Research Article

Ameliorative Effect of GSPE Against AFB_1 Induced Immunotoxicity and Hepatotoxicity in Japanese Quail

Maha Khalil[1], Gamal Shams[2], Hosny Abdel Fadil[2], Nagah Edrees[2], Mostafa Abonorag[1], Nasser El-Sabbagh[3], Eman A. Ahmed[4]*

[1]Animal Health Research Institute, Agriculture Research Center, Ismailia 41511, Egypt; [2]Pharmacology department, Faculty of Veterinary Medicine, Zagazig University, Egypt; [3]Pharmacology department, Faculty of Veterinary Medicine, Alexandria University, Egypt; [4]Pharmacology department, Faculty of Veterinary Medicine, Suez Canal University, 41522 Ismailia, Egypt.

Abstract | This study was conducted to assess the ability of grape seed proanthocyanidin extract (GSPE) to alleviate the detrimental effects triggered by aflatoxin B_1 in Japanese quail. Four groups of Japanese quail birds were fed either a basal diet (control), basal + 1mg/kg AFB_1 (AFB_1 diet), basal+ 500 mg/kg GSPE (GSPE diet), or basal+ 1mg/kg AFB_1+ 500 mg/kg GSPE (AFB_1+GSPE diet) for 5 weeks. Biochemical parameters, lipid peroxidation, pro-inflammatory cytokines, and histopathological investigations were assessed. GSPE supplementation alleviated AFB_1-induced hepatotoxicity as reflected in diminishing alanine transaminase, aspartate aminotransferase, alkaline phosphatase, lipid peroxidation, and raising TNF-α and IL-6 as pro-inflammatory cytokines. Moreover, elevating the reduced levels of glutathione peroxidase, catalase, and superoxide dismutase were reported. Conclusively, the results of our experiment suggest that that GSPE is a good feed additive for alleviating the negative impacts induced by aflatoxin B_1.

Keywords | Aflatoxin B_1, Hepatotoxicity, Liver apoptosis, Proanthocyanidin, Quails

Received | October 03, 2021; **Accepted** | December 30, 2021; **Published** | March 24, 2022
***Correspondence** | Eman Ahmed, Pharmacology department, Faculty of Veterinary Medicine, Suez Canal University, 41522 Ismailia, Egypt; **Email:** eman_ahmed@vet.suez.edu.eg
Citation | Khalil M, Shams G, Fadil HA, Edrees N, Abonorag M, El-Sabbagh E, Ahmed EA (2022). Ameliorative effect of GSPE against AFB_1-induced immunotoxicity and hepatotoxicity in Japanese quail. Adv. Anim. Vet. Sci. 10(4):898-904.
DOI | https://dx.doi.org/10.17582/journal.aavs/2022/10.4.898.904
ISSN (Online) | 2307-8316

INTRODUCTION

Mycotoxins, mostly aflatoxins (AF), immensely threaten human and animal health alike because of their teratogenic, carcinogenic, mutagenic, and immunosuppressive effects resulting from consumption of foods of animal origin such as eggs, milk, meat, cheese, with toxic residues (Nazhand et al., 2020). Moreover, AF exposure has been reported to elicit impaired productive performance, hepato-renal injury, or even mortality, paving the way for a substantial economic loss (Guo et al., 2021; Saleemi et al., 2020). Several studies thus far have linked AFB_1 with liver damage via oxidative stress, cell apoptosis, and inflammatory response (Limaye et al., 2018; Wang et al., 2019). Recently, researchers have evinced growing interest in plant-derived natural agents as low toxic and potential therapeutic approaches. GSPE has appropriate antioxidant efficacy and free radical capturing amplitude (Abdou et al., 2021; Hussein et al., 2020). GSPE has a prophylactic effect on the cellular membrane versus oxidative damage, and as a consequence, it prohibits hepatic lipid peroxidation (Deng et al., 2020). Not to mention anti-microbial activity, GSPE has been indicated to have anti-cancer, a cardio-protective, anti-inflammatory, and hepatoprotective effect, antidiabetic effect, neuroprotective effect, anti-obesity, as well as lipid-lowering activities (Liu

et al., 2016; Othman et al., 2020). Noteworthy, GSPE has proven to ameliorate oxidative stress and liver injury in the zearalenone-intoxicated liver (Long et al., 2016a; Taranu et al., 2020). Furthermore, it was reported that the AFB_1-triggered toxicity in pigs could be mitigated by dietary grape seed meal through retrieving the inflammatory markers and oxidative status of AFB_1-treated animals (Taranu et al., 2019). It prevents ROS-induced DNA damage and provides a potential protective effect against oxidative stress and free radical-induced pathological conditions (Liu et al., 2016; Sheng et al., 2020). Therefore, this study evaluate the protective impact of GSPE as an active additive in counteracting the deleterious effects of AFB_1 in quails that are exposed to AFB_1-intoxicated diets regarding biochemical, lipid peroxidation, pro-inflammatory cytokines, and histopathological parameters.

MATERIALS AND METHODS

Ethical approval

Study experimental protocol was conducted according to Local Experimental Animal Care Committee and approved by the ethics of the institutional committee of the Faculty of Veterinary medicine, Zagazig University, Egypt.

Chemicals

Aflatoxin B_1 is obtained from Animal Health Research Institute and added to the diet to get a concentration of 1 mg/kg diet (Rajput et al., 2017). GSPE is purchased from Green and healthy company at a dose of (500 mg/kg) (Rajput et al., 2019).

Animals and dietary treatments

A total of sixty (14 days-old) Japanese quail (*Coturnix japonica*) were obtained from a local hatchery. With free access to feed and water. Post 7 days of acclimatization, birds (average body weight = 128.56 g) were housed in stainless steel cages and randomly allotted to four treatment groups; 15 birds each. The 15 birds were alocated into three replicates of five birds per replica. A basal diet was formulated in order to meet the nutritional needs of Japanese quails (NRC, 1994; Table 1). The treatments were as the follows: The control group; diet free from feed additive or AFB_1, AFB_1 group; 1 mg/kg diet, GSPE group; 500 mg/kg diet and the 4th group fed diet containing aflatoxin and GSPE (500 mg/kg). Supplementation was continued for 5 weeks and the parameters for growth performance were recorded for all groups, taking into account body feed intake, weight gain, and weekly weight gain and feed conversion ratio (FCR).

Collection of samples and measurements

At the end of the trial, nine quails per group (3 from each replicate) were randomly selected, and blood samples were collected in a serum-separating tube. Samples were centrifuged for 15 min at 3000 ×g and kept for biochemical analyses. The liver was washed with normal saline and directed for oxidative biomarkers estimation and histological examination.

Serum biochemical and pro-inflammatory cytokines analysis

Serum samples were used for assessment of Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities. In addition, uric acid and creatinine analyses were performed to assess renal function. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were also analyzed using Enzyme-linked Immunosorbent Assay (ELISA) kits. All procedures were performed using commercial kits according to the manufacturer's instructions (CUSABIO BIOTECH CO. Ltd., Houston, TX 77054, USA)

Table 1: Ingredients and nutrient composition of the experimental diets for quail.

Ingredients	%
Yellow corn (8.9%)*	56.81
Soybean meal (44.1%)*	33.20
Corn gluten meal (62%)*	6.90
Dicap	0.86
Limestone	1.35
Lysine	0.19
Methionine	0.09
Salt	0.30
Premix	0.30
Calculated composition	
Crude protein (%)	24
ME (Kcal kg-1)	2900
Calorie/protein	120.8

*Determined values of crude protein %. #High mix premix, each 3 kg provide: Vitamin A - 12 mIU, vitamin D3-2 mIU, vitamin E - 1000 mg, vitamin K3-1000 mg, vitamin B1-1000 mg, vitamin B2-5000 mg, vitamin B6-1500 mg, vitamin - B12 10 mg, biotin - 50 mg, pantothenic acid - 10,00 mg, nicotinic acid - 30,000 mg, folic acid - 1000 mg, manganese - 60,000 mg, zinc - 50,000 mg, iron - 30,000 mg, copper - 4000 mg, iodine - 300 mg, selenium - 100 mg, cobalt - 100 mg, carrier ($CaCO_3$) to 3 kg.

Oxidative stress markers evaluation

Malondialdehyde concentration (MDA) and Superoxide Dismutase activities (SOD), and GSH-Px, and Catalase content (CAT) were measured for estimation of antioxidant activity in liver tissues, using commercial kits (CUSABIO BIOTECH CO. Ltd., Houston, TX 77054, USA) following the assay kits instructions.

Histopathological analysis

Dissected and cleaned liver samples fixed in 10% neutralized buffered formalin, dehydrated in different alcohol percentages, cleared in xylene, and finally made as paraffin blocks. Tissue section (5 µm) stained with hematoxylin-eosin (H and E) for demonstration of histopathological alterations.

Statistical analysis

The statistical analysis was performed using the GraphPad Prism software (version 9, San Diego, USA). Kruskal Wallis was used to evaluate the difference between groups. Dunn's multiple comparisons test as a Post hoc test was carried. The differences were significant when adjusted $p < 0.05$. The results are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Growth performance

The results of productive performance are summarized in Table 2. During the experiment, the birds that received AFB_1 diet (1 mg/kg) showed significantly lower body weight and weight gain than other birds. The adverse signs of AFB1 were mitigated by including 500 mg/kg of GSPE into AFB1-contaminated diets compared with the birds fed AFB1 alone. Besides, FCR of birds was adversely affected by AFB_1 along the trial ($p < 0.05$). Adding 500 mg/kg of GSPE caused marked improvement in FCR ($p < 0.05$) in AFB_1 fed group. Remarkably, GSPE alone has no effect on the performance of quails.

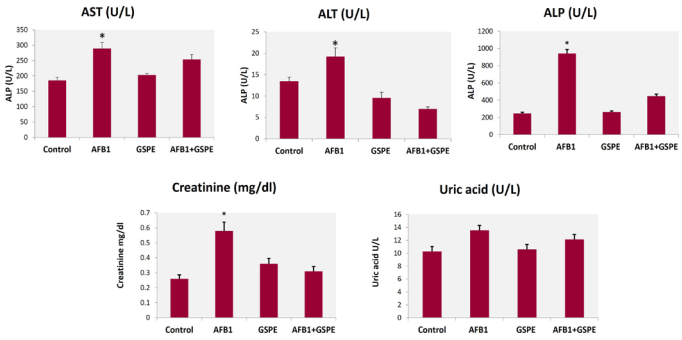

Figure 1: Effect of GSPE on quails' liver and kidney functions of fed diets contaminated with AFB1. Values are represented as the mean±SE (n=9). Means within the column with different stars are significantly different, $p<0.05$

Serum biochemical indices

As shown in Figure 1, birds fed the AFB_1 diet recorded a significant increase in serum ALP and AST activities ($p < 0.05$). GSPE supplementation could mitigate AFB_1-triggered liver damage by decreasing the ALT, AST, and ALP levels significantly. Creatinine level was elevated in AFB1 group in comparison to the control group ($p < 0.05$), whereas they were reserved in (GSPE+ AFB1) birds concerning the AFB1group ($p < 0.05$) (Figure 1).

Serum inflammatory cytokines

The inflammatory activity is estimated by the production of pro-inflammatory cytokine in serum, as shown in Figure 2. Notably, AFB_1 treatment significantly increased serum IL- 6 and TNF-α levels ($P < 0.05$), whereas GSPE supplementation decreased the levels of these two inflammatory markers in comparison with the AFB_1 birds ($P < 0.05$). The results proved that GSPE is able to mitigate the inflammatory response induced by AFB_1.

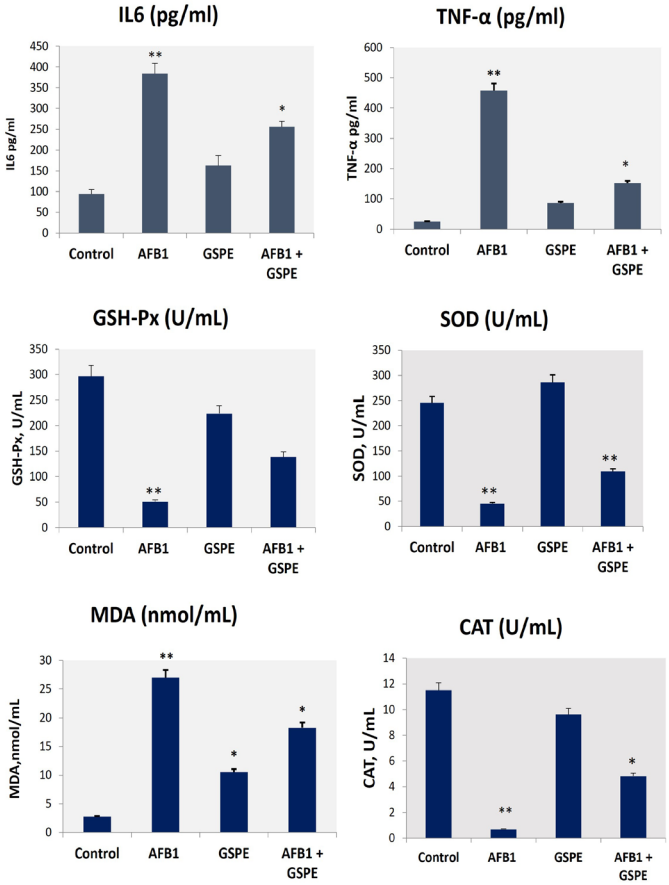

Figure 2: Effects on AFB1-induced liver oxidative stress markers and serum inflammatory cytokines. Values are represented as the mean ± SE (n = 9). *column with different superscript letters were significantly different ($p < 0.05$). AFB1, aflatoxin B1); GSPE, grape seed proanthocyanidin; SOD, total superoxide dismutase; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; CAT, catalase, IL6, interleukin-6; and TNF-α, Tumor necrosis factor-alpha.

Liver oxidative stress biomarkers

The effects of GSPE on hepatic antioxidant parameters of quails challenged with aflatoxin are presented in Figure 2. Data showed that AFB_1 administration increased liver Malondialdehyde (MDA) content ($p < 0.05$), this content of MDA was almost restored in AFB1+GSPE group in comparison to the control level. The scavenging ability of antioxidant enzymes (SOD, GPx, and CAT) were decreased following exposure of quails to AFB_1 ($P > 0.05$). Whereas GSPE counteracts this effect in the liver of AFB1

Table 2: Effects of grape seed proanthocyanidin extract (GSPE) on growth performance of quails fed AFB1contaminated diet.

FCR (%)	Feed intake (g)	Weight gain (g)	Final weight (g)	Initial weight (g)	Groups
1.49± 0.821	273.92±12.40	184.67±10.13	307.56±9.19	122.89±5.40	G1 (CON)
1.71±0.100*	270.35±5.91	156.67± 5.37*	287. 33±7.58*	130.67±5.08	G2 (AFB1)
1.34±0.095	271.51±4.93	204.89±6.87	329.56±5.56	124.67±3.28	G3 (GSPE)
1.41±0.945	280.1± 6.13	199.78±7.55	330.67±4.56	130.89 ± 5.70	G4 (AFB1+GSPE)

Values are represented as the mean ± SD (n = 9). * Mean values within a column were significantly different (p < 0.05). AFB1, aflatoxin B1; GSPE, grape seed proanthocyanidin extract; FCR, feed conversion ratio.

treated group (P > 0.05). Co-administration of GSPE and AFB_1 relieved AFB_1-induced oxidative stress and lowered the MDA level.

Histopathological changes in liver

Microscopic examination showed no histopathological alterations in liver sections of control and GSPE group as presented in Figure 3. In contrast, AFB_1 treatment-induced noticeable pathological alterations in hepatic tissues, such as marked fibrosis of portal triad area associated with focal dilatation of hepatic sinusoids, compared to the control group. Markedly, results clarified that the addition of GSPE (500 mg/kg) to AFB_1-challenged diets alleviated liver injury elicited by AFB_1. Whereas slight hydropic/fatty degeneration was noticed in the liver of GSPE + AFB_1 group. Therefore, administration of GSPE (500mg/kg) can partially adverse AFB_1 effect.

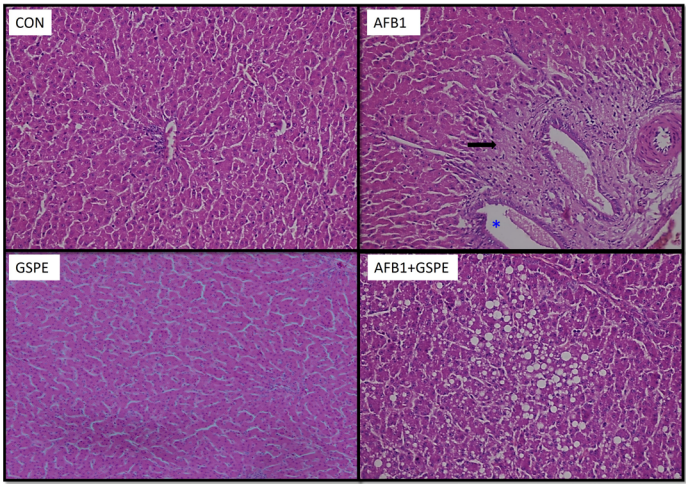

Figure 3: Photomicrographs of liver sections from quails in the different groups: Con, control quails showing normal hepatocytes arranged in plates radiating from the central vein and separated by blood sinusoids; AFB1, quails treated with aflatoxins alone showing marked fibrosis of portal area (→) associated with focal dilation of hepatic sinusoids; GSPE, quails treated with GSPE revealing normal hepatocytes and D, quails treated with (AF+GSPE) showing large intra-hepatic fat droplets.

The present study was designed with the aim of assessing the ability of GSPE to alleviate the negative effects of AFB_1 on growth performance, antioxidant capacity, biochemical parameters, and liver injury evoked by aflatoxin. In this study, after five weeks, AFB_1 administration (1mg/kg) significantly decreased the AWG and increase the FCR (p < 0.05). On the other hand, AFB1did not influence the daily feed intake along with the experiment. Overall, the results obtained regarding reduced final body weight and weight gain observed in quail chicks fed on AFB_1 alone also accords with the earlier reported findings by (Mahrose et al., 2021) Previously, broilers exposure to AFB1 (0.5 mg/kg) significantly reduced weight gain and increase FCR and in turn economic losses (Rashidi et al., 2020). In another research, ducks fed an AFB_1-contaminated diet revealed a significant diminished average daily gain and FCR (Han et al., 2008). The possible explanation for these results might be ascribed to the adverse effects of AFB_1 on the metabolism of protein, lipid, carbohydraten, and the pancreatic enzymes activity, as well as bile acid concentration (Tessari et al., 2010). Previous results are in contrast to earlier findings reported by Manafi and Khosravinia (2013), who found that broilers fed diet contaminated by AFB_1 (500 µg of /Kg) had no significant impact on weight gain along with the whole experiment (8 weeks) and no mortality was observed. This negative impact of AFB_1 on growth performance could be alleviated by the addition of the GSPE. These results align with previous studies that revealed that supplementation of GSPE to AFB1-fed birds could improve growth performance compared to the AFB1-fed birds (Long et al., 2016c; Rajput et al., 2017).

The outcomes of determination of biochemical indices showed an elevation in serum ALT, AST, ALP, uric acid, and creatinine after the exposure of quails to AFB_1. Increased AST, ALT, and ALP activities serve as diagnostic biomarkers of liver injury (Hussain et al., 2016). Additionally, elevated levels of uric acid and creatinine could indicate impairment of protein catabolism and renal impairment (Abdel-Wahhab and Aly, 2005). In sum, these findings support the idea of (Ismail et al., 2020), who reported that AFB_1 feeding exhibited deleterious influences on the liver and kidney function in rabbits and broiler chickens. Conversely, quails received AFB_1+GSPE diet (500 mg/kg) showed marked decreased in serum AST, ALT, and ALP as compared to the AFB_1 birds.

These findings match those observed in earlier studies by Rajput et al. (2017), who reported that AFB_1 provoked hepatic damage while GSPE administration alleviated these adverse effects. Besides, (Deng et al., 2020) reported that ProcyanidinB2 treatment could partially mitigate the acute liver injury triggered by AFB_1.

MDA regarded as a key marker that measured for determining the level of lipid peroxidation and cellular damage (Li et al., 2021). In addition, SOD, GSH-PX, and CAT act as pivotal endogenous elements in the antioxidant defense system. These markers play a crucial role in intracellular redox balance through scavenging the free radicals (Deng et al., 2020). In our experiment, AFB1 inducing oxidative stress in the liver, expressed as increased level of MDA and decreased antioxidant markers, including SOD, GSH-PX and CAT. These findings are in line with previous investigations of Li et al. (2021) and Wang et al. (2018), who reported that exposure to AFB_1 might result in oxidative stress by reducing levels of antioxidant enzymes. Nevertheless, GSPE suppressed lipid peroxidation and enhanced the activity of antioxidant enzymes in quail liver via effective removal of ROS. Likewise, previous studies have demonstrated that GSPE could overcome the oxidative damage triggered by AFB_1 (Rajput et al., 2018; Yousef et al., 2018).

Serum levels of pro-inflammatory cytokines are considered markers of cellular immunity. Currently, AFB_1 group showed a significant elevation in serum pro-inflammatory cytokines such as TNF-α and IL-6. These results align with recent research indicating that AFB_1 might lead to inflammation and alter the immune response (Hassan et al., 2020; Li et al., 2014; Long et al., 2016d). Nevertheless, GSPE supplementation significantly mitigated increased levels of TNF-α and IL-6 in the serum of AFB_1-fed quails. In accordance, Rajput et al. (2017) and Rajput et al. (2019) reported that GSPE could significantly modify the elevated levels of inflammatory cytokines in serum broiler following ingestion of AFB1 diet. All these results also confirmed the anti-inflammatory and antioxidant action of GSPE that could be responsible for its decisiveness effect.

Aflatoxins adversely affect birds, referring to alterations of relative edible organ weights and histopathological changes (Fan et al., 2015; Liu et al., 2016; Rashidi et al., 2020). Histological results proved the protective role of GSPE against injuries provoked by AFB_1. Interestingly, GSPE feed additives alleviated AFB_1 histopathological changes in the liver. These results match those observed in earlier studies that found a strong protective effect of GSPE against AFB_1-induced liver damage (Rajput et al., 2017; Long et al., 2016b). Our findings also accord with earlier observations, which showed that supplementation of grape seed meal (GSM) into the AFB_1-contaminated diet mitigated liver injuries and reversed liver oxidative stress and inflammation of intoxicated piglets (Taranu et al., 2020).

CONCLUSIONS AND RECOMMENDATIONS

The present study, GSPE inhibited AFB_1-induced oxidative stress by reducing lipid peroxidation and up-regulating the activity of the antioxidant enzymes.

NOVELTY STATEMENT

This study has demonstrated for the first time, that adverse effects of aflatoxin in Japanese quail can be alleviated by dietary supplementation of GSPE

AUTHOR'S CONTRIBUTION

The authors confirm contribution to the paper as follows: study conception and design: EA, MK, and HA; experiment: MK, GS, and HA; data collection: NE and MA; analysis and interpretation of results: MA; All authors draft manuscript Preparation, reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdel-Wahhab MA, Aly SE (2005). Antioxidant property of Nigella sativa (black cumin) and Syzygium aromaticum (clove) in rats during aflatoxicosis. J. Appl. Toxicol. Int. J., 25: 218–223. https://doi.org/10.1002/jat.1057

Abdou HM, Abd Elkader H-TAE, El-Gendy AH, Eweda SM (2021). Neurotoxicity and neuroinflammatory effects of bisphenol A in male rats: the neuroprotective role of grape seed proanthocyanidins. Environ. Sci. Pollut. Res., pp. 1–12. https://doi.org/10.1007/s11356-021-16311-1

Deng ZJ, Zhao JF, Huang F, Sun GL, Wei GAO, Li LU, De Qiang X (2020). Protective effect of procyanidin B2 on acute liver injury induced by aflatoxin B1 in rats. Biomed. Environ. Sci., 33: 238–247. https://doi.org/10.3390/toxins13110806

Fan Y, Zhao L, Ji C, Li X, Jia R, Xi L, Zhang J, Ma Q (2015). Protective effects of Bacillus subtilis ANSB060 on serum biochemistry, histopathological changes and antioxidant enzyme activities of broilers fed moldy peanut meal naturally contaminated with aflatoxins. Toxins (Basel). 7: 3330–3343. https://doi.org/10.3390/toxins7083330

Guo HW, Chang J, Wang P, Yin QQ, Liu CQ, Xu, XX, Dang XW, Hu XF, Wang QL (2021). Effects of compound probiotics and aflatoxin-degradation enzyme on alleviating aflatoxin-induced cytotoxicity in chicken embryo primary intestinal epithelium, liver and kidney cells. AMB Express, 11: 1–12. https://doi.org/10.1186/s13568-021-01196-7

Han XY, Huang QC, Li WF, Jiang JF, Xu ZR (2008). Changes

in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B1 levels. Livest. Sci., 119: 216–220. https://doi.org/10.1016/j.livsci.2008.04.006

Hassan AA, Mansour MK, Sayed-ElAhl RMH, El-Din HAT, Awad MEA, Younis EM (2020). Influence of selenium nanoparticles on the effects of poisoning with aflatoxins. Adv. Anim. Vet. Sci., 8: 64–73. https://doi.org/10.17582/journal.aavs/2020/8.s2.64.73

Hussain Z, Rehman HU, Manzoor S, Tahir S, Mukhtar M (2016). Determination of liver and muscle aflatoxin B1 residues and select serum chemistry variables during chronic aflatoxicosis in broiler chickens. Vet. Clin. Pathol., 45: 330-334. https://doi.org/10.1111/vcp.12336

Hussein SA, Elsenosi Y, Esmael TEA, Amin A, Sarhan EAM (2020). Evaluation of renoprotective effect of grape seed proanthocyanidin extract on Cyclosporine A-induced Nephrotoxicity by mitigating inflammatory response, oxidative stress and apoptosis in rats. Benha Vet. Med. J., 39: 167–172. https://doi.org/10.21608/bvmj.2020.33392.1218

Ismail IE, Farag MR, Alagawany M, Mahmoud HK, Reda FM (2020). Efficacy of some feed additives to attenuate the hepato-renal damage induced by aflatoxin B1 in rabbits. J. Anim. Physiol. Anim. Nutr. (Berl). 104: 1343–1350. https://doi.org/10.1111/jpn.13359

Li SW, Chang MH, Zhao WJ, Li HL, Sun HJ, Hong HC, Wang WQ (2021). Oxidative stress and lipid peroxidation with exposure of emerging disinfection byproduct 2, 6-dichlorobenzoquinone in mice. https://doi.org/10.21203/rs.3.rs-672041/v1

Li Y, Ma QG, Zhao LH, Wei H, Duan GX, Zhang JY, Ji C (2014). Effects of lipoic acid on immune function, the antioxidant defense system, and inflammation-related genes expression of broiler chickens fed aflatoxin contaminated diets. Int. J. Mol. Sci., 15: 5649–5662. https://doi.org/10.3390/ijms15045649

Limaye A, Yu RC, Chou CC, Liu JR, Cheng KC (2018). Protective and detoxifying effects conferred by dietary selenium and curcumin against AFB1-mediated toxicity in livestock: A review. Toxins (Basel), 10: 25. https://doi.org/10.3390/toxins10010025

Liu T, Ma Q, Zhao L, Jia R, Zhang J, Ji C, Wang X (2016). Protective effects of sporoderm-broken spores of ganderma lucidum on growth performance, antioxidant capacity and immune function of broiler chickens exposed to low level of aflatoxin B1. Toxins (Basel). 8: 278. https://doi.org/10.3390/toxins8100278

Liu W, Xu C, Sun X, Kuang H, Kuang X, Zou W, Yang B, Wu L, Liu F, Zou T (2016). Grape seed proanthocyanidin extract protects against perfluorooctanoic acid-induced hepatotoxicity by attenuating inflammatory response, oxidative stress and apoptosis in mice. Toxicol. Res. (Camb). 5: 224–234. https://doi.org/10.1039/C5TX00260E

Long M, Yang SH, Han JX, Li P, Zhang Y, Dong S, Chen X, Guo J, Wang J, He JB (2016a). The protective effect of grape-seed proanthocyanidin extract on oxidative damage induced by zearalenone in Kunming mice liver. Int. J. Mol. Sci., 17: 808. https://doi.org/10.3390/ijms17060808

Long M, Yang SH, Han JX, Li P, Zhang Y, Dong S, Chen X, Guo J, Wang J, He JB (2016b). The protective effect of grape-seed proanthocyanidin extract on oxidative damage induced by zearalenone in kunming mice liver. Int. J. Mol. Sci., 17: 808. https://doi.org/10.3390/ijms17060808

Long M, Zhang Y, Li P, Yang SH, Zhang WK, Han JX, Wang Y, He JB (2016c). Intervention of grape seed proanthocyanidin extract on the subchronic immune injury in mice induced by aflatoxin B1. Int. J. Mol. Sci., 17: 516. https://doi.org/10.3390/ijms17040516

Long M, Zhang Y, Li P, Yang SH, Zhang WK, Han JX, Wang Y, He JB (2016d). Intervention of grape seed proanthocyanidin extract on the subchronic immune injury in mice induced by aflatoxin B1. Int. J. Mol. Sci., 17: 1–10. https://doi.org/10.3390/ijms17040516

Mahrose KM, Michalak I, Farghly M, Elokil A, Zhang R, Ayaşan T, Mekawy A, Fazlani S (2021). Role of clay in detoxification of aflatoxin B1 in growing Japanese quail with reference to gender. Vet. Res. Commun., pp. 1–9. https://doi.org/10.1007/s11259-021-09817-z

Manafi M, Khosravinia H (2013). Effects of aflatoxin on the performance of broiler breeders and its alleviation through herbal mycotoxin binder. https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=283785

Nazhand A, Durazzo A, Lucarini M, Souto EB, Santini A (2020). Characteristics, occurrence, detection and detoxification of aflatoxins in foods and feeds. Foods, 9: 644. https://doi.org/10.3390/foods9050644

NRC, N. R. C. 1994. Nutritional requirements of poultry. 9th Rev. Ed. National Academy Press, Washington, DC.

Othman SNN, Lum PT, Gan SH, Mani S, Sekar M (2020). Protective effect of natural products against chemotherapy-induced cardiotoxicity: A review. Pharmacogn. J., 12: 1180-1189. https://doi.org/10.5530/pj.2020.12.166

Rajput SA, Sun L, Zhang N, Khalil MM, Gao X, Ling Z, Zhu L, Khan FA, Zhang J, Qi D (2018). Correction: Rajput SA, *et al*. Ameliorative effects of grape seed proanthocyanidin extract on growth performance, immune function, antioxidant capacity, biochemical constituents, liver histopathology and aflatoxin residues in broilers exposed to aflatoxin. Toxins (Basel), 10: 366. https://doi.org/10.3390/toxins10090366

Rajput SA, Sun L, Zhang N, Mohamed KM, Gao X, Ling Z, Zhu L, Khan FA, Zhang J, Qi D (2017). Ameliorative effects of grape seed proanthocyanidin extract on growth performance, immune function, antioxidant capacity, biochemical constituents, liver histopathology and aflatoxin residues in broilers exposed to aflatoxin B1. Toxins (Basel). 9: 371. https://doi.org/10.3390/toxins9110371

Rajput SA, Sun L, Zhang NY, Khalil MM, Ling Z, Chong L, Wang S, Rajput IR, Bloch DM, Khan FA (2019). Grape seed proanthocyanidin extract alleviates aflatoxinB1-induced immunotoxicity and oxidative stress via modulation of NF-κB and Nrf2 signaling pathways in broilers. Toxins (Basel), 11: 23. https://doi.org/10.3390/toxins11010023

Rashidi N, Khatibjoo A, Taherpour K, Akbari-Gharaei M, Shirzadi H (2020). Effects of licorice extract, probiotic, toxin binder and poultry litter biochar on performance, immune function, blood indices and liver histopathology of broilers exposed to aflatoxin-B1. Poult. Sci., 99: 5896–5906. https://doi.org/10.1016/j.psj.2020.08.034

Saleemi MK, Ashraf K, Gul ST, Naseem MN, Sajid MS, Mohsin M, He C, Zubair M, Khan A (2020). Toxicopathological effects of feeding aflatoxins B1 in broilers and its ameliosration with indigenous mycotoxin binder. Ecotoxicol. Environ. Saf., 187: 109712. https://doi.org/10.1016/j.ecoenv.2019.109712

Sheng K, Zhang G, Sun M, He S, Kong X, Wang J, Zhu F, Zha X, Wang Y (2020). Grape seed proanthocyanidin extract ameliorates dextran sulfate sodium-induced colitis through

intestinal barrier improvement, oxidative stress reduction, and inflammatory cytokines and gut microbiota modulation. Food Funct., 11: 7817–7829. https://doi.org/10.1039/D0FO01418D

Taranu I, Hermenean A, Bulgaru C, Pistol GC, Ciceu A, Grosu IA, Marin DE (2020). Diet containing grape seed meal by-product counteracts AFB1 toxicity in liver of pig after weaning. Ecotoxicol. Environ. Saf., 203: 110899. https://doi.org/10.1016/j.ecoenv.2020.110899

Taranu I, Marin DE, Palade M, Pistol GC, Chedea VS, Gras MA, Rotar C (2019). Assessment of the efficacy of a grape seed waste in counteracting the changes induced by aflatoxin B1 contaminated diet on performance, plasma, liver and intestinal tissues of pigs after weaning. Toxicon, 162: 24–31. https://doi.org/10.1016/j.toxicon.2019.02.020

Tessari ENC, Kobashigawa E, Cardoso ALSP, Ledoux DR, Rottinghaus GE, Oliveira CAF (2010). Effects of aflatoxin B1 and fumonisin B1 on blood biochemical parameters in broilers. Toxins (Basel), 2: 453–460. https://doi.org/10.3390/toxins2040453

Wang X, Li W, Wang X, Han M, Muhammad I, Zhang X, Sun X, Cui X (2019). Water-soluble substances of wheat: A potential preventer of aflatoxin B1-induced liver damage in broilers. Poult. Sci., 98: 136–149. https://doi.org/10.3382/ps/pey358

Wang X, Muhammad I, Sun X, Han M, Hamid S, Zhang X (2018). Protective role of curcumin in ameliorating AFB 1-induced apoptosis via mitochondrial pathway in liver cells. Mol. Biol. Rep., 45: 881–891. https://doi.org/10.1007/s11033-018-4234-4

Yousef MI, Khalil DKAM, Abdou HM (2018). Neuro and nephroprotective effect of grape seed proanthocyanidin extract against carboplatin and thalidomide through modulation of inflammation, tumor suppressor protein p53, neurotransmitters, oxidative stress and histology. Toxicol. Rep., 5: 568–578. https://doi.org/10.1016/j.toxrep.2018.04.006