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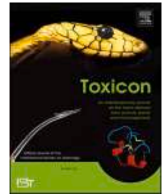

Spirulina platensis ameliorates hepatic oxidative stress and DNA damage induced by aflatoxin B1 in rats

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ABSTRACT

Aflatoxin B1 (AFB1) is a widely distributed mycotoxin, causing hepatotoxicity and oxidative stress. One of the most famous unicellular cyanobacteria is *Spirulina platensis* (SP) which is well known for its antioxidant characteristics against many toxicants. Therefore, this study aimed to investigate the antioxidant potential and hepatoprotective ability of SP against oxidative stress and cytotoxicity in male Wistar albino rats intraperitoneally injected with AFB1. Rats were separated into five groups as follows: negative control administered with saline; SP (1000 mg/kg BW) for two weeks; AFB1 (2.5 mg/kg BW) twice on days 12 and 14; AFB1 (twice) + 500 mg SP/kg BW (for two weeks) and AFB1 (twice) + 1000 mg SP/kg BW (for two weeks). Liver and blood samples were assembled for histological and biochemical analyses. AFB1 intoxicated rats showed a marked elevation in serum biochemical parameters (ALP, ALT, and AST), hepatic lipid peroxidation (MDA and NO), and proliferating cell nuclear antigen (PCNA) indicating DNA damage. Moreover, AFB1 caused suppression of antioxidant biomarkers (SOD, GHS, GSH-Px, and CAT). However, the elevated serum levels of biochemical parameters and PCNA expression were reduced by SP. Moreover, SP lowered oxidative stress and lipid peroxidation markers in a dose-dependent manner. To sum up, SP supplementation is capable of decreasing AFB1 toxicity through its powerful antioxidant activity.

1. Introduction

Aspergillus parasiticus and *Aspergillus flavus* generate the most famous mycotoxins which are Aflatoxins (AFs). AFs are one of the major powerful toxins which are distributed in different crops usually utilized to prepare various foods and are taken into consideration by the Food

Abbreviations: Afs, Aflatoxins; AFBO, AFB1-8,9-epoxide; AFB1, Aflatoxin B1; AFB1, -8,9-epoxide; ALT, alanine aminotransferase; ALP, Alkaline phosphatase; ANOVA, analysis of variance; AST, aspartate aminotransferase; CAT, catalase; CYP, Cytochrome P-450; GSH, reduced-glutathione; GPx, glutathione peroxidase; H and E, Hematoxylin and Eosin; NO, nitric oxide; PAS, Periodic acid Schiff; PCNA, proliferating cell nuclear antigen; ROS, reactive oxygen species; SOD, superoxide dismutase; SP, *Spirulina platenesis*.

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and Drug Administration as hazards that reflects on animals' and humans' health (Gell and Carbone, 2019). AFB1, AFB2, AFG1, and AFG2 are classes of AFs that occur naturally in a wide diversity of feeds and foods. AFB1 has immunosuppressive and carcinogenic activities in humans and animals (Cao et al., 2022). The pathologic symptoms of hepatotoxicity initiated by AFB1 are hemorrhage, bile duct damage, and periportal hepatocellular necrosis (Yilmaz et al., 2020).

Liver microsomal enzymes (Cytochrome P-450 (CYP) 3A4 and 1A2) metabolize AFB1 into the toxic metabolites AFB1-8,9-epoxide (AFBO) (Roze et al., 2013). For making aflatoxin B1–N7-guanine, AFBO is linked to the RNA and DNA, especially guanine N7 location (Aiko and Mehta, 2015) which suppresses the synthesis of RNA, DNA, and protein (Xu et al., 2017). Then, the AFBO detoxification is made by the glutathione S-transferase system of the liver into AFBO non-toxic metabolites which are excreted in urine and bile (Iqbal et al., 2019). Oxidative stress is a pivotal mechanism participating in the induction and pathogenesis of hepatic damage in AFB1-induced hepatotoxicity (Theumer et al., 2010). After the consumption of foods containing AFB1 by humans or animals, the metabolization of AFB1 generates highly reactive chemicals which give rise to lipid peroxidation, free radical production, and cell damage (Ingawale et al., 2014) by increasing catalase (CAT), reduced-glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx) which consume intracellular antioxidant system. Furthermore, free radicals can contribute to a developmental reduction in immune system functions (El Denshary et al., 2012).

It has been well-notarized that AFB1-adduct formation and drug-metabolizing enzymes could be ameliorated by active antioxidant contents of the nutrients, xenobiotics, and phytochemicals (Abdel-Aziem et al., 2011). The recent common trends have concentrated on the preventive functions of antioxidants in natural products resisting oxidative stress. The fibrosis in the liver might be lowered by antioxidants which remove free radicals (Abdel-Moneim et al., 2015). Recently, our group showed that fucoidan has antioxidant potency against AFB1 intoxication in rats (Abdel-Daim et al., 2021).

One of the most famous unicellular cyanobacteria is *Spirulina platenesis* (SP) which has a wide variety of medicinal purposes and rich nutritional value due to its high protein content and powerful antioxidant content. C-phycocyanin is the active constituent of SP which has neuroprotective, antioxidant, hepatoprotective, and anticancer activities (Gargouri et al., 2018).

Moreover, the toxicities stimulated by heavy metals have been mentioned to be treated by SP (Paniagua-Castro et al., 2011). The elevated focus on SP is due to its safety and has marked prevention against various drug-induced toxicity in many organs (Lu et al., 2010). We suggested that SP can protect liver against aflatoxicosis. Therefore, we studied the hepatoprotective and antioxidant influences of SP versus hepatotoxicity stimulated by AFB1 in rats.

2. Material and methods

2.1. Chemicals

In this investigation, all used chemicals were pure of analytical grade. The source of pure SP powder was HerbaForce, UK. Sigma-Aldrich Chemical Inc. Co. (St. Louis, MO, USA) produced high pure AFB1 (purity ≥99%). We bought all detection kits of liver injury biomarkers, tissue antioxidants and lipid peroxidation from Biodiagnostics Co. (Cairo, Egypt).

2.2. Animals and experimental design

The Egyptian Organization for Biological Products and Vaccines is the place where we obtained forty (175 ± 25 g in weight) male Albino Wistar rats. The rearing of rats was in planned laboratory circumstances of temperature (25 ± 2 °C), well ventilation, free supplementation of water and food, and a normal light-dark cycle (12 h light/dark). The Research Ethical Committee of Faculty of Veterinary Medicine, Damanhour University's Ethics of Animal Use Research Committee in Egypt (DMU/VetMed-2023/002). Following adaptation time for one week, rats have separated into five various groups where we distributed eight rats per group. The 1st group was receiving normal phosphate buffer saline as a negative control group. The 2nd group was administrated SP dissolved in phosphate buffer saline (1000 mg/kg BW) per oral for 14 days. The 3rd group was given an intraperitoneal dose of AFB1 dissolved in phosphate buffer saline (50 μg/kg BW) twice only on the 12th and 14th days according to Abdel-Daim et al. (2021). The same times and doses of AFB1 in the 3rd group have been received by the 4th and 5th groups with administrated SP (500 and 1000 mg/kg BW) per oral, respectively for 14 days according to Abdel-Daim et al. (2013) before AFB1 intoxication.

2.3. Serum collection and tissue preparation

On the 15th day, blood was taken from the retro-orbital under isoflurane anesthesia. We maintained blood samples at room temperature till be coagulated, and then we centrifuged blood samples at 3000×*g* for 15 min. For more biochemical analysis, we isolated and stored sera as liquor at −20 °C.

Then we sacrificed rats by decapitation, and we quickly extirpated livers and rinsed them with 0.9% NaCl solution. After that, livers were sectioned into two parts: one part preserved in neutral buffered formaldehyde for histopathology and immunohistochemistry for 24 h. The other parts were immersed in a medium containing a mixture of 0.1 mM ethylene diamine tetra-acetic acid and 50 mM sodium phosphate buffer saline. After that, we homogenized 1 g of hepatic tissue in 10 ml buffer and the homogenate centrifugation was for a half hour at 5000×*g*. We transferred the supernatants into Eppendorf tubes which were conserved at −80 °C until the utilization in a different biochemical assay.

2.4. Biochemical analysis of serum

We estimated liver injury biomarkers in serum such as alanine aminotransferase (ALT), Alkaline phosphatase (ALP), and aspartate aminotransferase (AST), based on Tietz et al. (1983).

2.5. Estimation of tissue antioxidant and lipid peroxidation enzymes

According to Mihara and Uchiyama (1978), we measured hepatic lipid peroxidation content by assessment of MDA. Moreover, we estimated oxidative stress markers; CAT, GSH-Px, SOD, and GSH according to Aebi (1984), Paglia and Valentine (1967), Nishikimi et al. (1972), and Beutler et al. (1963) respectively. The estimation of nitric oxide (NO) was based on Tracey et al. (1995).

2.6. Histopathological examination

The conventional paraffin embedding technique was utilized and Hematoxylin and Eosin (H and E) technique and Periodic acid Schiff (PAS) were the used stains which were based on Bancroft and Layton (2013) and Layton and Bancroft (2013) respectively. The used micrographs in our study were obtained by a microscope (Leica DM500) with an attached digital camera (Leica EC3, Leica, Germany). Semi-quantitative scoring of liver lesions was calculated based on Gibson-Corley et al. (2013).

2.7. Immunohistochemical analysis

An immunohistochemical study was done following the method of Wasef et al. (2021). 1:50 dilution of Anti-PCNA was used as the primary antibody obtained from Genemed Biotechnologies Inc. (CA, USA).

We utilized the Image J software (National Institutes of Health, Bethesda, MD, USA) to quantify the area percentage of

immunohistochemical and PAS reactions in ten randomly chosen fields from eight rats in each group (Sysel et al., 2013).

2.8. Statistical analysis

We expressed all data as means ± S.E.M. and we analyzed all data utilizing SPSS version 22.0 (Statistical Package for the Social Sciences) (SPSS Inc, Chicago, IL, USA). One-way analysis of variance (ANOVA) was the estimation method for the statistical significance of differences between various study groups ($P \leq 0.05$). Moreover, we utilized Duncan's multiple range test to distinguish among means (to define variations among averages of treatments at significance rates of 0.05).

3. Results

3.1. Serum biochemical analysis

Table 1 reveals the toxic AFB1 influences and the protective SP influences on the biochemical parameters of serum. In AFB1 intoxicated rats marked elevations ($P \leq 0.05$) in (ALP, ALT, and AST) were detected in comparison to the control negative group (186.42, 251.19, and 181.70% respectively).

500 and 1000 mg/kg SP had effective prevention against AFB1 intoxication and effectively lowered the alternations in the investigated serum parameters of hepatic functions. The serum hepatic biomarkers were markedly ($P \leq 0.05$) lowered by the pre-administration of 500 mg/kg SP; ALP, ALT, and AST (about 71.94, 66.57, and 70.56%, respectively). Moreover, the serum hepatic biomarkers were markedly ($P \leq 0.05$) decreased by pre-administration of 1000 mg/kg SP; ALP, ALT, and AST (about 57.53, 45.08, and 56.72%, respectively). Administration of 1000 mg/kg SP did not markedly change the serum biomarkers in comparison to the control negative group, suggesting SP safety.

3.2. Hepatic lipid peroxidation and antioxidant status

The AFB1 toxic influences and SP protective influences on hepatic homogenate antioxidant and lipid peroxidation parameters are revealed in Table 2. A marked elevation ($P \leq 0.05$) in hepatic NO and MDA (174.76 and 219.02%, respectively) was detected in AFB1 group compared to the negative control group. However, liver GSH-Px, GSH, CAT, and SOD were markedly ($P \leq 0.05$) reduced (45.40, 47.23, 52.19, and 48.81% respectively). In the AFB1+SP500 group, hepatic MDA and NO were lowered (71.12 and 79.12%, respectively) but GSH, GSH-Px, SOD, and CAT were elevated (151.07, 175.14, 150.78, and 135.87%, respectively) in comparison to AFB1-intoxicated group. Furthermore, hepatic MDA and NO, in the AFB1+SP1000 group were markedly lowered (48.21 and 57.83%, respectively), but GHS, GSH-Px, SOD, and CAT were significantly elevated (about 193.09, 216.69, 122.26, and 120.78%, respectively).

Table 1
Serum enzyme activity and biochemical parameters in control and different treated groups.

Parameters	Experimental groups				
	Control	SP1000	AFB1	AFB1-SP500	AFB1-SP1000
AST (U/L)	61.26 ± 0.91^a	59.98 ± 1.44^a	111.31 ± 2.40^b	78.55 ± 1.99^c	63.14 ± 1.28^a
ALT (U/L)	27.77 ± 1.22^a	27.58 ± 1.12^a	69.98 ± 3.37^b	46.59 ± 2.40^c	31.55 ± 1.38^a
ALP (U/L)	69.15 ± 2.75^a	67.02 ± 3.19^a	128.91 ± 3.03^b	92.74 ± 2.55^c	74.17 ± 3.62^a

Data are expressed as means ± SE (n = 8).
AFB; Aflatoxin, SP; *Spirulina platensis*, AST; aspartate aminotransferase, ALT; alanine aminotransferase and ALP; alkaline phosphatase.
Values having different superscripts within the same raw are significantly different ($P \leq 0.05$).

Table 2
Liver oxidative stress marker and antioxidant parameters in control and different treated groups.

Parameters	Experimental groups				
	Control	SP1000	AFB1	AFB1-SP500	AFB1-SP1000
MDA (nmol/g)	92.05 ± 1.37^a	89.83 ± 2.10^a	201.61 ± 3.01^b	143.40 ± 3.98^c	97.21 ± 1.42^a
NO (nmol/g)	81.96 ± 1.51^a	81.03 ± 1.76^a	143.16 ± 5.18^b	113.27 ± 2.59^c	82.80 ± 1.62^a
GSH (mg/g)	106.39 ± 2.69^a	111.67 ± 1.93^a	50.24 ± 1.86^b	75.90 ± 2.30^c	97.01 ± 1.36^d
GSH-Px (mol/g)	85.11 ± 2.15^a	89.33 ± 1.54^a	38.64 ± 1.43^b	60.72 ± 1.84^c	83.73 ± 1.90^a
SOD (U/g)	28.80 ± 1.07^a	31.98 ± 0.85^b	14.06 ± 0.52^c	21.20 ± 0.72^d	25.92 ± 0.61^e
CAT (U/g)	2.51 ± 0.08^a	2.61 ± 0.09^a	1.31 ± 0.05^b	1.78 ± 0.06^c	2.15 ± 0.06^d

Data are expressed as means ± SE (n = 8).
AFB; Aflatoxin, SP; *Spirulina platensis*, MDA; malondialdehyde, NO; nitric oxide, GSH; reduced glutathione, GSH-Px; glutathione peroxidase, SOD; superoxide dismutase and CAT catalase.
Values having different superscripts within the same raw are significantly different ($P \leq 0.05$).

3.3. Histopathological study

The rat livers of the control group had normal architecture (Fig. 1A and B). Significant histopathological lesions such as necrotic changes and hydropic degeneration in hepatocytes were detected in all rats of the AFB1 group (Fig. 1C). We observed vacuolar-hydropic degenerative or cloudy swelling hepatocytes located around the central veins and recognized by large foci of hepatocytes interrupted with congested hepatic sinusoids (Fig. 1D). In the periportal regions, hepatocytes had excessive cytoplasmic vacuolation or lysis and the nuclei were pyknotic (Fig. 1E). The sinusoids were retracted due to swollen hepatocytes. The hepatic cords were disarranged due to the severe degenerative cell foci. In the bile ducts, epithelial hyperplasia, focal mononuclear cell infiltration, and slight periportal fibrosis were observed (Fig. 1F). Normal hepatocytes were detected in the periportal areas (Fig. 1F). Extensive hemorrhage was found between hepatocytes (Fig. 2A). We detected changes in the liver tissues obtained from the AFB1+SP500 group, while these alterations were markedly lower than in the AFB1 group. Livers revealed slight necrotic changes and hydropic degenerations (Fig. 2B). In the portal area, some congested enlarged veins could be detected and surrounded by mononuclear cell infiltration (Fig. 2C). The architectures of lobules in the AFB1+SP1000 group were intact lobules with normal hepatocytes (Fig. 2D). On the other hand, a few lobules revealed a focus of degenerative cells foci with fewer cells had a mild cytoplasmic degeneration (Fig. 2E). The study of hepatic lesions scores by semi-quantitative statistical analysis declared that the AFB1 intoxicated animals had a markedly more elevated hepatic lesions score than the control and SP1000 groups. On the other hand, AFB1+SP500 and AFB1+SP1000 groups revealed a marked reduction ($P \leq 0.05$) in the hepatic lesions score in comparison with the AFB1 group (Fig. 2F).

Livers of rats in the control and SP1000 groups showed the highest PAS distribution (Fig. 3A and B). Decreased glycogen could be detected in hepatic samples obtained from rats intoxicated with AFB1 and stained with PAS (Fig. 3C). Livers of AFB1-intoxicated rats protected with SP500 had a moderate PAS reaction (Fig. 3D). Furthermore, AFB1+SP1000 exposed the best PAS reaction among the treated groups (Fig. 3E). AFB1 revealed a marked reduction ($P \leq 0.05$) in PAS distribution compared to the control and SP1000 group, however, the AFB1 group protected with SP500 and SP1000 revealed a high regular PAS distribution (Fig. 3F).

Rats of the control and SP1000 groups revealed negative PCNA

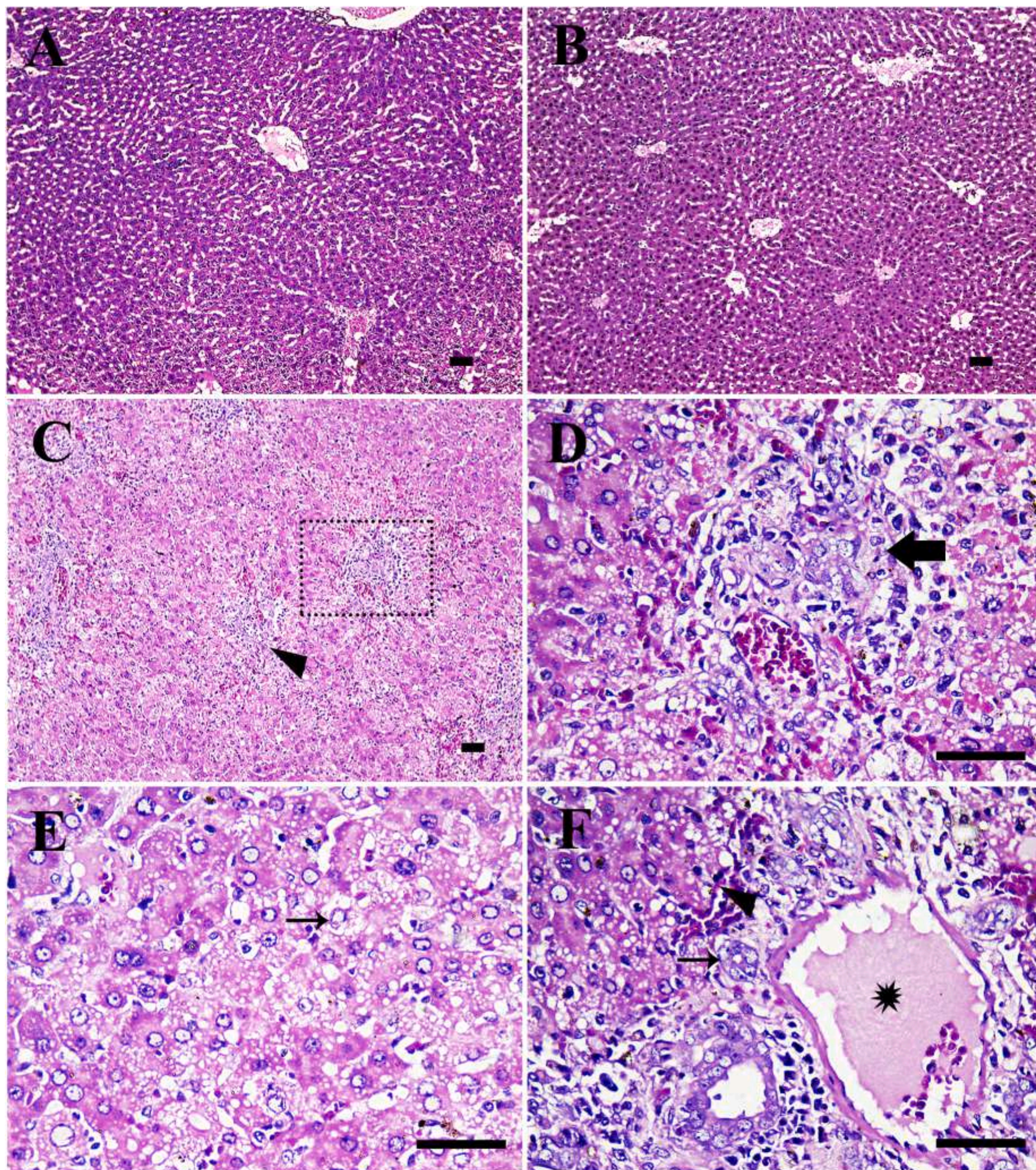

Fig. 1. Histopathological examination of rat liver. **A)** negative control group and **B)** SP1000 group showing the normal liver architecture. **C)** AFB1 group revealing necrotic foci of hepatocytes contains extensive hemorrhages and is distributed around central veins (arrowheads). **D)** is the inset of the dotted square in the previous micrograph showing aggregation of necrotic hepatocytes (thick arrow). **E)** In the periportal regions, the AFB1 group showed hepatocytes with moderate to severe cytoplasmic vacuolation (thin arrow). **F)** AFB1 group revealing epithelial hyperplasia in the bile ducts (asterisk) surrounded by mononuclear cell infiltration. Many bile ducts (arrow) and normal-looking hepatocytes (arrowhead) are observed around the periportal area. H and E stain. *Scale bar* 50 μm.

reactions (Fig. 4A and B). Interestingly, rats of the AFB1 group revealed a powerful PCNA reaction in most hepatocytes' nuclei (Fig. 4C). However rats that obtained a mixture of SP500 and AFB1 displayed lower PCNA reaction than the AFB1 alone group (Fig. 4D). Moreover, AFB1+SP1000 exposed nearly negative PCNA reaction in hepatocyte nuclei (Fig. 4E). Semiquantitative analysis of PCNA immunohistochemistry showed a significantly large area ($P \leq 0.05$) for the PCNA distribution in the AFB1 group. However this high distribution was decreased in the control, SP1000, AFB1+SP500, and AFB1+SP1000 groups (Fig. 4F).

4. Discussion

Cells constantly produce reactive oxygen species (ROS) as an output of numerous endogenous metabolic mechanisms such as bioenergetic electron transport mechanism and redox enzymes and/or environmental exposure to several xenobiotics and exogenous drugs (Reuter et al., 2010). In normal conditions, the endogenous antioxidants neutralize the produced ROS and there is a balance between the existing antioxidants and the created ROS (Reuter et al., 2010). ROS causes mischievous influences which take place as a result of imponderables among the generation and ROS inhibition resulting in various pathological conditions

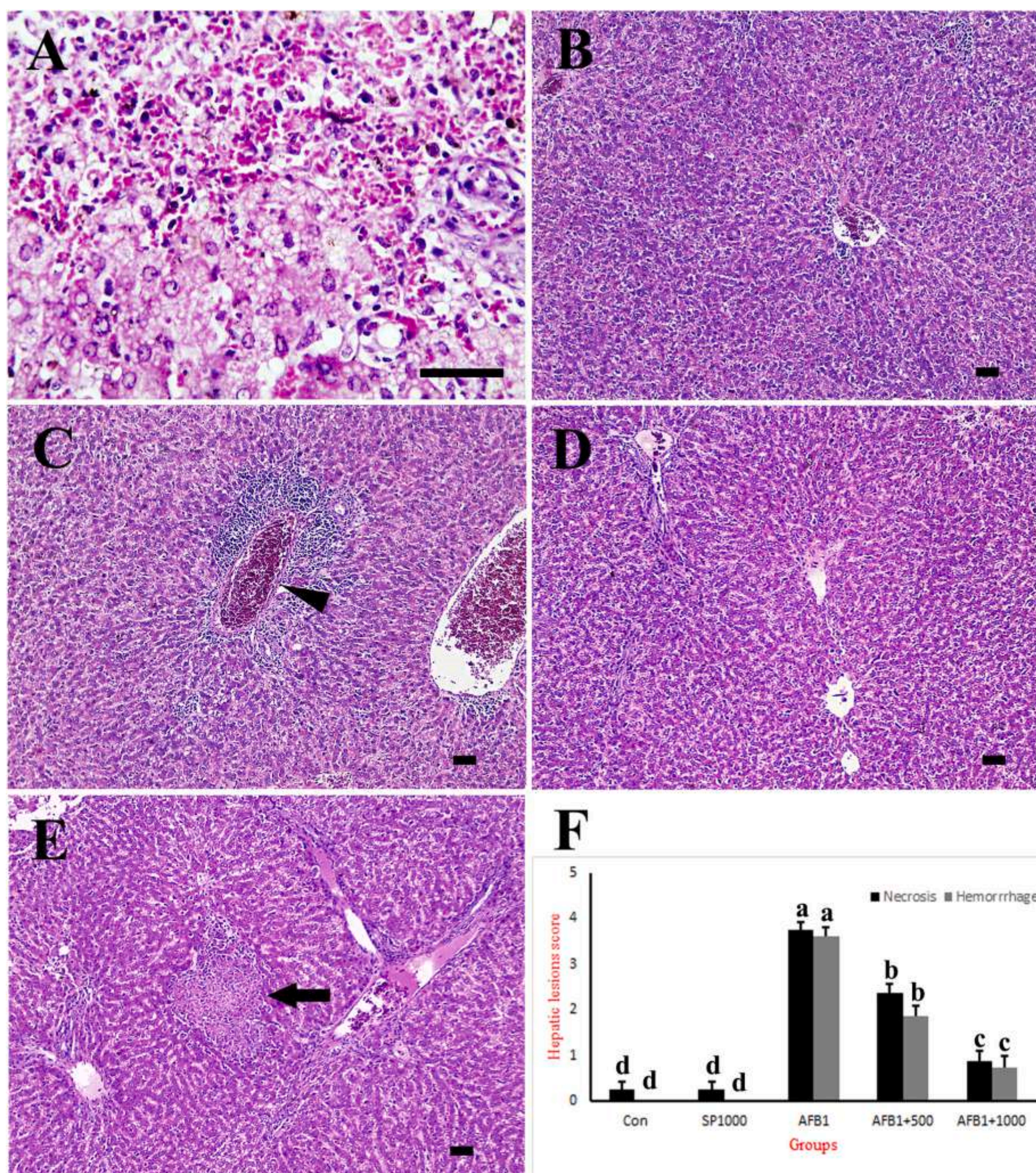

Fig. 2. A) AFB1 group showing severe hemorrhage between hepatocytes. **B)** AFB1+SP500 group showing a slightly normal hepatic architecture as a negative control group. **C)** The AFB1+SP500 group has some congested enlarged veins that could be detected and surrounded by mononuclear cell infiltration (arrowhead). **D)** AFB1+SP1000 group showing normal hepatic lobule. **E)** AFB1+SP1000 group has few degenerative cell foci with fewer cells having a normal nuclear appearance and mild cytoplasmic degeneration (arrow). H and E stain. *Scale bar* =50 μm. **F)** hepatic lesions scoring of necrosis and hemorrhage. Data showed as Mean ± SE and analyzed utilizing one-way ANOVA at $p \leq 0.05$, *significance in comparison with control. Aflatoxin (AFB1) and *Spirulina platensis* (SP).

(Gulcin, 2020; Reuter et al., 2010). The ROS enhances the oxidative destruction of many biomolecules such as lipids, proteins, lipoproteins, and DNA (Adjimani and Asare, 2015; Cetin Cakmak and Gülçin, 2019; Gülçin, 2012).

In this investigation, we detected the standard biochemical and histopathological findings of AFB1 toxicity in rats. The protection against cancer by the addition of SP to the diet has been investigated previously in rats and mice (Braune et al., 2021). Although well-established proof is reporting the biological activities and health-enhancing influences of SP (Kusmayadi et al., 2021), the possible SP function in the hepatic defense system against aflatoxicosis is not well illustrated. This investigation was designed to elucidate the potential role of SP in resisting the oxidative stress of the liver caused by aflatoxicosis. Because AFB1 is mutagenic and hepatotoxic, the liver was chosen.

AFB1 is a powerful hepatocarcinogenic and hepatotoxic mycotoxin. It has been detected to cause many harmful influences in tissues by oxidative stress enhancement (Abdel-Daim et al., 2021). AFB1 hepatotoxic influences have been well investigated which indicated that ROS is the main mechanism of AFB1 hepatotoxicity (Yilmaz et al., 2020). The cell membrane damage caused by AFB1 might be through enhancing phospholipid A2 which finally causes lipid peroxidation (Cao et al., 2022). Furthermore, AFB1 initiates the production of 8-hydroxydeoxyguanosine contributing to lipid peroxidation (Cao et al., 2022; Güven

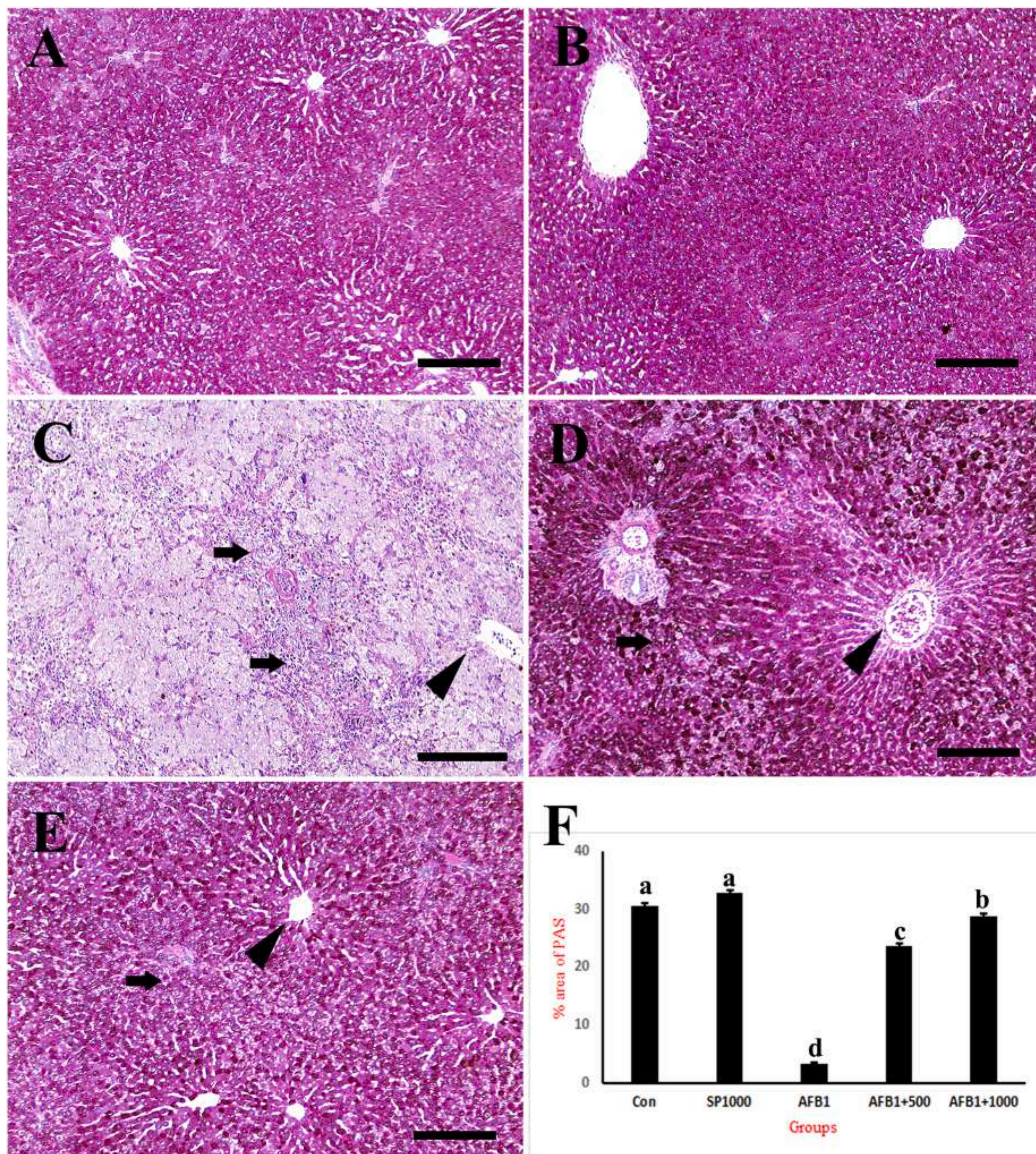

Fig. 3. Periodic acid Schiff (PAS) staining of rat liver. **A)** negative control group and **B)** SP1000 group showing the normal distribution of PAS reaction in the hepatic lobules. **C)** The Aflatoxin group revealed no PAS reaction in all hepatic lobules including the central vein (arrowhead), and a weak reaction in the periportal area (arrow). **D)** AFB1+SP500 group showing massive PAS reaction, especially around the periportal area (arrow), and weak reaction in the central vein (arrowhead). **E)** AFB1+SP1000 group showing massive PAS reaction surrounding the central vein (arrowhead) and the periportal area (arrow). *Scale bar* =200 μm. **F)** PAS quantification in the various groups' livers. Data displayed as Mean ± SE and analyzed utilizing one-way ANOVA at $p \leq 0.05$, *significance in comparison to control.

et al., 2023). The AFB1 capability to induce cellular oxidative stress and AFB1-DNA adduct production may have an essential function in the pathogenesis of cancer caused by AFB1 (Engin and Engin, 2019).

As revealed in Table 1, the AFB1 group had a marked ($P \leq 0.05$) elevation in the levels of ALP, AST, and ALT in comparison to the control group. The elevated serum levels of cytoplasmic AST, ALP, and ALT could be explained by the destroyed structural integrity of the hepatocytes which are full of these enzymes (Xu et al., 2021). SP treatment lowered the high levels of serum ALP, AST, and ALT compared to the AFB1-intoxicated group, suggesting the effectiveness of SP in the suppression of AFB1-induced hepatocyte injury.

Lipid peroxidation is a pivotal index of oxidative destruction, and it has an essential role in carcinogenicity and toxicity. Membrane lipids are sensitive to the mischievous activities of ROS. Peroxidization of polyunsaturated fatty acids produces MDA. Elevated MDA content is a pivotal signal of oxidative membrane destruction (Apak et al., 2022; Wang et al., 2021). Concentrations of MDA and NO were markedly elevated in the livers of the AFB1 group, which could be attributed to an elevation of ROS in the AFB1-intoxicated rats. However, SP protection prevented the elevation of MDA contents in the liver of rats, which is attributed to the success of SP in preventing the free radicals thereby preventing oxidative damage of the membrane lipids and suppressing lipid peroxidation. This detection is harmonious with the study by Abdel-Wahhab et al. (2015) which have mentioned that AFB1 can

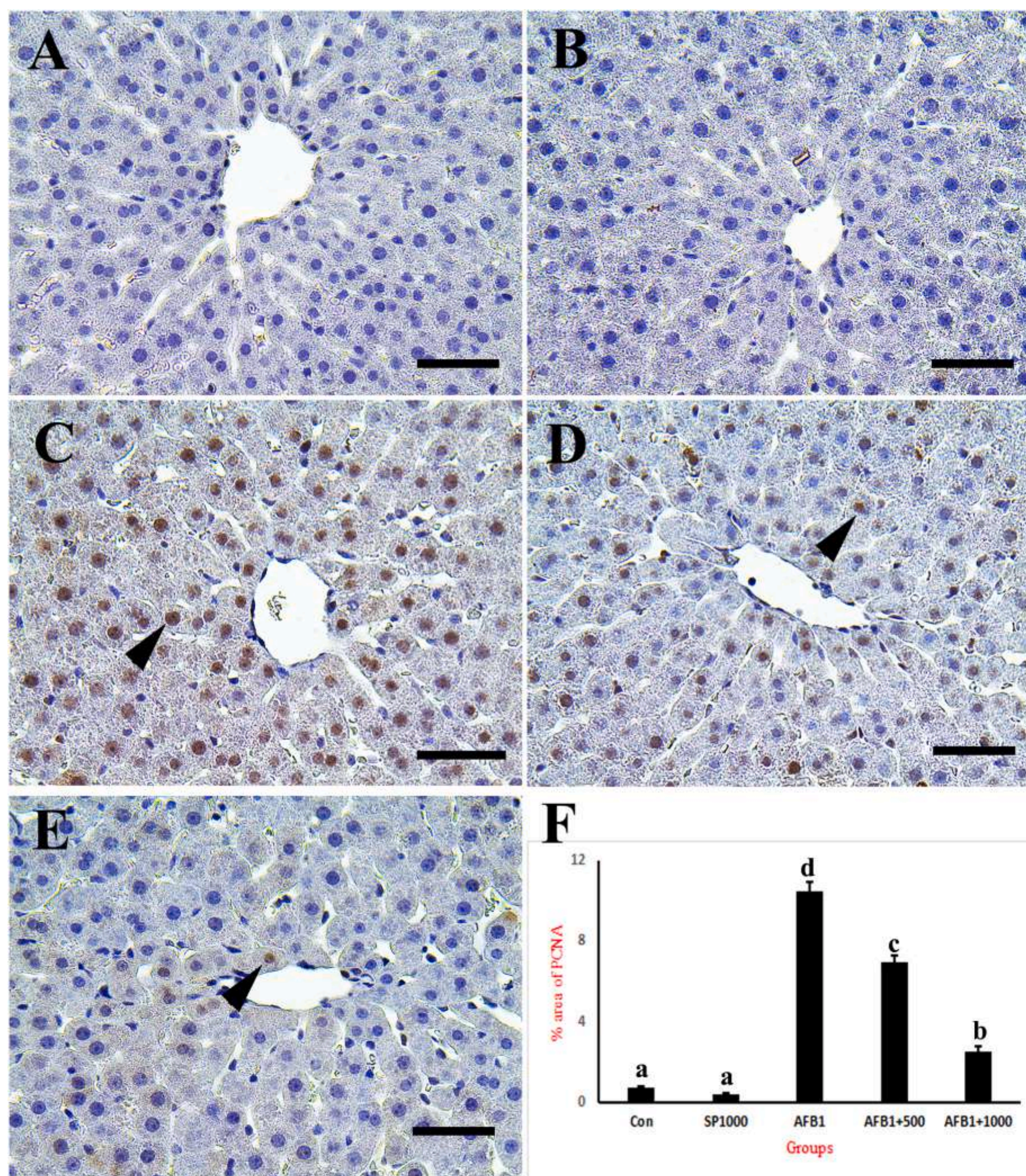

Fig. 4. Proliferating cell nuclear antigen (PCNA) immunohistochemical staining of hepatic tissues. **A)** negative control group. **B)** SP1000 group. **C)** AFB1 group. **D)** AFB1+SP500 group. **E)** AFB1+SP1000. Arrowheads show the PCNA reaction. *Scale bar* =50 μm. **F)** PCNA quantification of various groups' livers. Data displayed as Mean ± SE and analyzed utilizing one-way ANOVA at p ≤ 0.05, *significance compared to control.

initiate lipid peroxidation. The hepatic MDA level was increased in the group injected with 2.5 mg/kg BW of AFB1. On the other hand, SP prevented the effects of AFB1, suggesting the capability of SP to alleviate lipid peroxidation made by AFB1. Therefore, the ROS suppression or the antioxidant activity of SP is pivotal in the prevention of hepatotoxicity caused by AFB1 (Bin-Jumah et al., 2021).

To prevent lipid peroxidation, the body possesses efficacious protection mechanisms through endogenous antioxidant enzymes like SOD, CAT, GSH-Px, and GSH. Thus, the reduced GSH level accompanied by the marked elevation in lipid peroxidation might be because of a marked decrease in the antioxidants' actions (Choi et al., 2010; El-Bahr, 2015) which could be also attributed to the elevation in the utilization of GSH in the elimination of lipid peroxide (Abdel-Wahhab et al., 2015). A similar report was mentioned previously, where Liu et al. (2013) detected marked decreases in SOD, CAT, GPX, and GST. Therefore, co-administration of SP with AFB1 led to the normalization of CAT, SOD, GSH-Px, and GSH levels in a dose-dependent manner in comparison to AFB1-injected rats.

It is well established that glycolysis and glycogen storage is one of the most important hepatic functions. We detected a significant reduction in hepatic glycogen levels in AFB1-exposed rats compared with controls. This decrease in hepatic glycogen has also been revealed by Zhang et al. (2011). Zhang et al. (2011) attributed the reduced glycogen to the accelerated rates of glycolysis and glycogenolysis by AFB1 exposure. Moreover, increased glucose use was suggested as one of the metabolic influences of acute mycotoxin intoxication. In agreement with our

finding, elevated glucose usage was detected as a pivotal metabolic influence resulting from acute intoxication caused by mycotoxicosis (Wu et al., 2022). Furthermore, Zhang et al. (2011) reported the up-regulation of many metabolizing glycogen enzymes like glucose 6-phosphate dehydrogenase after AFB1 intoxication.

Cao et al. (2022) mentioned the destructive effect of AFB1 on DNA by directly connecting guanine residue to AFB1-8,9-epoxide to form AFB1-N7-guanine. Several proofs indicated the possible ROS inclusion in DNA oxidation. So, DNA integrity could be indirectly influenced by AFB1 through AFB1-induced ROS production (Abdel-Daim et al., 2020). Our study confirmed the DNA destruction by AFB1 toxicity which was detected by increased expression of PCNA, the indicator for DNA repair after damage (Strzalka and Ziemienowicz, 2011). Our finding is in agreement with Abdel-Daim et al. (2021) and Li et al. (2019), who detected upregulated PCNA expression in AFB1-intoxicated hepatocytes.

The preventive and antioxidant influences of SP are because of their antioxidant active principles like minerals, carbohydrates, C-phycocyanins, vitamins, β carotene, lipids, and proteins reported in SP (Bortolini et al., 2022). Numerous reports revealed the hepatoprotective influences of SP against xenobiotics, chemicals, and drugs (Gentscheva et al., 2023). Furthermore, the SP antioxidant influences have been mentioned versus sodium fluoride-induced oxidative changes in the offspring of pregnant rats (Banji et al., 2013). Moreover, brain damage caused by lead in newborns was mitigated by SP-fed pregnant rats' (Gargouri et al., 2012). In addition, SP treatment could have a pivotal effect on lowering the toxic influences of cadmium and SP making its preventive influences either direct by the reduction of NO and MDA or indirect through the increased levels of GSH-Px, CAT, SOD, and GSH in the liver (Karadeniz et al., 2009).

Toxic influences of chemicals mainly manifest primarily in the liver tissues because the major different metabolic pathways occur in the liver (Azab et al., 2013). The severe destruction of hepatic architecture and necrotic alterations in AFB1-intoxicated rats were detected in the current investigation suggesting acute hepatic injury. SP-treated animals revealed markedly fewer histological abnormalities such as dysplastic hepatocytes, hydropic degeneration, and periportal fibrosis in comparison with AFB1-intoxicated rats, herewith highlighting its preventive activity in resisting the AFB1 hepatotoxicity (Table 3). Our study outcomes are in harmony with Abdel-Daim et al., (2013), who have mentioned that SP has a hepatoprotective role against deltamethrin-induced hepatotoxicity (Abdel-Daim et al., 2013). Thus, the histopathological findings emphasized the SP prevention against hepatotoxicity caused by AFB1.

5. Conclusion

Our findings revealed that AFB1 induced severe hepatotoxic influences on the rat through activation of oxidative stress pathway and DNA damage. These toxic effects could be prevented by SP at 500 mg/kg BW or 1000 mg/kg BW. Moreover, the hepatoprotective activities of SP could be achieved *via* the improvement of hepatic histoarchitecture and amelioration of oxidative stress and lipid peroxidation markers. Further studies are recommended for more understanding of SP effects on the signaling pathways of AFP1.

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Table 3
Histopathological parameters of liver lesions in control and different treated groups.

	Experimental groups				
Parameters	Control	SP1000	AFB1 control	AFB1-SP500	AFB1-SP1000
Necrosis	0.25 ± 0.16[a]	0.25 ± 0.16[a]	3.75 ± 0.16[b]	2.375 ± 0.18[c]	0.875 ± 0.23[a]
Hemorrhage	0±0[a]	0±0[a]	3.625 ± 0.18[b]	1.875 ± 0.23[c]	0.75 ± 0.25[a]

Data are expressed as means ± SE (n = 8).
AFB; Aflatoxin, SP; *Spirulina platensis*.
Values having different superscripts within the same raw are significantly different (P ≤ 0.05).

Author contributions

Conceptualization, G.M.A, O.A.K, A.E.A, and M.M.A-D; methodology, G.M.A, A.E.N, A.A.S., A.E.A, and M.M.A-D; software, G.M.A, A.E.N, A.A.S., A.E.A, Z.M.M., M.Q.A, N.G. and M.M.A-D; validation, G.M.A, A.E.N, A.A.S., F.M.E, Z.M.M., A.E.A, and M.M.A-D; formal analysis, G.M.A, A.E.N, F.M.E, O.A.K, Z.M.M., A.E.A, and M.M.A-D; investigation, G.M.A, O.A.K, A.E.A, and M.M.A-D.; resources, G.M.A, A.E.A, and M.M.A-D data curation, G.M.A, A.E.A, Z.M.M., M.Q.A, N.G. and M.M.A-D writing—original draft preparation, G.M.A, O.A.K, A.E.A, F.M.E, M.Q.A, N.G. and M.M.A-D.; writing—review and editing, G.M.A, M.P., Z.M.M., A.E.A, and M.M.A-D.; visualization, G.M.A, M.P., A.E.A, Z.M.M., M.Q.A, N.G. and M.M.A-D supervision, F.M.E, M.P., A.E.A, and M.M.A-D project administration, G.M.A, O.A.K, A.E.A, and M.M.A-D; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board statement

The National Institutes of Health (NIH) recommendations were followed in all animal-related investigational steps, and the protocol was approved by the Faculty of Veterinary Medicine, Damanhour University's Ethics of Animal Use Research Committee in Egypt (DMU/VetMed-2023/002).

Informed consent statement

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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