content and CYP450 activity and downregulated CYP1A1, CYP1A2 and CYP3A4 mRNA expression in the liver of chickens fed an AFB$_1$-contaminated diet. Similar to our studies, Gan et al. [45] reported that the polysaccharides from bush sophora root downregulated CYP1A5 expression, which, in turn, helped to prevent AFB$_1$-induced hepatotoxicity in primary chicken hepatocytes. Zhao et al. [39] demonstrated that dietary polysaccharide-rich medicinal plants decreased the AFBO-DNA content of the livers of AFB$_1$-exposed broilers. Additionally, other dietary natural phytochemicals showed an inhibitory effect on CYP450-involved bioactivation of AFB$_1$ into AFBO. For instance, Zhang et al. [20] found that curcumin reduced the hepatic activity of CYP1A1, CYP1A2, CYP2A6 and CYP3A4, thus inhibiting the production of toxic adduct AFBO-DNA in broilers exposed to AFB$_1$. However, the exact mechanism by which natural antioxidants regulate CYP450-
mediated AFB$_1$ bioactivation remains unclear. In-depth research is imperative to elucidate the mode of action of dietary SDP with respect to CYP450.

AFB$_1$-induced broiler liver injury is closely related to oxidative stress and phase II detoxification enzymes, both of which are regulated by the Nrf2 signaling pathway [46]. Nrf2 is a key transcription factor that responds to oxidative stress and regulates phase II detoxification enzymes, and is a sensor of exogenous toxic substances and oxidative stress [47]. Nrf2 is a basic leucine bZIp transcription factor; under normal physiological conditions, Nrf2 binds to Kelch-like ECH-associated protein 1 (Keap1) and anchors it in the cytoplasm (Nrf2/Keap1), so that Nrf2 is in a non-free inactive state [46]. Nrf2 can be activated by bioactive compounds and released to undergo nuclear translocation into the nucleus, and it modulates the gene expression of phase II detoxification enzymes (e.g., GSH-Px, GSTs, HO-1), thereby promoting the detoxification process [48]. The Nrf2-mediated phase II detoxification enzyme pathway is also regulated by MAPKs, a class of protein kinases including the subclasses of p38MAPK. Existing studies have confirmed that p38MAPK promoted Nrf2 activation and nuclear translocation and upregulated the expression of downstream phase II detoxification enzymes [49]. The phase II detoxification enzymes can enhance the combination of GSH and AFBO and excrete the AFBO from the body [16]. In the current study, it was found that dietary SDP improved the activity of GSH-Px, GSTs and HO-1 and their corresponding mRNA expression, upregulated the protein expression of p38MAPK and Nrf2 and promoted nuclear translocation of Nrf2 in the liver of broilers after being fed an AFB$_1$ diet. Similarly, Guo et al. [36] suggested that dietary SDP alleviated chicken bursal damage triggered by AFB$_1$, which was related to the upregulated mRNA expression of GSTs and the activation of the p38MAPK/Nrf2 signaling pathway. Liu et al. [35] reported that dietary SDP could improve the gut mRNA expression of GPx1, GSTT1 and HO-1 by activating the Nrf2 signaling pathway in broilers. Wang et al. [50] demonstrated that dietary natural antioxidants could relieve the uterine injury caused by toxins in chickens and was associated with the activation of the p38MAPK/Nrf2-mediated phase II detoxification enzyme pathway. Meanwhile, other well-known natural antioxidants, such as medicinal plants, curcumin and lycopene, have been reported to alleviate AFB$_1$-induced hepatotoxicity by activating the Nrf2-mediated phase II detoxification enzyme pathway [19,22,25,39]. Therefore, it can be concluded that, in addition to suppressing the bioactivation of AFB$_1$ by inhibiting CYP450 isozymes, dietary SDP could also activate the phase II detoxification enzyme pathway involved in p38MAPK/Nrf2 signaling, thereby ultimately promoting the hepatic detoxification of AFB$_1$ in broilers (Figure 8).

![Proposed Model of Protective Effects of Seaweed](image-url)

**Figure 8.** Proposed model of the protective effects of seaweed (*Enteromorpha prolifera*)-derived polysaccharides against aflatoxin B$_1$-induced hepatotoxicity in broiler chickens.
5. Conclusions

To summarize, dietary SDP improved the liver-function-related biochemical indicators in serum and reversed the increase in the relative liver weight of broilers exposed to AFB$_1$. Histological and apoptosis results suggested that AFB$_1$-induced liver injury was partially ameliorated by SDP supplementation. Additionally, SDP treatment enhanced the activity of GSH-Px and GST while reducing the activity of CYP450, MDA and AFBO-DNA levels in the liver of broilers exposed to AFB$_1$. Dietary SDP downregulated the hepatic mRNA expression of CYP1A1, CYP1A2 and CYP3A4 in broilers after being subjected to AFB$_1$ exposure. Moreover, SDP supplementation upregulated the hepatic mRNA expression of GPx1, GSTT1, GSTO1, GSTP1 and GSTM2 in AFB$_1$-exposed broilers, and this regulation was involved in the activation of p38MAPK/Nrf2 signaling. Therefore, this study revealed, for the first time, that dietary SDP from *E. prolifera* promoted hepatic detoxification of AFB$_1$ by inhibiting phase I detoxification enzymes and upregulating the p38MAPK/Nrf2-mediated phase II detoxification enzymes pathway, which provided a reference and a mechanistic basis for the use of SDP as a novel antioxidant and AFB$_1$ decontamination agent in broiler chickens.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox11091757/s1. Table S1: The chemical and monosaccharide composition of seaweed-derived polysaccharides (SDP) from *Enteromorpha prolifera*; Table S2: The formulation and nutrient levels of the basal diet.

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