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Hepatoprotective effects of *Silymarin* in experimental aflatoxicosis in broiler chickens

Efectos hepatoprotectores de la Silimarina en la aflatoxicosis experimental en pollos de engorde

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ABSTRACT

Aflatoxicosis, a mycotoxicosis caused by aflatoxins, poses significant economic challenges in animal husbandry and presents serious health risks to humans. The current study aimed to evaluate the protective effects of *Silymarin*, an antioxidant, against aflatoxicosis in broiler chickens. A total of thirty–two broilers were randomly assigned to four experimental groups: control, *Silymarin*–treated, Aflatoxin B_1 (AFB_1)–treated, and AFB_1+*Silymarin*–treated. Broilers in control and *Silymarin*–treated groups were fed with a diet without Aflatoxin. AFB_1 and AFB_1+*Silymarin* treated groups received approximately 1 mg of Aflatoxin/broiler/total for 21 days. AFB_1+*Silymarin* group also received 10 g of *Silymarin* kg^{-1} of food as a supplementation diet for 21 days. Biochemically, liver and serum TAS, SOD, GSH–Px, CAT, and BcL–2 levels in the AFB_1 group were lower than the control, while serum TOS, and serum and liver Caspase–3 levels were high (P<0.001). In addition, in the AFB_1+*Silymarin* group, liver and serum GSH–Px, SOD and BcL–2, and serum CAT values were significantly higher than the AFB_1 group (P<0.001). Also, in the AFB_1+*Silymarin* group, there was a slight increase in CAT level in liver samples compared to the AFB_1 group (P>0.05). Furthermore, TOS and Caspase–3 levels of both serum and liver in the AFB_1+*Silymarin* group were low, while BcL–2 levels were quite high in serum samples compared to the AFB_1 group (P<0.001). Pathologically, the livers were larger and pale in the AFB_1 group. Histopathological analysis of the AFB_1–treated group revealed diffuse hydropic/vacuolar degeneration and fibrosis, characterized by the activation of perisinusoidal myofibroblastic (*Ito*) cells. These morphological changes were significantly reduced in the AFB_1+*Silymarin* group. These findings indicate that *Silymarin*, with its strong antioxidant effect, may be effective against aflatoxicosis in preventing liver damage in broilers.

Key words: Aflatoxicosis; antioxidant; broiler; histopathology; *Silymarin*

RESUMEN

La aflatoxicosis es una micotoxicosis causada por aflatoxinas en animales y humanos. La aflatoxicosis provoca importantes pérdidas económicas en los animales y representa una amenaza para la salud humana. El objetivo de este estudio fue investigar el efecto protector de la Silimarina, con propiedades antioxidantes, contra la aflatoxicosis en pollos de engorde. Treinta y dos pollos de engorde fueron divididos aleatoriamente en cuatro grupos experimentales: control, tratados con Silimarina, tratados con Aflatoxina–B_1 (AFB_1) y tratados con AFB_1+Silimarina. Los pollos en los grupos control y tratados con Silimarina fueron alimentados con una dieta sin aflatoxina. Los grupos AFB_1 y AFB_1+Silimarina recibieron aproximadamente 1 mg de aflatoxina/pollo/total durante veintiún días. El grupo AFB_1+Silimarina también recibió 10 g de Silimarina por kg de alimento como dieta suplementaria durante 21 días. Desde un punto de vista bioquímico, los niveles hepáticos y séricos de TAS, SOD, GSH–Px, CAT y BcL–2 en el grupo AFB_1 fueron inferiores al control, mientras que los niveles séricos de TOS y los niveles de Caspasa–3 en suero e hígado fueron altos (P<0,001). Además, en el grupo AFB_1+Silimarina, los valores de GSH–Px, SOD y BcL–2 en hígado y suero, y los valores de CAT en suero fueron significativamente más altos que en el grupo AFB_1 (P<0,001). También, en el grupo AFB_1+Silimarina, hubo un ligero aumento en el nivel de CAT en las muestras de hígado en comparación con el grupo AFB_1 (P>0,05). Además, los niveles de TOS y Caspasa–3 en suero e hígado en el grupo AFB_1+Silimarina fueron bajos, mientras que los niveles de BcL–2 en suero fueron significativamente más altos en comparación con el grupo AFB_1 (P<0,001). Patológicamente, los hígados eran más grandes y pálidos en el grupo AFB_1. En términos histopatológicos, se observaron degeneración hidrópica/vacuolar difusa y fibrosis caracterizada por la activación de células miofibroblásticas perisinusoidales (células de *Ito*) en el grupo AFB_1. Estos cambios morfológicos se redujeron significativamente en el grupo AFB_1+Silimarina. Estos hallazgos indican que la Silimarina, con su fuerte efecto antioxidante, puede ser eficaz contra la aflatoxicosis en la prevención del daño hepático en pollos de engorde.

Palabras clave: Aflatoxicosis; antioxidantes; pollo de engorde; histopatología; Silimarina

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INTRODUCTION

Aflatoxins are toxic secondary metabolites that were synthesized especially by *Aspergillus* spp. (*Aspergillus flavus, A. parasiticus*) strains. Aflatoxins (B_1, B_2, G_1, and G_2) can be found as contaminants in many animal feeds, especially peanuts and grains. It was demonstrated that Aflatoxin–B_1 (AFB_1) had hepatotoxic, hepatocarcinogenic, mutagenic, and teratogenic effects in many animal species [1, 2]. AFB_1 is metabolized by monooxygenases in the liver and converted to aflatoxin 8,9 epoxide by cytochrome P450 [3]. AFB_1 8,9 epoxide is inactivated by conjugation with glutathione and excreted through urine or bile [4].

Aflatoxicosis particularly affects turkeys (*Dacnis hartlaubi*), quails (*Coturnix coturnix*), ducklings (*Anas platyrhynchos*) and chickens (*Gallus gallus domesticus*) [5]. As a result of broilers taking feed contaminated with AFB_1, AFB_1 reaches the liver, kidney, gizzard, and pectoral muscle [6], while in layer breeds it is transferred to the eggs [7]). It has been reported that aflatoxins cause fatty degeneration of the liver (fatty liver syndrome) in these birds [8]. Aflatoxicosis in chickens is characterised by weakness, loss of appetite, failure to thrive, decrease in feed intake and egg production, fatty liver, death, and increased susceptibility to many diseases [5, 9]. This toxicity is one of the reasons for significant economic losses in broiler production. It is also claimed that the presence of aflatoxin metabolites in poultry tissues eaten by humans may pose a significant public health problem [1, 10]. For this reason, intensive studies are being carried out to reduce the effects of toxins. For this purpose, non–nutritive sorbents such as bentonite and hydrated sodium amoxylate have been added to poultry feed to reduce the effects of aflatoxins [11, 12, 13]. However, since the binding spectra of these substances are broad and non–specific, they have also been reported to remove nutritional components such as vitamins and minerals [14].

Today, current trends further increase the interest in extracts or active compounds obtained from plants for food production [15]. Historically, plants have been employed in the treatment of various diseases in both humans and animals, owing to their diverse bioactive compounds [16]. In recent years, plant–derived compounds have begun to be used more in the poultry industry to get better efficiency and control the toxic effects of aflatoxins [15, 17, 18]. The standardized extract of milk thistle (*Silybum marianum*), a medicinal plant, is *Silymarin*, and it is mostly found in milk thistle seeds. *Silymarin*, a potent antihepatotoxic agent, is widely used in the treatment of liver diseases and as a hepatoprotector in humans. *Silymarin* contains various bioactive compounds such as silybin, silychristin, dehydrosilybin, silidiyanin, and isosilybin. Among these bioactive compounds, the main component is silybin [19]. Studies have reported that *Silymarin* has anti–inflammatory, cytoprotective, hepatoprotective, and anticarcinogenic properties [20, 21].

Studies conducted on broilers showed that adding *Silymarin* to AFB_1 contaminated feed content stimulates the immune system and increases growth performance by reducing the toxic effects of AFB_1 [22]. Also, *Silymarin* phospholipid (*Silymarin* phytosome) complex counteracts the negative and toxic effects of AFB_1 on the broiler's performance provides protection [23], and facilitates the absorption of nutrients and regulates metabolism in the intestines [24]. It has been reported that combining *Silymarin* with AFB_1–contaminated feed in broilers had a macroscopically positive impact on growth rate, carcass quality, and liver morphology, although its histopathological effects on the liver have not been previously investigated [25]. Additionally, it has been reported that adding *Silymarin* to the AFB_1–contaminated diet in broilers has a positive effect on performance by suppressing bacterial proliferation and increasing the absorptive surface area in the ileum [26]. It has been stated that simply adding *Silymarin* to the feed reduces fatty liver in broilers and has a hepatoprotective effect by increasing the glycogen content in hepatocytes [27]. Prior research has largely focused on the protective effects of *Silymarin* against AFB_1 toxicity in terms of biochemical parameters such as catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GSH–Px), glutathione–S–transferase (GST), and malondialdehyde (MDA) [28, 29, 30]. According to the literature, no histopathological studies have explored *Silymarin*'s hepatoprotective potential against AFB_1 toxicity in broilers. The present study evaluated the hepatoprotective effect of *Silymarin* in broilers with aflatoxicosis, specifically examining fatty degeneration, necrosis, and fibrosis in the liver. Also, it biochemically evaluated oxidative stress, total oxidant status (TOS), total antioxidant status (TAS), antioxidant enzymes (SOD, CAT and GSH-Px), and apoptotic parameters (B–cell lymphoma 2 (BcL–2) and Caspase–3), which there are few studies on these parameters in experimental aflatoxicosis.

MATERIALS AND METHODS

Experimental animal grouping and nutrition plan

In this study, 32 one–day–old Avian breed broiler chicks, unvaccinated, were used. Ethical approval was granted by the Van Yuzuncu Yil University Experimental Animals Local Ethics Committee (approval protocol number: 2023–13/22). Chickens with similar body weights (Mettler Toledo, Switzerland) were randomly allocated into four groups of equal size. Initially, all groups received water containing 5% sugar for the first 4–5 hours, followed by 21 days (d) of free access to water and a standard feed diet. The chickens were housed in temperature–controlled compartments (maintained at 22°C) with a 12–hour light/dark cycle.

The experimental groups were as follows:

1. **Control Group (n=8):** Control group (n=8): This group consisted of broilers fed a standard diet throughout the study.
2. **AFB_1 Group (n=8):** Chicks in this group received a diet containing 1 $mg \cdot kg^{-1}$ AFB_1 for 21 d.
3. ***Silymarin* Group (n=8):** Chicks were fed a diet supplemented with 10 $g \cdot kg^{-1}$ *Silymarin* for 21 d.
4. **AFB_1+*Silymarin* Group (n=8):** This group was provided with a diet containing both 1 $mg \cdot kg^{-1}$ AFB_1 and 10 $g \cdot kg^{-1}$ *Silymarin* for 21 d.

Preparation of AFB_1–contaminated feed

Aflatoxin B_1 (1 mg) was mixed into 1 kg of commercial feed that was confirmed to be free of aflatoxins by laboratory analysis.

Histopathological examination

At the end of the study, necropsies were performed, and liver samples were collected as the liver is the primary organ affected by aflatoxin toxicity. Macroscopic changes were recorded. Liver tissues were fixed in 10% formalin, embedded in paraffin, sectioned to a thickness of 4 μm using a rotary microtome (Leica RM2135, Germany), stained with hematoxylin–eosin (HE), and examined under a light microscope (Nikon 80i–DSRI2, Made in Japon), with images captured for documentation. Hepatocellular degeneration was microscopically evaluated and classified into three categories. In the mild grade, mild hepatocellular swelling and fatty changes were observed, primarily localized around the periacinar areas. The moderate grade was characterized by noticeable hepatocellular swelling in both the periacinar and midzonal regions. In the severe grade, diffuse and extensive hepatocellular swelling, accompanied by cytoplasmic pallor and rupture, was observed throughout the liver tissue [31].

Serum samples collection

At the end of the experimental period, blood samples were drawn intracardially from anaesthetized chicks and placed in biochemistry tubes. To obtain serum, these samples were centrifuged (Centurion Scientific K3 Series, UK) at 3000 g at 4°C for 15 min which was then stored for biochemical analysis.

Liver tissue homogenization and supernatant preparation

Phosphate buffer (50 mM, pH 7.4) was added to liver tissues (200 mg), homogenized (Ultra Turrax–T25, IKA Turkey Laboratory and Process Technologies Inc.) for 15 s, and centrifuged at 3000 g at 4°C for 30 min. The supernatant was collected and stored at -80°C (Qingdao Antech Scientific, NDF–86U588T, Made in China) for subsequent analysis.

Biochemical analysis

Levels of glutathione peroxidase (GSH–Px), superoxide dismutase (SOD), total oxidant status (TOS), catalase (CAT), total antioxidant status (TAS), B–cell lymphoma 2 (BcL–2), and Caspase–3 were measured in serum and liver homogenates using ELISA kits from YL Biotech Co., Ltd. (Shanghai, China), following the manufacturer's protocols.

Statistical analysis

Statistical analyses were performed using SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation. Group differences were analyzed using one–way ANOVA, and post–hoc comparisons were made with the Tukey HSD test. A *P*–value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Gross pathological examination revealed pale, yellow–discolored, enlarged, delicate, and rounded livers in the AFB_1–treated group (FIG. 1–C), in contrast to the normal liver appearances in the control and *Silymarin* groups (FIG. 1–A, B). However, the livers of broilers in the AFB_1+*Silymarin* group appeared nearly normal, similar to the control group (FIG. 1–D).

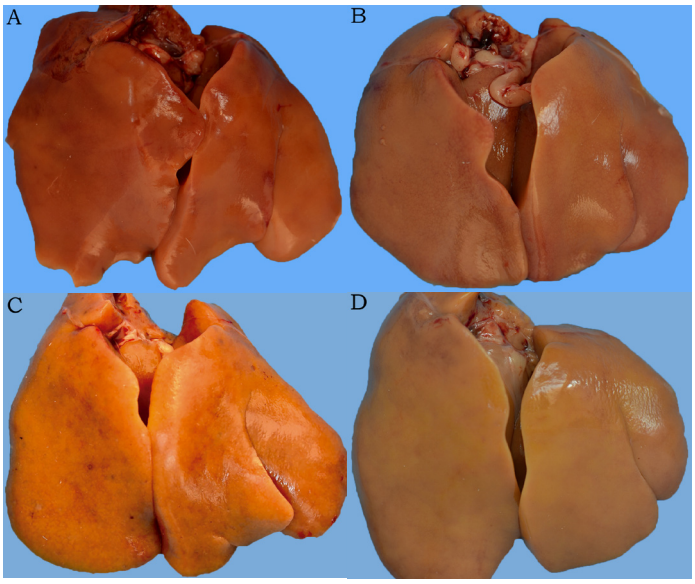

FIGURE 1. The normal view of the broiler liver of the (A) control and (B) *Silymarin* group. (C) AFB_1 group has a distinct yellowish colour change (hydropic/fat degeneration) in the broiler liver. (D) AFB_1+*Silymarin* group shows almost the normal appearance of the broiler liver

Livers in the control and *Silymarin* groups were found to have normal histologic appearance (FIG. 2–A). Microscopic examination of the liver in the AFB_1 group revealed that the most remarkable basic morphological changes were degeneration and fibrosis. These lesions were mostly hydropic/vacuolar and to a lesser extent, fatty degeneration of hepatocytes, and these changes were especially prominent in centrilobular regions. Fibrosis, indicated by the activation of perisinusoidal myofibroblastic (*Ito*) cells, was prominent in the liver parenchyma of the AFB_1 group. Additionally, focal–disseminated heterophil and mononuclear cell infiltration, as well as bile duct proliferation, were observed in the parenchyma. These morphological changes led to the dissociation of hepatic cords and the narrowing and obstruction of sinusoids. The less frequent observed lesions were perivascular infiltration, multifocal haemorrhages, and necrotic foci (FIG. 2–B and C). Additionally, a large number of proliferated small bile ducts were noticed around the vena porta (FIG. 3). In the AFB_1+*Silymarin* group, however, it was noted that these morphological changes were very distinctly mild (FIG. 2–D).

Exposure to Aflatoxin B_1 (AFB_1) in broiler chickens has been linked to considerable liver damage, including the disruption of hepatic lobule structure, hydropic and fatty degeneration, and compromised hepatocellular membrane integrity, as noted in earlier studies [32, 33]. Aflatoxicosis has also been macroscopically linked to liver changes such as pallor, enlargement, and congestion [25]. In the current study, similar pathological changes were observed in the livers of broilers treated with AFB_1. However, in the group receiving both AFB_1 and *Silymarin*, the liver morphology was nearly identical to that of the control group. These findings align with previous research [33, 34], supporting the conclusion that *Silymarin*, when included in the diet, effectively protects the liver from AFB_1–induced damage, likely due to its potent antioxidant properties.

A comparison of histopathological lesions between groups is provided in TABLE I, with the severity of morphological changes graded as mild (*), moderate (**), or severe (***).

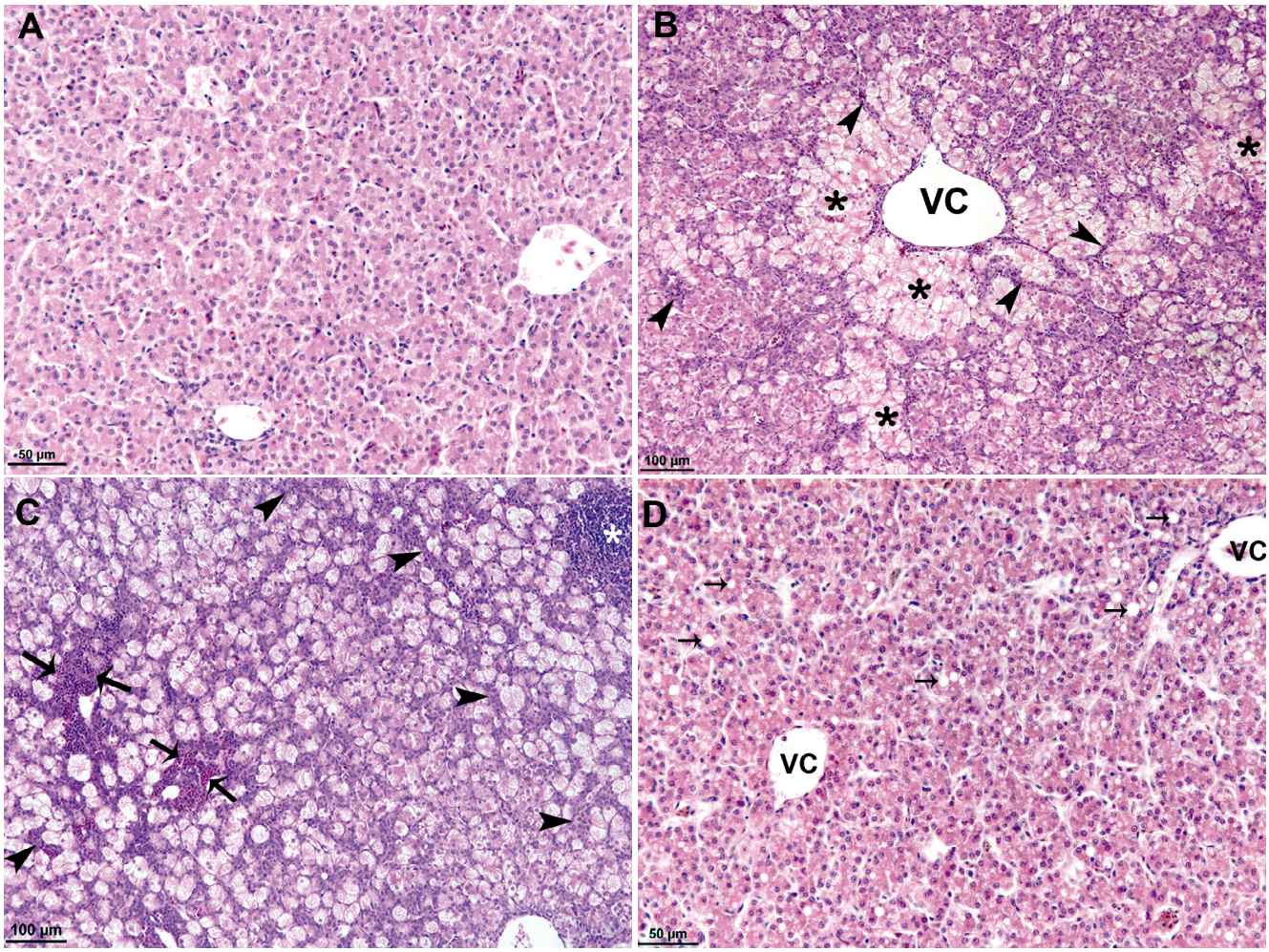

FIGURE 2. Composite representation of histological liver images across experimental groups. (A) The control group exhibits normal liver histology (H&E stain, scale bar: 50 µm). (B) The AFB_1 group shows widespread, severe hydropic degeneration (stars) in hepatocytes, particularly in the periacinar (vc) regions, along with marked activation of perisinusoidal cells (arrowheads) (H&E stain, scale bar: 100 µm). (C) Liver tissue from the AFB_1 group also demonstrates fibrotic changes extending in thin strands (arrowheads) throughout the parenchyma, in addition to perisinusoidal cell activation, parenchymal mononuclear cell aggregation (arrows), heterophil infiltration (star), and widespread severe hydropic degeneration in hepatocytes (H&E stain, scale bar: 100 µm). (D) In the AFB_1+*Silymarin* group, no significant widespread degeneration of hepatocytes is observed, aside from vacuolar degeneration (arrows) in some hepatocytes, while perisinusoidal cell activation remains evident (H&E stain, scale bar: 50 µm)

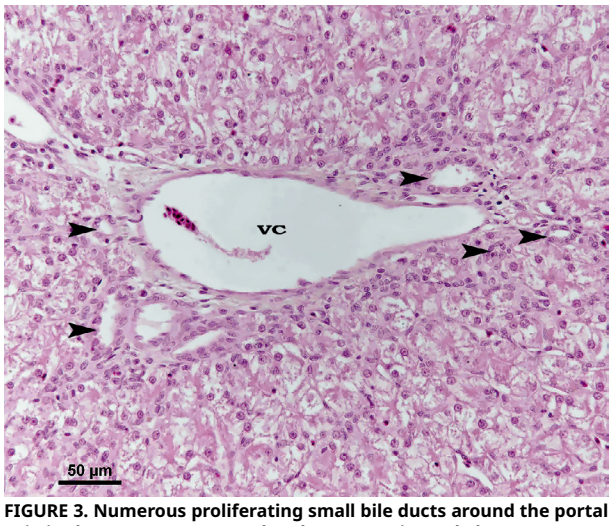

FIGURE 3. Numerous proliferating small bile ducts around the portal vein in the AFB_1 group (arrowheads) (H&E stain, scale bar: 50 µm)

TABLE I
Effects of *Silymarin* on the AFB1 – induced liver injury (affected broiler/total number of broiler)

Liver lesions	Control	AFB1	*Silymarin*	AFB1+ *Silymarin*	P Values
Degeneration	–/8[b]	8/8[a]	–/8[b]	5/8[a]	
Mild	–	0	–	5	**
Moderate	–	2	–	–	
Severe	–	6	–	–	
Fibrosis	–/8[b]	8/8[a]	–/8[b]	3/8[ab]	
Mild	–	0	–	3	**
Moderate	–	3	–	–	
Severe	–	5	–	–	
Inflammatory cell infiltration	–/8[b]	8/8[a]	–/8[b]	3/8[b]	
Mild	–	0	–	3	**
Moderate	–	1	–	–	
Severe	–	7	–	–	
Bile duct proliferation	–/8[b]	5/8[a]	–/8[b]	–/8[b]	
Mild	–	5	–	–	**
Moderate	–	–	–	–	
Severe	–	–	–	–	

[a, b:] Values marked by different letters on the same row indicate statistically significant differences. $^{**}P<0.01$

In aflatoxicosis, hepatocellular degeneration has been localized primarily in the periacinar regions, with bile duct hyperplasia occurring in portal areas [34, 35]. It was noteworthy that similar lesions were observed in this study.

In liver fibrosis, *Ito* cells in the space of Disse undergo myofibroblastic transformation, proliferate, and secrete collagen, leading to fibrosis as a result of parenchymal damage [36]. *Ito* cell activation in response to aflatoxicosis has been previously reported [37], and this study observed significant activation of perisinusoidal cells in the AFB_1–treated group, whereas such activation was not significant in the AFB_1+*Silymarin* group. It is thus suggested that the bioactive components of *Silymarin* may prevent degenerative–necrotic changes and fibrosis caused by AFB_1.

TAS levels in serum samples and liver homogenates from the AFB_1 group were significantly lower than in the control and *Silymarin* groups, while TOS levels were substantially higher ($P \leq 0.019$). However, in the AFB_1+*Silymarin* group, TAS levels were higher and TOS levels were lower compared to the AFB_1 group ($P \leq 0.019$), (FIG. 4). Antioxidant enzyme levels, including SOD, GSH–Px, and CAT, were significantly reduced in the liver homogenates and serum samples of the AFB_1 group compared to the control and *Silymarin* groups ($P \leq 0.022$). Nevertheless, these values were restored in the AFB_1+*Silymarin* group ($P \leq 0.019$), (FIG. 4).

In terms of apoptosis markers, Caspase–3 levels in both serum and liver were elevated in the AFB_1 group compared to the control and *Silymarin* groups, while serum BcL–2 levels were lower ($P \leq 0.004$). However, interestingly, liver BcL–2 values in the AFB_1 group were increased according to the control, *Silymarin,* and partially AFB_1+*Silymarin* group ($P \leq 0.004$). In the AFB_1+*Silymarin* group, Caspase–3 levels were partially reduced, and BcL–2 levels were partially restored ($P \leq 0.004$), (FIG. 5).

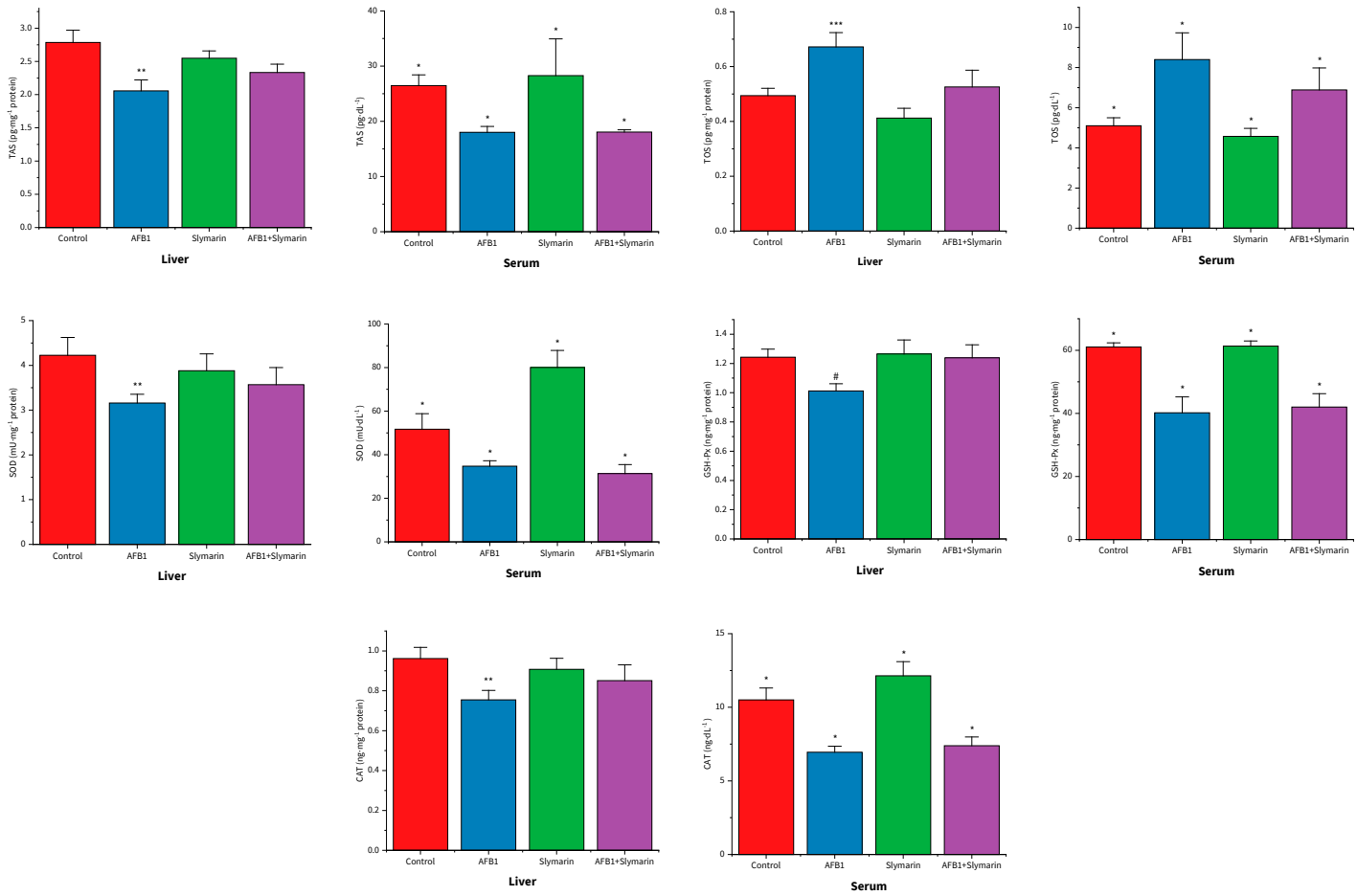

FIGURE 4. Comparison of antioxidant capacity and antioxidant enzyme levels in the liver and serum samples. TAS: total antioxidant capacity, TOS: total oxidative stress, GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase, GSH–Px: glutathione peroxidase. ****P*: It is significant compared to other experimental groups. *****P*: It is lower than the control group. ******p*: It is significant according to the control and *Silymarin* groups. #*P*: It is lower than the control and aflatoxin+*Silymarin* groups

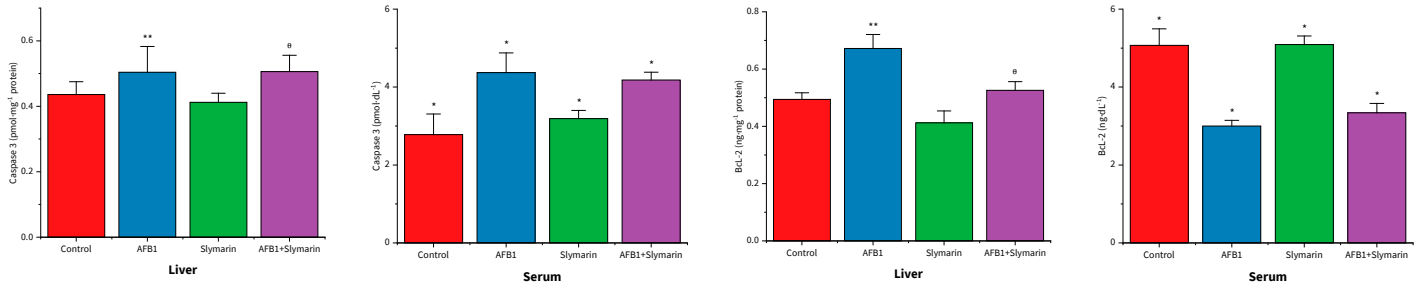

FIGURE 5. Comparison of Caspase–3 and BcL–2 levels in the liver and serum samples. **P*: It is higher than the other experimental groups. ***P*: It is significant according to the control and *Silymarin* groups. $^{\theta}$*P*: It is higher than the *Silymarin* group

AFB_1 toxicity manifests in acute or chronic forms depending on species, age, gender, dosage, duration of exposure, metabolism, and overall health and nutrition [38]. AFB_1 is recognized for its toxic, mutagenic, carcinogenic, and genotoxic effects [39, 40]. In poultry, these effects result in biochemical, hematological, reproductive, and pathological alterations, as well as mortality [41, 42].

Aflatoxins are metabolized in the liver in two phases. Initially, AFB_1 is metabolized by cytochrome P450 (CYP450) enzymes into reactive metabolites such as aflatoxin B1 8,9–epoxide. In the second phase, both the parent compound and its toxic metabolite, aflatoxin B1 8,9–epoxide, are conjugated with glutathione, glucuronides, and sulfonides and subsequently excreted via urine [43].

Oxidative stress occurs when the capacity of antioxidant enzymes (SOD, GSH–Px, CAT, GST) and non–enzymatic antioxidants (GSH, vitamin C, etc.) is overwhelmed, resulting in the accumulation of reactive oxygen species (ROS) [44]. AFB_1 exposure has been linked to the generation of ROS, such as hydroxyl radicals and hydrogen peroxide, under both in vitro and in vivo conditions [45, 46], leading to an imbalance between oxidant and antioxidant systems.

Oxidative stress in the liver, induced by dietary AFB_1, is characterized by elevated levels of nitric oxide and hepatic lipid peroxidation (LPO) products in the serum, alongside decreased levels of hepatic antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) [32]. On a molecular level, AFB_1 has been shown to downregulate the expression of CAT, glutathione peroxidase (GSH–Px), and glutathione S–transferase (GST) mRNA, while upregulating inducible nitric oxide synthase (NOS) protein expression [43].

In this study, a significant increase in TOS levels, indicative of oxidative stress, was observed in AFB_1–treated broilers. However, in the AFB_1+*Silymarin* group, TOS levels in both serum and liver homogenates were restored to levels approaching those of the control group. This suggests that *Silymarin* may alleviate oxidative stress induced by AFB_1 exposure.

Additionally, in this study, in parallel with the above studies, GSH–Px and CAT values of the liver homogenate and serum samples in the AFB_1 group were low dramatically compared to the control and *Silymarin* groups. However, serum SOD, serum and liver GSH–Px, and liver CAT values were significantly restored and increased in the AFB_1+*Silymarin* group compared to the AFB_1 group. Additionally, TAS levels, an indicator of total antioxidant capacity, were lower in the AFB_1 group than in the control and *Silymarin* groups. These results revealed that aflatoxin toxicity caused a decrease in antioxidant enzyme levels and total antioxidant status, however, *Silymarin* application could significantly restore these parameter values.

AFB_1–induced hepatocellular apoptosis has been associated with the upregulation of pro–apoptotic proteins such as p53, Caspase–3, and Bax. These molecular alterations correlate with the morphological changes observed in the liver [6]. AFB_1–induced liver damage appears to involve multiple mechanisms, including oxidative stress, inflammation, apoptosis, disruption of hepatic lobule structure, impaired autophagy, and altered macromolecule metabolism [47].

For this reason, in this study, we investigated how oxidative stress, antioxidant enzymes and total antioxidant status, as well as Caspase–3 and BcL–2 levels, which are markers of apoptosis, change with the use of *Silymarin* along with AFB_1. In this study, serum and liver caspase 3 values in the AFB_1 group were higher than in the control and *Silymarin* groups, while serum BcL–2 values were lower ($P \leq 0.004$). However, interestingly, liver BcL–2 values in the AFB_1 group were increased according to the control, *Silymarin* and partially AFB_1+*Silymarin* group ($P \leq 0.004$). In addition, while serum caspase 3 values in the AFB_1+*Silymarin* group were partially lower than in the AFB_1 group, BcL–2 values were partially restored and increased ($P \leq 0.004$). This result shows that AFB_1 administration increased caspase 3 levels in both serum and liver tissue, whereas *Silymarin* restored Caspase–3 levels and brought them closer to the control group.

CONCLUSION

The results of this study demonstrate that AFB_1 exposure increased oxidative stress, elevated Caspase–3 levels, reduced antioxidant enzyme levels, and caused significant liver damage, including pallor, enlargement, congestion, and myofibroblastic activation. However, *Silymarin* supplementation mitigated these effects, restoring antioxidant enzyme activity and reducing histopathological damage in the liver. Therefore, it was shown that *Silymarin* used together with AFB_1 may play a hepatoprotective role against the negative effects caused by AFB_1 in the liver. However, to confirm these results, new studies at the molecular level are needed on the effects of using *Silymarin* together with AFB_1 on the liver.

Conflicts of interest

The authors declare no conflicts of interest.

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