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RESEARCH ARTICLE

Ameliorative Effects of Cholestyramine and Oxihumate on Aflatoxicosis in Broiler Chickens

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ABSTRACT

Aflatoxin B1 (AFB1) is widely available mycotoxin that is secreted by certain types of *Aspergilli.* In this research the ameliorative efficacy of two mycotoxin binders in broilers was evaluated; cholestyramine which was used for the first time in the poultry and oxihumate. A total of 64 one-day-old chicks were divided into four equal groups: birds of group A, B & C were fed on AFB1 contaminated diet at a rate of 2 ppm for 36 days either alone, with cholestyramine at a dose rate of 340µg/kg ration or with oxihumate at a dose rate of 3.5g/kg ration. Group D was kept as control with basal diet of neither toxin nor drug treatment. Morality was highest and the lesions of AFB1 intoxication were pronounced among birds of group A with marked degenerative and necrotic changes in different examined organs. Variable degrees of ameliorative effects of AFB-induced toxic lesions were observed in both treated groups (group B & C) with beneficial effects for cholestyramine. Mild expression of the apoptosis-related marker (p53) was encountered in group B and C relative to AFB1 intoxicated group. Aflatoxin residues were significantly reduced in the bird liver and kidney tissues in the instance of the two antimycotoxin binders. It could be concluded that both cholestyramine and oxihumate have an ameliorative effect for controlling aflatoxicosis with the superiority of cholestyramine in its protective effect. This the first *in vivo* trail to use cholestyramine as anti-AFB1 agent in poultry.

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INTRODUCTION

Mycotoxins are significant global issue with strong economically negative impact on livestock, poultry and humans. The big issue associated with mycotoxin-contaminated animal feed is not only acute disease outbreaks (Murugesan *et al.*, 2015; Haque *et al*., 2020), but also severe metabolic disorders contributing to poor animal productivity (Bryden, 2012). Aflatoxins are chemically difuranocoumarin derivatives with a bifuran group attached to the coumarin nucleus with a pentanone ring (in case of AFBs) or a lactone ring (in case of AFGs). Among the 20 identified AFs, the most important members are AFB1, AFB2, AFG1 and AFG2 (Kumar *et al.*, 2017; Abdel-Sattar *et al*., 2019).

Liver is the main site for AFB1 accumulation and metabolization (Yunus *et al.*, 2011). A survey was conducted on a total of 300 samples (100 feed stuff, 100 liver 100 and breast muscle samples) from different farms at age of marketing. The results revealed higher levels of AFB1 residues in liver samples than those detected in muscle tissues (Khaled *et al.*, 2019). In livers, AFB1 induced hepatic lipidosis or necrosis accompanied by bile duct hyperplasia and fibrosis beside portal inflammatory cell infiltrates (Mendieta *et al.*, 2018).

A mycotoxin binder are nutritionally inert materials that are added to animal feed in a limited amount to firmly bind mycotoxins restraining them from entering the blood stream. The supplementation of mycotoxin binders to contaminated diet has been deemed a promising result in counteracting the mycotoxicosis (Chen *et al.*, 2014; Pappas *et al.*, 2016: Saleemi *et al*., 2020). Cholestyramine is a synthetic chemical drug contains high doses from insoluble quaternary ammonium anion exchange resins which strongly combines with anionic compounds, bile acid and some cationic compounds (Underhill *et al.*, 1995). It exerted efficient adsorption capacity for ochratoxin A, fumonisins and zearalenone on many *in vitro* and

laboratory models but not in poultry (Avantaggiato *et al.*, 2005). Oxihumate is a specific bituminous carbon humic acids by reversing the mechanism by which the coal was produced (Bergh *et al.*, 1997). It is physical antimycotoxins had a high adsorption capacity for several mycotoxins especially AFB1. Oxihumate adsorbed many concentrations of aflatoxin at different pH levels. Its supplementation at a concentration of 3.5 g/kg ration was potent in reducing the growth inhibitory effects of aflatoxicosis.

However, published data regarding the uses of cholestyramine as a mycotoxin binder for aflatoxicosis in poultry feed have so far not been available. Furthermore, it is not known if it could alleviate the mycotoxicosis lesion in different organs. Therefore, the present work was aimed to evaluate the protective role of cholestyramine against aflatoxicosis in broiler chickens in a comparative study with oxihumate. The study evaluated some parameters such as clinical signs, mortalities, pathological and immunohistochemical analysis as well as toxin residues in different organs.

MATERIALS AND METHODS

Birds: Sixty-four healthy, one-day-old, baby chicks of Cubb breed, were purchased from the Alkahira Poultry Company. The birds were housed separately in animal house research facility at the Faculty of Veterinary Medicine, Zagazig University and were kept under standard hygienic conditions. All the bird procedures were performed in strict accordance with the recommendations in the guidelines of the Institutional Animal Care and Use Committee (ZU-IACUC) of Zagazig University.

Preparation of Mycotoxin (Aflatoxin): *Aspergillus flavus* MD 341 strain was kindly obtained from the Central Laboratory of Residues of Agricultural Product, Agriculture Pesticides Residues Centre, Dokki, Egypt. The *Aspergillus flavus* MD 341 strain was incubated at 28°C for 8 days on liquid media containing 2% yeast extract and 20% sucrose. AFB1 concentration in the culture was determined using High performance liquid chromatography (HPLC) according to the method described by (AOAC, 2000). The media was sprayed and mixed in the chicken home-made diet at a concentration of 2 ppm which was rechecked using HPLC (Kumar *et al.*, 1993).

Antimycotoxin drugs: Cholestyramine, it was manufactured by Bristol- Myers Squibb (Questran ®) 4 gm/sachet. Oxihumate, it was purchased from El-Nasr pharmaceutical Chemical Company, Egypt.

Experimental design: A total of 64, one-day-old, Cubb broiler chicks were divided evenly into four groups. In the group (Gp) A treatment, birds were fed on home-made maize-soyabean meal diet contaminated with AFB1 at 2ppm. In Gp B treatment, broilers were fed on the Gp A contaminated diet plus cholestyramine at a dosage of 170 µg/ mg mycotoxin (340 µg / kg diet). Birds on Gp C were fed on the Gp A contaminated diet with 3.5 g/kg ration of oxihumate, whereas Gp D was fed on a home-made maize–soybean meal diet with neither mycotoxins nor drug treatment. Hence, the diet ingredients had no detectable AFB1 mycotoxin level. The clinical signs and mortalities were recorded daily in all the experimental groups. Postmortem lesions of dead birds were recorded. At the end of the experiment (36) days, all the live birds were sacrificed and examined for any gross lesions. Tissue specimens from livers and kidneys were homogenized and frozen at -20ºC in the dark till the time of the analysis of the AFB1 residues. Furthermore, tissue specimens from livers, kidneys, spleens, bursa of fabricius and intestine were fixed in 10% buffered neutral formalin. Paraffin sections 5 micron thick was prepared and stained with hematoxylin and eosin stain and examined microscopically (Survarna *et al.*, 2013). Lesion scores of different examined organs in the experimental groups were estimated based on the examination of ten fields for each investigated section.

Immunohistochemical (IHC) analysis: To estimate the DNA integrity, further sections on positively charged coated slides were used for IHC technique using Anti-P53 antibody (Abcam, Cambridge, UK; ab131442) and IHC kits were purchased from Dako (Glostrup, Denmark) according to the manufactures' instructions.

Residual investigation: Residues of AFB1 were determined in livers, kidneys and breast muscles at the end of the experiment in all the experimental groups, 36 days post initial feeding. High performance liquid chromatography (HPLC), Agilent Series 1200 using fluorescence (FLD) detector with excitation at 360 nm and emission at 440 nm was used. The procedures were performed according to the method described by (Beg *et al.*, 2006).

Statistical analysis: The obtained data were analyzed using the statistical package for social science (SPSS, version 18 software, 2011) for obtaining means and standard deviation. The data were statistically analyzed using one-way ANOVA. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity (Tamhane and Dunlop, 2000).

RESULTS

Chickens of AFB1 intoxicated group (Gp A) showed general signs of an illness, mainly in the form of ruffled feather, droopy head and wings with depression, lameness and yellowish diarrhea. The cumulative mortality rate was 25% (4 birds/group) during the whole experiment. Chickens orally supplemented with cholestyramine at a dosage of 170 µg/ mg mycotoxin (Gp B) appeared clinically healthy with mild diarrhea and mortalities reached 12.5% (2 birds/group). Five chicks of Gp C, orally supplemented with mycotoxin contaminated diet plus 3.5 g/kg ration of oxihumate, had poor performance and depression beside the mortality rate in this group was 18.75% (3 birds/group). While neither clinical signs nor mortalities among chickens of Gp D could be detected.

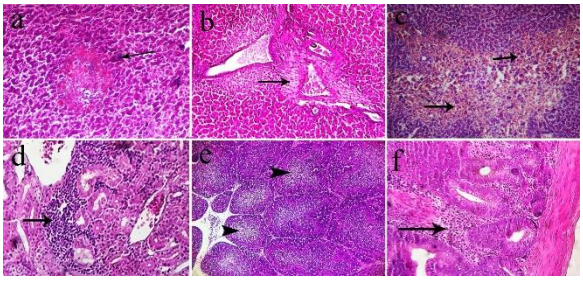

Fig. 1: Photomicrographs of H&E stained sections of chicken organs from dead and sacrificed chickens group A (AFB1 intoxicated) (a): Liver showing necrotic area with a few inflammatory cells infiltration (thin arrow) (X 200). (b): liver showing severe portal fibrosis (arrow) (X 200). (c): Liver showing necrotic area replaced by extravasated blood (arrows) (X 400). (d): kidney showing focal intertubular leukocytic cells infiltration (arrow) (X 200). (e): Bursa of Fabricius showing mild depletion of lymphocytes from the medulla of lymphoid follicles (arrowhead) (X 200) (f): Intestine showing submucosal leukocytic cells infiltration (arrow) (X 400).

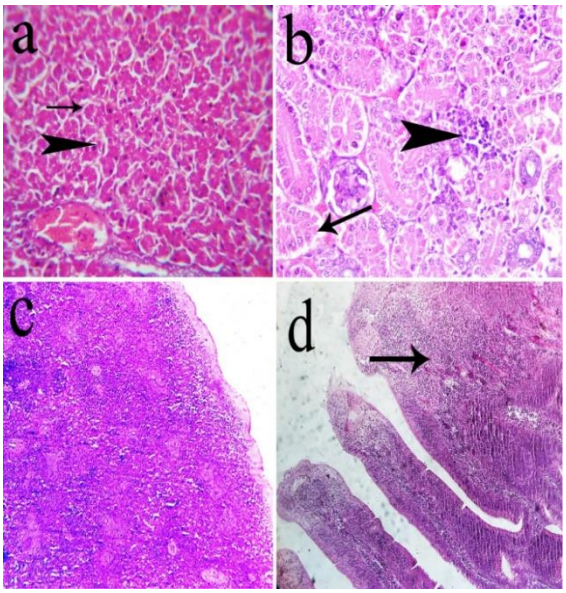

Fig. 2: Photomicrographs of H&E stained sections of chicken organs from group B (AFB1 treated with cholestyramine) (a): Liver showing apparently normal parenchyma with slightly dilated sinusoids and proliferation of Von kupffer's cells (arrowhead) (X400). (b): Kidney showing mild leukocytic cells infiltrations (arrowhead) with apparently normal renal tubules (arrow) (X 400). (c): Spleen showing normal lymphoid tissue (X200). (d): Intestine showing fusion of some intestinal villi and massive mucosal lymphocytic cells infiltration (arrow) (X 400)

Birds fed on AFB1 contaminated diet have friable enlarged livers with yellow coloration and greyish necrotic areas on their cut surface. The kidneys were enlarged or pale in color and four birds had urates deposition. The intestinal mucosal surface showed severe congestion with presence of undigested food particles. Atrophied bursa of fabricius was observed in twelve cases (75%) while splenomegaly was also noticed in six cases (37.5%). The detectable gross lesions were mildest in chickens treated with cholestyramine (Gp B) that were apparently normal. Three dead birds (18.75%) of Gp C had mild to moderate congestion of their livers and kidneys with a few extravasated blood from their cut surfaces. The spleens and bursa of fabricius were normal in size.

The histological lesions of the examined organs are summarized in Table 1. Briefly, samples from AFB1 treated group (Gp A) had moderate to severe lesions. Random areas of coagulative necrosis of the hepatocytes

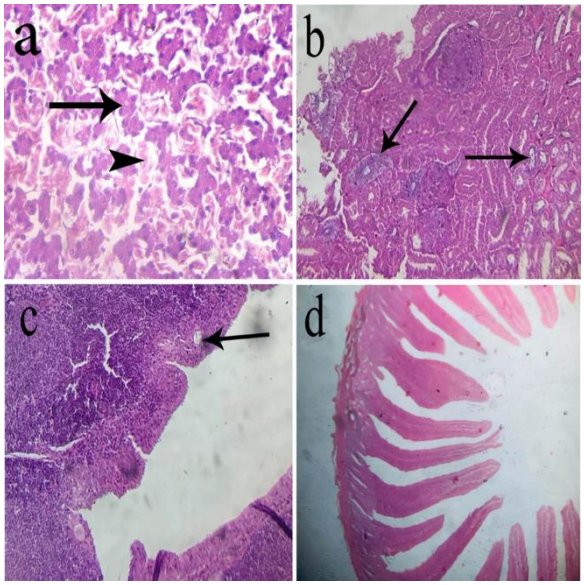

Fig. 3: Photomicrographs of H&E stained sections of chicken organs from group C (AFB1 treated with oxihumate) (a): liver showing shrunken hepatocytes (arrow) and dilated sinusoids (arrowhead) (X 400). (b): kidney showing regenerative attempts of some renal tubules (arrows) (X 200). (c): Bursa of fabricius showing hyperplastic lymphoid follicles beside vesicle formation in covering epithelium (arrow) (X 400). (d): intestine showing apparently normal intestinal coats (X 200)

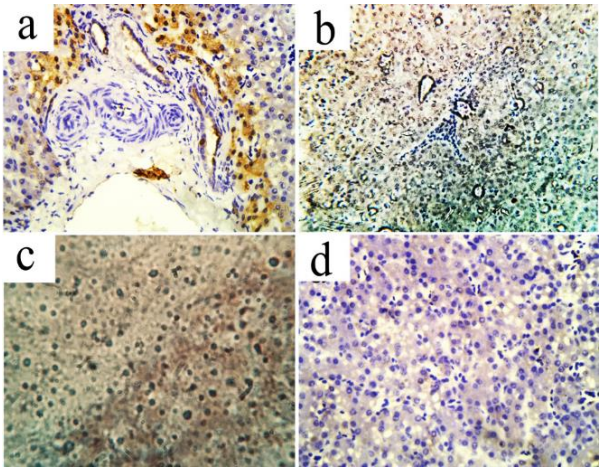

Fig. 4: Immunohistochemical photomicrographs of chickens livers of groups (A, B, C and D) at 36th days of age (a): liver of Gp A showing intense immune staining for p53. (b): liver of Gp B showing mild immune staining for p53. (c): liver of Gp C showing mild to moderate immune staining for p53. (d): liver of Gp D showing no immune staining for p53

with or without a few inflammatory cells were abundant (Fig. 1a). Portal fibrosis, cholestasis, leukocytic infiltration and numerous bile ductules were prevalent (Fig. 1b). Some cases showed focal extravasated erythrocytes replacing the hepatic parenchyma (Fig. 1c). Some apoptotic hepatic cells were intermingled with degenerated cells. Besides the hepatic lesions, renal lesions were also included such as individualization and dissociation of other renal epithelium with massive leukocytic cells infiltration (Fig. 1d). The lymphoid organs (bursa of fabricius and spleen) were characterized by mild to moderate depletion of lymphocytes from the medulla of lymphoid follicles and white pulps (Fig. 1e). Moreover, enteric lesions in group A represented by destruction of villous epithelium with severe periglandular leukocytic cells infiltration in submucosa (Fig. 1f).

Table 1: Lesion scores of different examined organs in the experimental groups

Organs	Main lesions	Lesions score in different examined organs among experimental groups		
		A	B	C
	Apoptosis	++	+	+
Liver	Coagulative necrosis	+++	+	++
	Portal fibrosis	+ + +	+	+ +
	Portal leukocytic cells infiltrations	+ + +	+	+
	Extravasated erythrocyte	+ +	-	+
	Hyperplastic biliary epithelium and cholestasis	++	+	+
	Kupffer cell proliferation	+ +	+ +	+
	Renal hemorrhage	++		+
Kidney	Coagulative necrosis and dissociation	++	-	+
	Regenerative attempts	+	+	++
	Urate deposition	++	++	+
			-	
	Lymphocytic depletion and necrosis	+ +	+	+
Spleen	Hyperplasia of RES	+ +	+	+
Bursa of	Follicular necrosis and depletion		+	+
Fabricius	Vesicle formation in the epithelium	++	-	+
	Normal lymphoid follicle zones	+ +	++	+
		-		
Intestine	Villous epithelium destruction	+ +	+	+
	Submucosal leukocytic cells infiltration	+ +	+	+
	Villous epithelium hyperplasia	++	+	+

+++ (severe); ++ (moderate); + (mild); - (absent).

Table 2: AFB1 residues in different examined organs of the experimental groups

Organ groups	Liver*	Kidney*	Muscle*
A	77.58±2.56[a]	39.28±3.17[a]	10.28±1.70[a]
B	14.74±.0.79[b]	23.19±2.86[b]	6.93±1.04[a]
C	18.70±1.72[b]	27.80±0.23[b]	8.21±1.54[a]
D	----	------	------

*Results showed as (mean ±SD): Superscripts within the same column are significantly different at P<0.05, based on Duncan's multiple comparison.

Addition of cholestyramine to AFB1 contaminated diet led to milder histological lesions in comparison to Gp A and C in all the examined organs. The majority of hepatic parenchyma appeared normal with slightly dilated hepatic blood vessels and sinusoids beside proliferation of Von kupffer's cells (Fig. 2a). Minimal perivascular fibrosis could be seen in few cases. Moreover, kidneys suffered from mild leukocytic cells infiltrations with mild reversible degenerative changes of some renal tubules, meanwhile the majority of renal tissues were within the normal histomorphological picture (Fig. 2b). Spleen almost exhibited normal lymphoid tissue architecture (Fig. 2c). Mild vacuolation of the epithelial covering the lymphoid follicles of bursa of fabricius with apparently normal follicular zones were common. Mild to moderate intestinal lesions in Gp B characterized by restore of the absorptive surface with hyperplastic villous epithelium and fusion of some intestinal villi beside massive mucosal lymphocytic cells infiltration (Fig. 2d). Regarding supplementation with oxihumate (Gp C), the microscopical lesion became moderate compared to Gp A and represented by dilated hepatic sinusoids and shrunken hepatocytes (Fig. 3a). Kidneys of some cases exhibited congestion of renal blood vessels with mild perivascular fibrosis. Regenerative attempts in some renal tubules were prevalent in the majority of cases (Fig. 3b). Spleen of some cases showed mild lymphoid depletion, while others showed apparently normal lymphoid tissues. Bursa of fabricius revealed regenerative tissues particularly in the medulla with or without vesicle formation in the covering epithelium (Fig. 3c). A few cases exhibited normal or partial destruction of intestinal mucosa meanwhile, the majority of birds had apparently normal intestinal coats (Fig. 3d).

In order to assess the ameliorative effects of the used antimycotoxin binders on AFB1contaminated ration, the AFB1 residues were measured in liver, kidney and breast muscles using high performance liquid chromatography (Table 2). The highest residues level was detected in the liver tissues of Gp A then kidneys while both residues levels of liver and kidneys were significantly higher than AFB1 residues levels in muscles. Highly significant lowering of residual concentrations of AFB1 in all in kidney and liver were detected in Gp B and C due to inclusion of either cholestyramine or oxihumate in birds ration. No detectable value of AFB1 residues could be detected in chickens of Gp D.

DISCUSSION

The present study assessed the efficacy of two antimycotoxin binders to reduce or eliminate the toxic effects of AFB1 on mortality, gross & histological lesions, DNA damage and AFB1 residues in different organs of broiler chickens. AFB1 is one of the most abundant mycotoxin that contaminate bird ration. Intoxication of broiler chickens with AFB1 leads to liver impairment, weight loss, immune suppression, and increasing mortality (Yunus *et al.*, 2011). In accordance with the previous, chickens of AFB1 intoxicated group (Gp A) showed AFB1 intoxication clinical signs as well as the highest cumulative mortality rate, 25%. Consistently, an earlier study reported that feeding of broiler chickens with diet containing 0.1 mg/kg AFB1 led to 10% cumulative mortality throughout the experimental period (Pappas *et al.*, 2016). The variation in the mortality rate between the two experiments could be attributed to the difference in the dosage of the mycotoxin included in the chicken diet. Hence, there is established a dose-effect relationship between aflatoxin B_1 level and broiler performance (Yunus *et al.*, 2011).

In the present work, chickens orally supplemented with cholestyramine at a dosage of 170 µg/ mg mycotoxin (Gp B) appeared clinically healthy and were in better clinical conditions compared with oxihumate treated birds or AFB1 only intoxicated birds with lower mortality rate.

Reduction of both clinical signs and mortality, particularly in group B could be attributed to the ameliorative effect of the used antimycotoxin binders, cholestyramine or oxihumate. The variation in mortality declared the beneficial effect of sequestering agent (cholestyramine), which was reported here for the first time as antimycotoxin for poultry. Sequestering agents are non-absorbable materials with large surface area and high adsorptive capacity. They are capable of binding toxins in the gastrointestinal tract, thereby reducing enterohepatic recirculation and ultimately the body burden of toxins (Hope, 2013). Additionally, these agents are nonspecific and can bind additional toxins, helping to reduce overall body burden of toxins.

In a previous work, oxihumate (humic acid) could alleviate some of the toxic effects of aflatoxin in growing broilers, especially when used in combination with other mycotoxin management practices (Jansen van Rensburg *et al.*, 2006).

Necropsy of bird of Gp A revealed remarkable liver injuries. In addition, variable gross lesions were recorded in kidneys, intestinal mucosal surface and immune organs (bursa of fabricius and spleens). Similar pathological changes were observed earlier when broiler chickens were fed on AFB1 at 0.5 ppm for 6 weeks (Kumar and Balachandran, 2014). Similar to the gross lesions, variable degenerative and necrotic alterations in the hepatic and renal ultrastructures were detected, which were concurred with those of (Kumar and Balachandran, 2014). The toxic effects of AFB1in the kidney were clearly studied in details in HEK 293 cells and mice model (Li *et al.*, 2018). Results of the aforementioned study showed that AFB1 (0.5 mg/kg) caused nephrotoxicity by activating oxidative stress via reducing the expression of proline dehydrogenase and L-proline levels which led to downstream apoptosis. Additionally, aflatoxicosis had a toxic effect on all the lymphoid organs and severe lymphoid depletion, which was obvious in our study (Yunus *et al.*, 2011; Chen *et al.*, 2014).

Addition of cholestyramine to AFB1 contaminated diet led to milder gross and histological lesions in comparison to Gp A and C in all examined organs. Reduced lesions in Gp B due to the ability of cholestyramine to combine with the mycotoxins in the gut and then, the toxin binder complex passes outside and removed through feces. This minimizes and prevents the exposure to mycotoxins (EFSA, 2009). Cholestyramine drug act like 'chemical sponge' and adsorb AFB1 in the gastrointestinal tract, thus preventing toxin uptake in the blood and subsequent distribution to target organs. It was proved to be an effective binder for AFB1 in vitro and in laboratory animal models only (Avantaggiato *et al.*, 2005). Adding of oxihumate to the initial composition of feedstuffs should alleviate aflatoxicosis efficiently by forming of an oxihumate-aflatoxin complex that inhibits aflatoxin absorption from the gastrointestinal tract (Jansen van Rensburg *et al.*, 2006).

Apoptosis is a process of programmed cell death that occurs in multicellular organisms. Mycotoxins induce apoptosis in chicken liver tissue, which can be easily revealed using apoptosis-related antibodies p21 and p53 that mediated p53/p21 apoptotic signaling pathway (Hussar *et al.*, 2018). As confirmed earlier, AFB1 mode of action lead to DNA adduct formation (AFB1-formamido-pyrimidine). This adduct causes mutation at codon 249 of the p53 tumour suppressor gene leading to malignant transformation (Liew and Mohd-Redzwan, 2018). In accordance with the previous, ingestion of maize-soybean meal diet contaminated with AFB1 at 2ppm for nearly five weeks (Gp A) led to an intense immune staining reaction for p53 marker in the immunohistochemical analysis. On the other hand, feeding of broilers on contaminated diet plus either cholestyramine or oxihumate at a dosage of 340µg/kg or 3.5g/kg ration resulted in only mild to moderate immune expressions for p53 marker, respectively. The previous confirms the value of the p53 Ab to estimate DNA integrity and apoptosis incidence results from AFB1 exposure. In addition, it verifies the induction of either liver necrosis or apoptosis via death receptor pathway in chickens, which fed on AFB1 contaminated diet only (Mughal *et al.*, 2017). Reduction of p53 expression in both groups (B&C) was arising from the ameliorative effect of mycotoxin-induced liver apoptosis via the two used antimycotoxin binders.

The absorption of mycotoxins in birds takes place in the proximal part of the jejunum into vascular circulation. The absorbed mycotoxins transfers via the portal vein and reaches the liver and other organs. In the present work the highest residues level was detected in the livers, kidneys, and then muscles. Constantly, metabolites of AFB1 were clearly appeared in livers of broilers fed on 2.5 or 5 mg of aflatoxin/ kg ration for a period of 32 days more than kidney and muscle tissues (Fernández *et al.*, 1994). However, Hussain and his colleagues (2010) detected AFB1 residue in muscle the highest AFB1 residue levels are usually detected in liver and muscles and residues clearance is slow from those tissues (Hussain *et al.*, 2010). On the other hand and consistent with the other findings, highly significant lowering of residual concentrations of AFB1 in all kidney and liver were detected in Gp B and C due to inclusion of either cholestyramine or oxihumate in birds ration.

Conclusions: In conclusion, cholestyramine by dose (340 µg/kg ration) had a pronounced ameliorative action to aflatoxicosis in broiler chickens by alleviating the majority of its adverse effects on vital organs particularly when compared with oxihumate (3.5g/kg ration).

Authors contribution: AMAA, MFF and MMM conceived and designed the study as well as executed the immunohistochemical works. HAA and REM executed the bird experiment and analyzed the ration & tissue samples. OH analyzed and revised the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES

Abdel-Sattar WM, Sadek KM, Elbestawy AR, *et al.*, 2019. Immunological, histopathological and biochemical protective effect of date pits (Phoenix dacrylifera seeds) feed additive against aflatoxicated broiler chickens. Int J Vet Sci 8:198-205.

AOAC, 2000. Association official analytical chemists, 15th Edition. Ass offic Analytic chemists, Washington, DC.

Avantaggiato G, Solfrizzo M and Visconti A, 2005. Recent advances on the use of adsorbent materials for detoxification of fusarium mycotoxins. Food Addit Contam 22:379-88.

Beg MU, Al-Mutairi M, Beg KR, *et al.*, 2006. Mycotoxins in poultry feed in kuwait. Arch Environ Contam Toxicol 50:594-602.

Bergh JJ, Cronjé IJ, Dekker J, *et al.*, 1997. Non-catalytic oxidation of water-slurried coal with oxygen: Identification of fulvic acids and acute toxicity. Fuel 76:149-54.

Bryden WL, 2012. Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. Anim Feed Sci Technol 173:134-58.

Chen X, Horn N and Applegate TJ, 2014. Efficiency of hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of graded levels of aflatoxin b1 in broiler chicks. Poult Sci 93:2037-47.

EFSA, 2009. Review of mycotoxin-detoxifying agents used as feed additives: Mode of action, efficacy and feed/food safety.

Fernández A, Verde MT, Gascón M, *et al.*, 1994. Aflatoxin and its metabolites in tissues from laying hens and broiler chickens fed a contaminated diet. J Sci Food Agric 65:407-14.

Haque MA, Wang Y, Shen Z, *et al.*, 2020. Mycotoxin contamination and control strategy in human, domestic animal and poultry: A Review. Microbial Pathog 142:104095.

Hope J, 2013. A review of the mechanism of injury and treatment approaches for illness resulting from exposure to water-damaged buildings, mold, and mycotoxins. The Sci World J 2013:767482.

Hussain Z, Khan MZ, Khan A, *et al.*, 2010. Residues of aflatoxin B1 in broiler meat: Effect of age and dietary aflatoxin b1 levels. Food Chem Toxicol 48:3304-7.

Hussar P, Järveots T, Pendovski L, *et al.*, 2018. T-2 mycotoxin induced apoptosis in broiler's liver tissue. Papers Anthropol XXVII:9-16.

Jansen van Rensburg C, Van Rensburg CE, Van Ryssen JB, *et al.*, 2006. In vitro and in vivo assessment of humic acid as an aflatoxin binder in broiler chickens. Poult Sci 85:1576-83.

Khaled A, Moselhy W, Ibrahim M, *et al.*, 2019. The effect of aflatoxin b1 contamination on the antioxidant status of broilers' liver and breast muscle. Adv Anim Vet Sci 7:492-7.

Kumar A, SK C, AT R, *et al.*, 1993. Clinicopathological changes in experimental aflatoxicosis in quail. Indian J Poult Sci 28:150-3.

Kumar P, Mahato DK, Kamle M, *et al.*, 2017. Aflatoxins: A global concern for food safety, human health and their management. Front Microbiol 7.

Kumar T and Balachandran C, 2014. Pathological effect of citrinin and aflatoxin in broiler chicken. Int J Life Sci Pharma Res 4:1-14.

Li H, Xing L, Zhang M, *et al.*, 2018. The toxic effects of aflatoxin b1 and aflatoxin m1 on kidney through regulating l-proline and downstream apoptosis. BioMed research international 2018:9074861.

Liew W-P-P and Mohd-Redzwan S, 2018. Mycotoxin: Its impact on gut health and microbiota. Front Cell Infec Microbiol 8:60.

Mendieta CR, Gómez GV, Del Río JCG, *et al.*, 2018. Effect of the addition of *saccharomyces cerevisiae* yeast cell walls to diets with mycotoxins on the performance and immune responses of broilers. The J Poult Sci 55:38-46.

Mughal MJ, Xi P, Yi Z, *et al.*, 2017. Aflatoxin b1 invokes apoptosis via death receptor pathway in hepatocytes. Oncotarget 8: 8239-49.

Murugesan GR, Ledoux DR, Naehrer K, *et al.*, 2015. Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. Poult Sci 94:1298-315.

Pappas AC, Tsiplakou E, Tsitsigiannis DI, *et al.*, 2016. The role of bentonite binders in single or concomitant mycotoxin contamination of chicken diets. Br Poult Sci 57:551-8.

Saleemi MK, Ashraf MK, Gul ST, *et al.*, 2020. Toxicopathological effects of feeding aflatoxins B1 in broilers and its amelioration with indigenous mycotoxin binder. Ecotoxicol Environ Safety 187:109712.

Survarna S, C L and JD B, 2013. Theory and pratice of histological techniques, 7 Edition. Elsevier, Churchill Livingstone, England.

Tamhane AC, Dunlop DD, 2000. Statistics and data analysis: From elementary to intermediate. Upper Saddle River, NJ: Prentice Hall, UK 722p.

Underhill KL, Rotter BA, Thompson BK, *et al.*, 1995. Effectiveness of cholestyramine in the detoxification of zearalenone as determined in mice. Bull Environ Contam Toxicol 54:128-34.

Yunus AW, Razzazi-Fazeli E and Bohm J, 2011. Aflatoxin b(1) in affecting broiler's performance, immunity, and gastrointestinal tract: A review of history and contemporary issues. Toxins 3:566-90.