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Spirulina platensis mediated the biochemical indices and antioxidative function of Nile tilapia (*Oreochromis niloticus*) intoxicated with aflatoxin B_1

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ABSTRACT

Aflatoxicosis is one of the threats that cause severe mortalities in fish farms. The dietary functional additives are a friendly approach attributed to beneficial effects on aquatic animals. The study aimed at evaluating the impact of *Spirulina platensis* (SP) on the biochemical indices and antioxidative function of Nile tilapia (*Oreochromis niloticus*) intoxicated with aflatoxin B_1 (AFB_1). A control diet and 3 test diets were enriched with 0% SP/0 mg AFB_1/kg (control), 1% SP (SP), 2.5 mg AFB_1/kg diet (AFB_1), and 1% SP+2.5 mg AFB_1/kg diet (SP/AFB_1). The diets were supplied to three aquaria for each group twice daily at the rate of 2.5% for 30 days. The blood alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST) were significantly increased by AFB_1 toxicity with regards to fish fed the control and SP diets ($P < 0.05$). The inclusion of SP in the diet of tilapia intoxicated with AFB_1 lowered the levels of ALT, AST, and ALP in comparison to fish contaminated with AFB_1 without SP ($P < 0.05$). The total blood protein and albumin were decreased in fish contaminated with AFB_1 ($P < 0.05$); however, the dietary SP resulted in improving the blood protein and albumin with similar levels with the control and SP diets. The urea and creatinine were increased in tilapia fed AFB_1 diet without SP ($P < 0.05$); however, the inclusion of SP reduced the levels of urea and creatinine with similar levels with the control and SP diets. The antioxidative capacity of Nile tilapia fed SP and contaminated with AFB_1 is expressed by superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) concentration. The activities of SOD and GSH were decreased by AFB_1 ($P < 0.05$); however, dietary SP increased the SOD and GSH in fish fed AFB_1. On the other hand, the concentration of MDA was increased in tilapia fed AFB_1 ($P < 0.05$); however, SP decreased the level of MDA in fish fed AFB_1. In conclusion, the application of SP in the aquafeed seems to be an innovative approach to relieve the toxic influences of AFB_1 on aquatic animals.

1. Introduction

Due to the increased consumption of animal proteins in the human food chain (FAO, 2018), the demand for ingredients used in the manufacture of animal's food has increased (Dawood, 2016; Ismail et al., 2020). The poultry and animal feed are primarily dependent on plant ingredients, while aquafeed requires some animal protein sources, including fishmeal (Ahmadifar et al., 2019; Rashidian et al., 2020; Tveterås and Tveterås, 2010). However, the lack of fishmeal and its high prices encouraged the nutritionists to apply cereals and plant

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ingredients as a replacer for fish meal in aquafeeds (Dawood et al., 2020b; Magouz et al., 2020; Zhou et al., 2018). Besides, some fish species, including Nile tilapia (*Oreochromis niloticus*) can accept the complete plant protein-based diets (Ogello et al., 2014). Nile tilapia is the second most consumed fish species around the globe and successfully can be reared in tropical and subtropical conditions (Abdel-Latif et al., 2020a; de Souza et al., 2019; Van Doan et al., 2019). The cereals and grains borne aflatoxins are produced by molds (*Aspergillus flavus* and *A. parasiticus*) which can grow in hot weather conditions (Zeng et al., 2019). Aflatoxin B_1 (AFB_1) is the main toxin that spoils the plant feedstuff, which used in the preparation of aquafeed (Gonçalves et al., 2018). The AFB_1 toxicity induced low feed intake, digestibility, and growth rate in Yellow River carp (Fan et al., 2018), Nile tilapia (*O. niloticus*) (Hussain et al., 2017), and grass carp (Zeng et al., 2019). Further, AFB_1 impaired the health condition in fish by weakening the immune and antioxidative responses (Abdel-Daim et al., 2020a). Therefore, AFB_1 is among the main obstacles for the development of the aquaculture sector, especially in hot and humid regions and countries (Santacroce et al., 2008). The accumulated AFB_1 can pass through the gastrointestinal tract to the bloodstream and interfere with the kidney and liver, and destroy the function of the organs (Verheecke et al., 2016). Mineral and clay adsorbents usually are included in the diets to solve this problem (Hussain et al., 2017); however, recent reports illustrated that the adsorbent binders are harmful to animals (Elliott et al., 2020). The mineral-based adsorbents may induce dysfunction of the antioxidative status and immunity. These clays also can bind with the micronutrients, vitamins, and drugs in diets and decrease their availability for absorption in the GIT. Additionally, they contain other elements (e.g. nontronite, erionite, quarts, cadmium, lead, copper) which are toxic for animals (Elliott et al., 2020). Hence, it is crucial to look for healthy and eco-friendly alternatives.

Spirulina platensis (SP) is successfully applied in aquafeed as herbal immunostimulant supplement with the potential impact to reduce the over oxidation (Rosas et al., 2019). AFB_1 causes the overproduction of free radicals which increases the lipid peroxidation, apoptosis, and DNA damage in the cells (Marin and Taranu, 2012). The inclusion of SP counteracted with the oxidation impacts of AFB_1 in broilers (Raju et al., 2005). However, no studies were tested the potential role of SP to cope with the influences of AFB_1 in aquatic animals. Hence, the present study aimed at the evaluation of SP as an eco-friendly supplement to mediate the biochemical blood indices and tissues antioxidative status of Nile tilapia.

2. Materials and methods

2.1. Experimental procedure

Juveniles of Nile tilapia (initial weight, 60 ± 6.1 g) were collected from a local farm located in Kafrelsheikh, Egypt, and adapted to the laboratory conditions for two weeks. Afterwards, fish were distributed randomly in twelve glass aquaria (60 L) (40 × 60 × 70 cm) with a rate of 10 fish per aquarium. The aquaria were filled with dechlorinated tap water and supplied with a continuous aeration system. The tap water was kept in storage tanks for 24 h before pouring it in the aquaria to remove the chlorine. The aquaria were syphoned daily, and around 50% of the water was replaced with fresh water from the storage. The water quality was checked regularly and recorded 6.6 ± 0.4 mg/L for dissolved oxygen, 7.13 ± 0.76 for pH, 25.8 ± 1.7 °C for temperature. The fish were kept under 12 h light:12 h dark during the trial.

The basal diet was formulated to provide the nutritional requirements for Nile tilapia, according to NRC (2011) (Table 1). The ingredients were well mixed in the presence of water and oil and then supplemented with SP (*Spirulina platensis*, HerbaForce, Lewes, UK) or/and AFB_1 (aflatoxin B_1 from *Aspergillus flavus*, Sigma-Aldrith™) and pelleted using a meat grinder. A control diet and 3 test diets were produced with 0% SP/0 mg AFB_1/kg (control), 1% SP (SP), 2.5 mg AFB_1/kg diet (AFB_1), and 1% SP+2.5 mg AFB_1/kg diet (SP/AFB_1). The diets were supplied for three aquaria for each group twice daily (8:00 a.m.; 3:30 p.m.) at a rate of 2.5% for 30 days.

Table 1
Ingredients and chemical analysis (% based on dry matter) of the basal diet.

Ingredients	(%)	Chemical analysis	(%)
Fish meal	26	Crude protein	31.78
Yellow corn	29	Ether extract	7.15
Soybean meal	20.5	Ash	8.14
Corn gluten meal	2	Crude fiber	5.66
Wheat bran	9	Gross energy (KJ/g)[c]	18.46
Rice bran	7.3		
Fish oil	3		
Gelatin	2		
Vitamin mixture[a]	0.5		
Mineral mixture[b]	0.5		
Di-Calcium phosphate	0.2		

[a] Vitamins premix (IU or mg/kg diet); vit. A 5000, Vit. D_3 1000, vit. E 20, vit. k_3 2, vit. B1 2, vit. B_2 5, vit. B6 1.5, vit. B_{12} 0.02, Pantothenic acid 10, Folic acid 1, Biotin 0.15, Niacid 30.

[b] Mineral mixture (mg/kg diet); Fe 40, Mn 80, Cu 4, Zn 50, I 0.5, Co 0.2 & Se 0.2.

[c] Gross energy was calculated based on the values of values for protein, lipid and carbohydrate as 23.6, 39.5 and 17.2 kJ/g, respectively.

2.2. Trial termination and sample collection

Before collecting the samples, all fish were deprived of feed for 24 h. The fish were euthanized with benzocaine (0.02%), then the blood was obtained from the caudal vein of 3 fish per aquarium (9 fish per treatment) and disposed in Eppendorf tubes (1.5 mL) and kept for 2 h, then centrifuged at 3000 rpm, 4 °C, for 15 min to collect the serum. After the blood collection, the liver, kidney, and gills were dissected gently and kept in cool physiological saline (PSA, NaCl 0.9%) until further processing.

2.3. Biochemical indices

The collected serum was used for the determination of biochemical indices including, alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST), total serum protein, albumin, creatinine, urea using commercial kits (Biodiagnostic Co., Cairo, Egypt) as per the manufacturer procedure.

2.4. Oxidative status-related indices

The collected liver, kidney, and gills tissues were homogenized in cool physiological saline (PSA), filtered, and centrifuged at 1500 rpm for 20 min at 4 °C with a fast cooling rotator (Type 3K-30, Sigma, Osterode-am-Harz, Germany). The clear supernatant was collected and stored at −80 °C for the analysis of superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) concentration. Supernatant protein content was estimated as mg protein/g of wet tissue (Lowry et al., 1951). The SOD, GSH, and MDA were assessed using the method of Aebi (1984), Nishikimi et al. (1972), and Uchiyama and Mihara (1978).

2.5. Statistical analysis

All the data were analyzed by one-way ANOVA (SPSS® version 22, SPSS Inc., IL., USA). When a significant treatment effect was observed, a Duncan post-hoc test was used to compare the means. Treatment effects were considered at a $P < 0.05$ level of significance.

3. Results

3.1. Biochemical indices

The blood ALT, AST, and ALP were significantly increased by AFB_1 toxicity with regards to fish fed the control and SP diets ($P < 0.05$). The inclusion of SP in the diet of tilapia intoxicated with AFB_1 lowered the levels of ALT, AST, and ALP in comparison to fish contaminated with AFB_1 without SP ($P < 0.05$) (Fig. 1).

The total blood protein and albumin were decreased in fish contaminated with AFB_1 ($P < 0.05$); however, the dietary SP resulted in improving the blood protein and albumin with similar levels with the control and SP diets (Fig. 2).

The urea and creatinine were increased in tilapia fed AFB_1 diet without SP ($P < 0.05$); however, the inclusion of SP reduced the levels of urea and creatinine with similar levels with the control and SP diets (Fig. 3).

3.2. Oxidative indices

The antioxidative capacity of Nile tilapia fed SP and contaminated with AFB_1 is expressed by SOD, GSH, and MDA concentration. The activities of SOD and GSH were decreased by AFB_1 ($P < 0.05$); however, dietary SP increased the SOD and GSH in fish fed AFB_1 (Figs. 4 and 5). On the other hand, the concentration of MDA was increased in tilapia fed AFB_1 ($P < 0.05$); however, SP decreased the level of MDA in fish fed AFB_1 (Fig. 6).

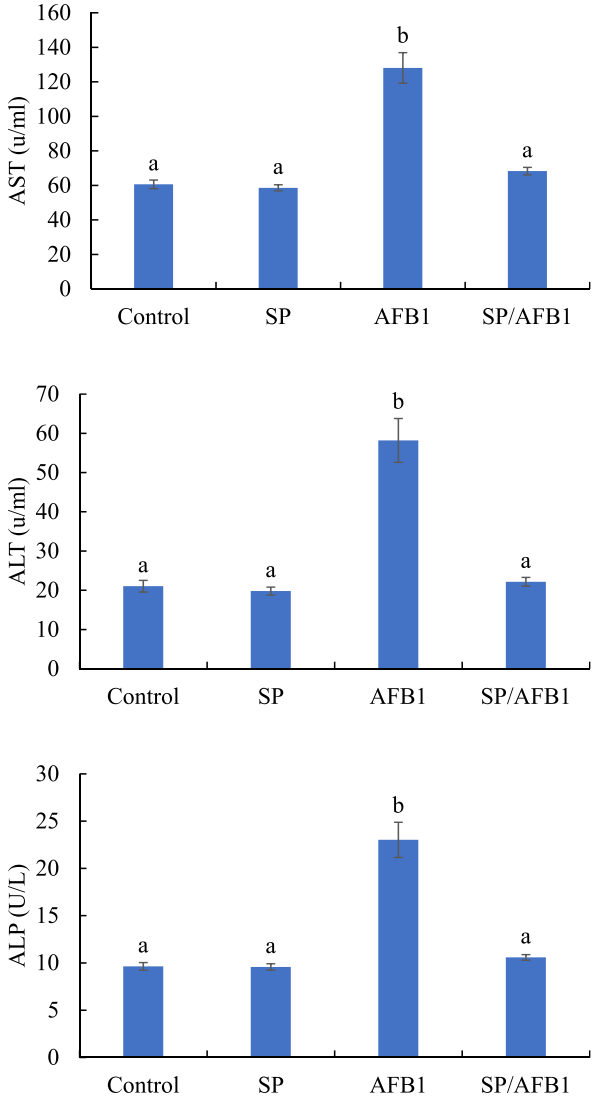

Fig. 1. Serum ALT, AST, and ALP enzyme activities in the experimental groups. SP: *Spirulina platensis*, AFB1: Aflatoxin B1. Data are expressed as means ± SE ($n = 8$). Bars with different superscripts are significantly different ($P < 0.05$).

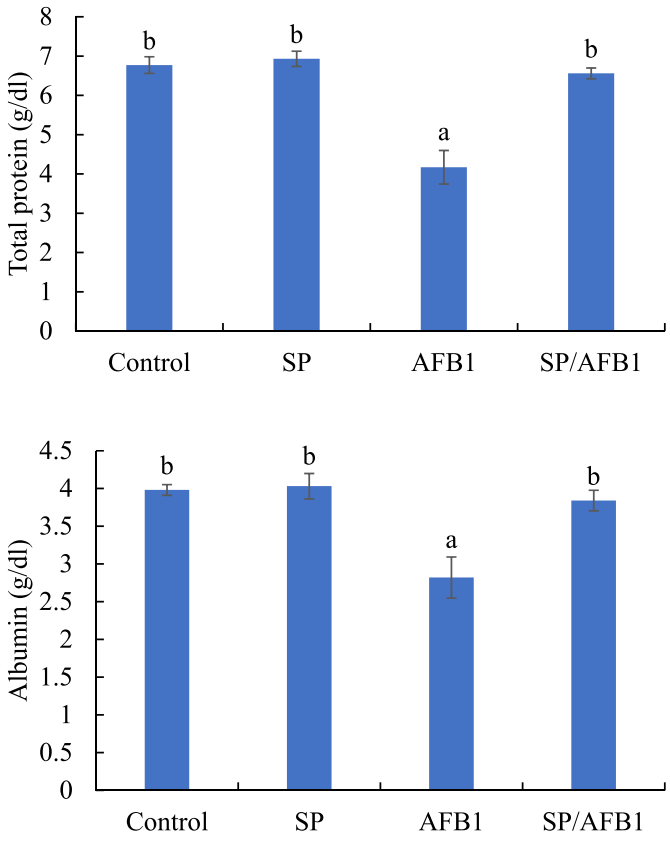

Fig. 2. Blood total protein and albumin levels in the experimental groups. SP: *Spirulina platensis*, AFB1: Aflatoxin B1. Data are expressed as means ± SE ($n = 8$). Bars with different superscripts are significantly different ($P < 0.05$).

4. Discussion

Aflatoxicosis is one of the common threats that cause severe mortalities in fish farms (Wu et al., 2019). The inclusion of clay adsorbents in aquafeed has been conventionally used to lower the levels of AFB_1 (Hussain, 2018). However, an innovative alternative option must be applied to avoid the side effects of clay minerals on aquatic animals. Dietary SP is a healthy supplement with potential immunostimulant and antioxidative activities (Soni et al., 2017). The present study revealed that dietary SP resulted in improving the liver function and antioxidative indices of fish exposed to dietary AFB_1 contamination.

A growing body of evidence suggested that the toxic metabolite, AFB_1-8,9-epoxide, formed by cytochrome P450 is the main modulator of aflatoxicosis in fish. It has the ability to form adducts with the cellular macromolecules, with an affinity in decreasing order of macromolecules of DNA > RNA > protein (Coppock et al., 2012). Inhibition of such molecules plays a crucial role in initiation of oxidative damage. There is substantial evidence documented the involvement of oxidative stress in AFB1-induced tissue injury (Marin and Taranu, 2012). As a sequel of oxidative stress, a variety of free reactive oxygen species (ROS) are generated along with depletion of the cellular antioxidant defense molecules; thereby, the cellular redox hemostasis is disrupted (Abdeen et al., 2019b; Abdel-Latif et al., 2020b; Elliott et al., 2020). Liver is well known as the main target for AFB_1-induced injury (Matejova et al., 2017). Consistently, our results revealed occurrence of hepatotoxicity

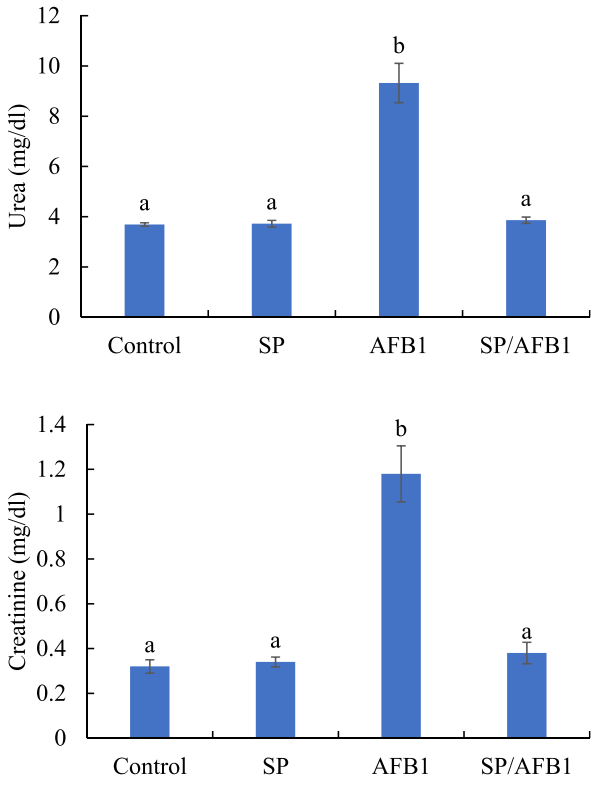

Fig. 3. Serum urea and creatinine levels in the experimental groups. SP: *Spirulina platensis*, AFB1: Aflatoxin B1. Data are expressed as means ± SE (n = 8). Bars with different superscripts are significantly different ($P < 0.05$).

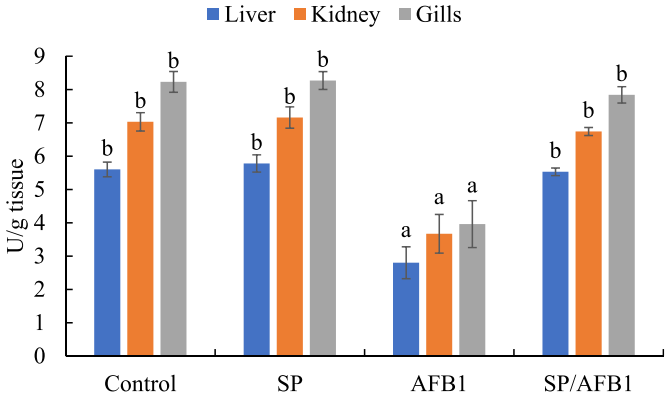

Fig. 4. Superoxide dismutase (SOD) levels of liver, kidney and gills in control and different groups. SP: *Spirulina platensis*, AFB1: Aflatoxin B1. Data are expressed as means ± SE (n = 8). Bars with different superscripts are significantly different ($P < 0.05$).

indicated by elevated levels of ALP, AST, and ALT enzymes fish fed on AFB_1-contaminated ration. Hydroxyl radical is classified as the most injurious radical among other ROS. It can directly breaks down the unsaturated fatty acids in the cell membrane in a process called lipid peroxidation; where, MDA is produced as a byproduct (Marin and Taranu, 2012). Therefore, the membrane damage indicated in this study by increased MDA level might have contribution in the release of the transaminases (AST and ALT) and ALP (a membrane-bounded protein) into the bloodstream increasing their serum levels. These data are in the same light of our previous reports which proposed a positive association between MDA and those enzymes (Abdeen et al., 2019a; Abdel-Daim and Abdeen, 2018). Interestingly, these levels were almost restored to normal levels when SP was included in AFB_1-contaminated ration. The

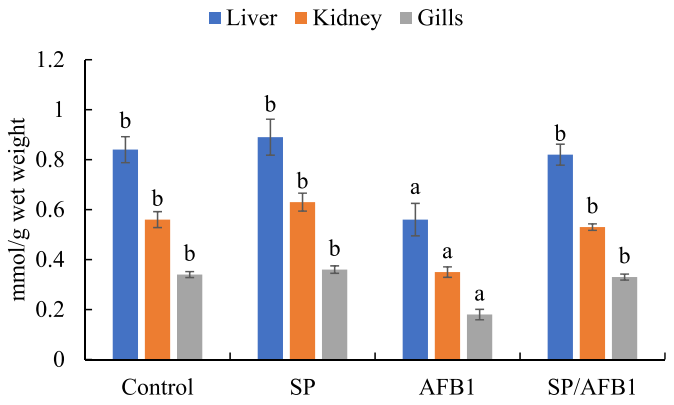

Fig. 5. Glutathione (GSH) levels of liver, kidney and gills in control and different groups. SP: *Spirulina platensis*, AFB1: Aflatoxin B1. Data are expressed as means ± SE (n = 8). Bars with different superscripts are significantly different ($P < 0.05$).

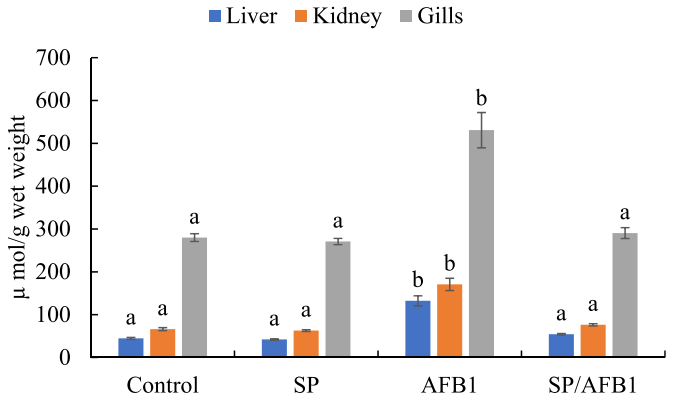

Fig. 6. Malondialdehyde (MDA) levels of liver, kidney and gills in control and different groups. SP: *Spirulina platensis*, AFB1: Aflatoxin B1. Data are expressed as means ± SE (n = 8). Bars with different superscripts are significantly different ($P < 0.05$).

pharmacodynamic role of SP in regulating the liver enzymes is associated with the high content of essential amino acids, fatty acids, vitamins, and minerals which are the main nutrient required for the optimal metabolic pathways in the hepatic tissue (Mahmoud et al., 2018). In line with the present study, reduced levels of ALP, AST, and ALT enzymes were observed in Nile tilapia fed SP and exposed to other toxins (Abdel-Daim et al., 2020b; Abdelkhalek et al., 2017).

The blood total protein refers to the amount of proteins in the blood, including globulin and albumin (Faggio et al., 2014; Trichet, 2010). It is strongly proposed that the observed decline in the total protein and albumin are attributed to the inhibited gene transcription and protein translation processes as a result of direct binding of AFB_1-8,9-epoxide to DNA forming AFB_1-N^7-Guanine adduct and to protein forming protein adducts as well as via direct damaging effect of the generated ROS (Coppock et al., 2012; Matejova et al., 2017). However, fish fed SP displayed increased total protein and albumin which can be attributed to the enhanced immunity of tilapia (Dawood et al., 2020a, 2020cbib_Dawood_et_al_2020abib_Dawood_et_al_2020c). The increased total protein and albumin in fish fed SP indicate the enhanced immunity to counteract with the impacts of AFB_1. In agreement with the present study, tilapia fed SP revealed increased blood total protein and albumin levels (Abdel-Daim et al., 2020b; Abdelkhalek et al., 2017). SP has several active compounds such as vitamins, minerals, carotenoids, polysaccharides, and γ-linolenic acid which act like immunostimulants to enhance the immune system (Rosas et al., 2019).

Urea level is known to monitor the amount of broken protein in the

liver and the capacity of the kidney to help in removing the excessive blood urea (Hester et al., 1992). In addition, the high levels of creatinine reflect the broken creatine content in the muscle and the ability of the kidney to filtrate it into creatinine to secret with the urine (Watson et al., 2002). The present study displayed increased urea levels together with elevated blood creatinine in tilapia exposed to AFB_1, which are associated with kidney dysfunction. Kidney is high-energy requiring organ contains huge abundance of mitochondria which is vulnerable to oxidative damage. The excessive production of ROS in renal cells causes mitochondrial dysfunction, ATP depletion, damage of membrane transporters, and interfere with the cytoplasmic protein (Abdel-Daim et al., 2019b). All these events ended by impaired renal tubular function observed in this study. That also could be another reason for increased protein loss decreasing the total blood protein levels. These findings are in consistence with our previous investigation where increased blood urea and creatinine along with decreased total protein levels were reported in oxidative-damaged renal cells in response to zinc oxide nanoparticle intoxication in Nile tilapia (Abdel-Daim et al., 2019a). In contrast, the dietary SP reduced the urea and creatinine levels in fish exposed to AFB_1. The antioxidant potential role of SP is also correlated with regulating the function of the liver and kidney decreasing the damage effects of AFB_1. However, further studies are required to reveal the possible modes of action of SP in alleviating the induced AFB_1 toxicity on liver and kidney function.

Moreover, the antioxidative capacity was measured in the gills, liver, and kidney tissues of tilapia fed SP and/or AFB_1-contaminated diet. The results presented impaired antioxidative status in fish contaminated with AFB_1 and increased antioxidative status in fish fed SP. As mentioned earlier, the impaired liver and kidney functions were associated with the altered antioxidative response of fish exposed to AFB_1. While fish fed on SP displayed high SOD and GSH with reduced MDA concentration reflecting activated antioxidative response. The dietary SP is well known by the increased antioxidative role, which is correlated to the high content of polyphenols and carotenes that are considered as antioxidant substances (Bhowmik et al., 2009). The proposed mechanisms underlying the protective effects of SP against AFB1 toxicity are summarized in Fig. 7.

5. Conclusion

Dietary SP regulated the liver and kidney functions and increased the antioxidative capacity of Nile tilapia. The application of SP in the aquafeed seems to be an innovative approach to relieve the toxic influences of AFB_1 on aquatic animals.

Declaration of competing interest

None of the authors has any conflicts of interest to declare.

CRediT authorship contribution statement

Mohamed M. Abdel-Daim: Conceptualization, Funding acquisition, Formal analysis, Investigation, Methodology, Supervision, Writing - original draft. **Mahmoud A.O. Dawood:** Conceptualization, Funding acquisition, Formal analysis, Investigation, Methodology, Supervision, Writing - original draft. **Abdullah A. :** Conceptualization. Conceptualization, Funding acquisition, Formal analysis, Investigation, Methodology, Supervision. **Ahmed Abdeen:** Conceptualization, Funding acquisition, Formal analysis, Investigation, Methodology, Supervision. **Hoda H. Senousy:** Conceptualization, Funding acquisition, Formal

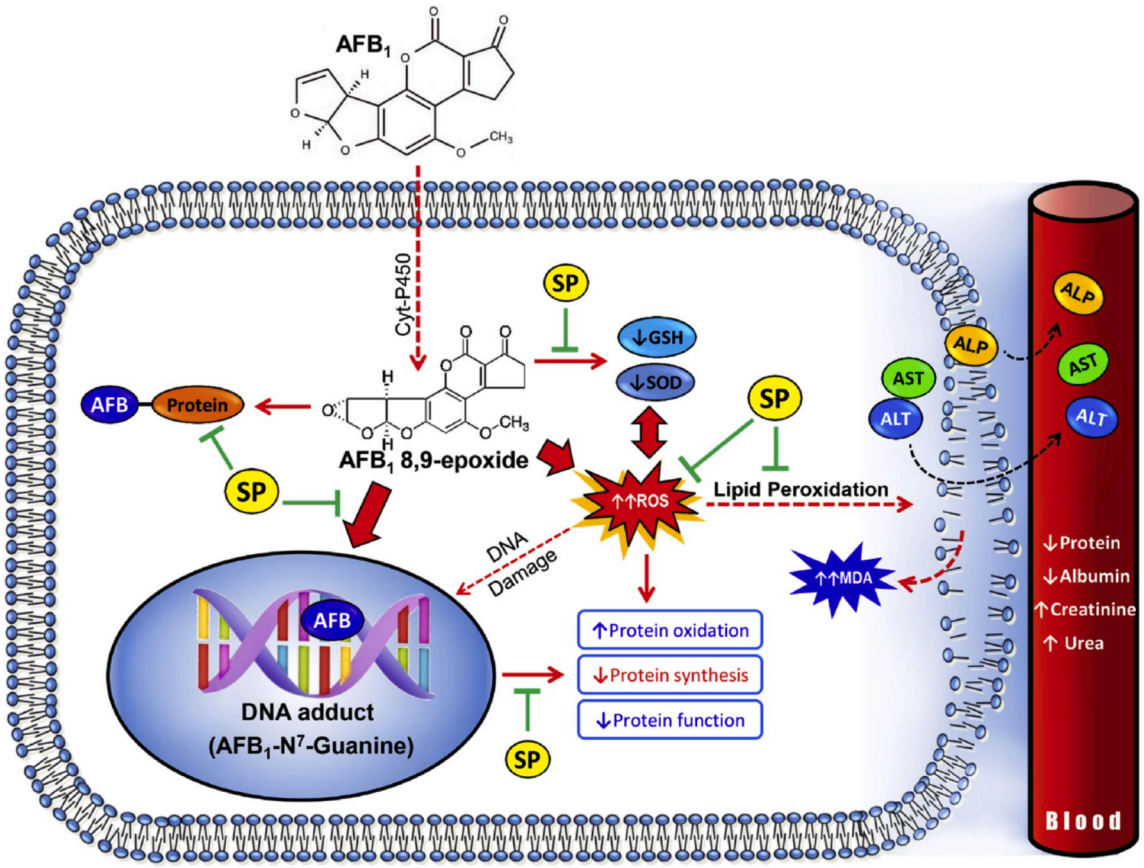

Fig. 7. The proposed mechanisms underlying the protective effects of SP against AFB1 toxicity in Nile tilapia. AFB_1: aflatoxin B_1, ALP: alkaline phosphatase, ALT: alanine transaminase, AST: aspartate transaminase, Cty-P450: cytochrome-P450, GSH: reduced-glutathione, MDA: malondialdehyde, ROS: reactive oxygen species, SOD: superoxide dismutase, SP: *Spirulina platensis*.

analysis, Investigation, Methodology, Supervision. **Lotfi Aleya:** Conceptualization, Funding acquisition, Formal analysis, Investigation, Methodology, Supervision.

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