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RESEARCH ARTICLE

Toxic Effects of Aflatoxin B1 on Hematobiochemical and Histopathological Parameters of Juvenile White Leghorn Male Birds and their Amelioration with Vitamin E and *Moringa oleifera*

Muhammad Kashif Saleemi[1]*, Ahmad Raza[1], Aisha Khatoon[1], Muhammad Zubair[2], Yongping Xu[3], Bilal Murtaza[3], Xiaoyu Li[3], Maria Jamil[1], Muhammad Imran[1], Faqir Muhammad[4], Khawar Zubair[1], Sheraz Ahmed Bhatti[4], Muhammad Kamran Rafique[5], Ishtiaq Ahmed[5], Farhan Azal[6], Farhat Jubeen[7] and Abdul Basit[8]

[1]Department of Pathology, University of Agriculture Faisalabad, Pakistan; [2]Department of Veterinary Clinical Sciences, University of Poonch, Rawalakot, Azad Kashmir, Pakistan; [3]School of Bioengineering, Dalian University of Technology, No. 2 Linggong Road, Dalian 10 116024, China; [4]Faculty of Veterinary Sciences, Bahauddin Zakariya University Multan, Pakistan; [5]Department of Pathobiology (Pathology Section), University of Veterinary and Animal Sciences, Lahore, Sub Campus Jhang, Pakistan; [6]Poultry Research Institute, Shamas Abad Murree Road Rawalpindi, Pakistan; [7]Department of Chemistry, Government College Women University Faisalabad, Pakistan; [8]HQ Military Farms, Remount Veterinary and Farm Corps, Okara, Pakistan
*Corresponding author: drkashif313@gmail.com

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A B S T R A C T

Mycotoxins are secondary metabolites produced by pathogenic fungi under suitable environmental conditions. *Moringa oleifera* and vitamin E are known antioxidants and immunostimulants. In present experimental study, *M. oleifera* and vitamin E were administered in birds feed to mitigate the toxico-pathological impacts of aflatoxin B1 in white Leghorn layer males. A total of 120 white Leghorn layer male birds were equally divided into six experimental groups. Group A was kept as negative control, group B was fed 400ppb of aflatoxin, while D and F groups were fed normal feed and supplemented with vitamin E (100ppm) and 1 %*M. oleifera*, respectively. The birds in C and E groups were given 400 ppb AFB1 in feed and supplemented with vitamin E and *M. oleifera*, respectively. During the 60 days of experimental trial, hematobiochemical and histopathological parameters were studied. The data obtained was analysed by analysis of variance test and group means were compared by DMR test using M STAT C statistical software. During and at the end of experimental trial, male layer birds were slaughtered, collected kidney and liver were fixed in 10% neutral buffered formalin to investigate gross and histopathological alternations. There were significant alterations in hematological and serum biochemical parameters of aflatoxin treated birds. Serum enzymes including ALT, AST and LDH values were significantly increased in group B in contrast to control group A. The histopathological changes in hepatic and renal parenchyma induced by AFB1 were prominent and no such changes were seen in A, D and F groups, while C and E group showed mild necrotic changes which was an indication of ameliorative effects of vitamin E and *M. oleifera*. It may be concluded that vitamin E and *M. oleifera* have potential to mitigate toxicity of aflatoxin B1.

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INTRODUCTION

Aflatoxin is considered as one of major contaminant in foods and has profound effects to change the physiology of animals and humans. Corn is major crop affected from aflatoxins (Richard, 2007). The permissible level of aflatoxins (AFs) in poultry feed is very low as compared to other mycotoxins and poultry feed is considered at higher risk of contamination with AFs. In 1960, aflatoxin was found as a causative agent in Turkey X disease that caused liver necrosis. At preharvest stage of corn *Asepergillus flavus* and *Aspergillus parasitcus* can grow and produce aflatoxins, drought conditions favor the fungus growth (Payne and Widstrom, 1992). Aflatoxins are secondary metabolites generated by pathogenic fungi especially *Aspergillus* species including *A. flavus* and *A. parasitcus*

during their metabolic processes (Ahmed *et al*., 2012a). Aflatoxins are hepatotoxic in nature, affected birds have enlarged liver and swollen kidneys (Benkerroum, 2020). Aflatoxin B1 (AFB1) is known hepatotoxic and carcinogenic mycotoxin (Prieto-Simón *et al*., 2007). Aflatoxins especially B1 has been observed to cause the negative effects on performance of broilers (Hussain *et al*., 2016; Nazarizadeh *et al.*, 2019).

In Pakistan from 2006-2009, three-year survey program was started to find out the amount of aflatoxin B1 in starter and finisher feed of birds. A total of 1021 samples were collected from different regions of the country. Overall, 61% samples were found positive for aflatoxin B1 (Khan *et al.*, 2011). The presence of mycotoxins in poultry feed is responsible for the deleterious effects on health and performance of birds (Anjum *et al.*, 2011). The absolute weights of liver and kidneys were increased and immune organs like bursa of Fabricius were reduced in size due to aflatoxin toxicity and resulted in lymphocytic depletion and ultimately immunosuppression (Verma *et al.*, 2004; Khan *et al*., 2014). Aflatoxin B1 (AFBI) is potent carcinogen in birds, human and rodents. Oxidative stress caused by AFBI further leads to tumorigenesis (Marin and Taranu, 2012). Eshak *et al*. (2015) reported that exposure of mice to AFB1 caused a significant increase of liver enzymes (alanine amino transferase, aspartate amino transferase). Use of high doses of AFB1 to different avian species like chickens, turkeys, ducks and quails exhibited various levels of mortality, reduced egg production and decreased body weights (Hoerr, 2020).

Alpha tocopherol or vitamin E is a potent antioxidant which safeguards the body cells from oxidative stress induced by aflatoxins (Schneider, 2005). The vitamin E is used as a salubrious agent to treat aflatoxin induced toxicity and oxidative stress (Yılmaz *et al*., 2017). It is used to reduce the immunotoxicity induced by aflatoxin in broilers and layers (Verma *et al*., 2004; Khan *et al*., 2014).

Moringa is a well-known ethnoveterinary plant. It is a rapidly growing plant and resistant to drought conditions. Moringa tree is an abound source of protein, ascorbic acid, iron, calcium and carotenoids. Moringa leaves contain protein 27.51%, fiber 19.25%, lipid 6.5%, ash 7.13%, carbohydrates 43.88%, ether extract 29.55% and nitrogen free extract 7.13% (Gakuya *et al.*, 2014; Uchendu *et al*., 2021). *M. oleifera* leaves are excellent source of antifungal and anti-inflammatory activity (Farag *et al.*, 2018; Ullah *et al*., 2022). It also helps in digestion and improvement of liver functions (Uchendu *et al*., 2021; Ullah *et al*., 2022) Deviated biochemical parameters can also be turned into normal by using *M. oleifera* (Afolabi *et al*., 2013). Based on these findings, our study proposed that use of these two antioxidants may be a novel approach by which these two antioxidants may ameliorate the toxic effects of AFB1. The objective of this study was to investigate toxico-pathological changes caused by AFB1 in white Leghorn males birds and mitigation of these deleterious effects by using Vitamin E and *M. oleifera*.

MATERIALS AND METHODS

Experimental design: The white leghorn breeder males of eight weeks of age were purchased from well reputed commercial layer breeder farm. These male birds were placed in properly designed pens in shed. A total of 120 birds were placed into 6 groups and each group contained 20 birds. Aflatoxin contaminated feeds with complaints of high mortality, swollen liver and kidneys were collected from different farms and that feed was used to prepare further feed by contaminating normal feed with aflatoxin affected feed. The aflatoxin was also experimentally produced in Avian Molecular Toxicologic Pathology lab to fulfill the experimental need. Aflatoxins were produced on rice (Hussain *et al*., 2008). The details of experimental lay-out have been given in Table 1. Spores of *A. flavus* (link: Fries. A. NRRL 6540 and CECT. 2687) were mixed into rice. Quantifications of aflatoxins were carried out by HPLC-FD method 990.33 (Anonymous, 2000). This experimental study was approved by Graduate Studies and Research Board (GSRB) of University of Agriculture Faisalabad. The *M. oleifera* powder was purchased from Department of Crop Physiology, UAF, where it was available for commercial sale with brand name of MORINGA leaves Powder a product of Virsa Agri Farms and Services. The vitamin E was purchased from AIMS Health Solutions Lahore with brand name of Selenex Plus® (20% Vitamin E) imported from ALBOVIT^{TM}, ALBORS Animal Health and Welfare Milan, Italy.

Hematobiochemical parameters: Blood without anticoagulant was collected at day 30 and 60 of the experiment. Blood (5ml) was collected in the glass tubes vacutainers for the separation of serum, and stored at -20^{o}C till further analysis. Collected serum samples were utilized for biochemical analysis. Serum total proteins were measured by the Biuret method (Oser, 1976). Serum albumin concentration was measured by the bromocresol green binding method (Varley *et al*., 1980) by using commercial kits of Merck France (CAT No. 5.17530.0001). Serum globulin concentration was calculated by deducting albumin values form the value of total serum protein. Serum creatinine level was analysed by the Jaffe reaction (Bosnes and Taussky, 1945) and serum alanine transferase (ALT) and aspartate aminotransferase (AST) was analysed by the colorimetric method of Reitman and Frankel (1957) using the commercially available kit (CAT No. 5.17530.0001 and 5.17520.0001 respectively)**.** The microhematocrit method was used to assess the hematological parameters, such as hematocrit (%) values, and the cyanmethemoglobin method (Drabkin's solution at 540nm wavelength) was used to assess the hemoglobin (Benjamin, 1978).

Gross and microscopic pathology: During experiment died or killed birds were necropsied for macroscopic changes. Liver and kidneys tissue specimen were preserved in 10% neutral buffered-formalin (NBF) to study the microscopic changes by tissue processing through paraffin embedding technique (Bancroft and Gamble, 2008).

Statistical analysis: Experimental data were analyzed by using one-way analysis of variance (ANOVA) test and group means were compared by DMR test by using M-STAT C statistical software.

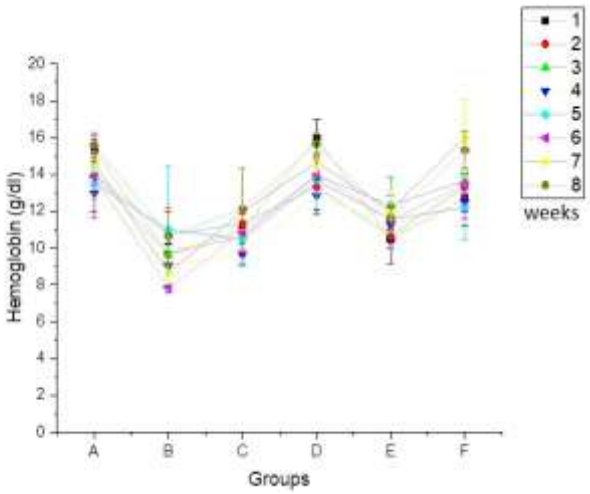

Fig. 1: Hemoglobin Concentration of white Leghorn male layers fed with different levels of Aflatoxin B1, *Moringa olifera* and Vitamin E (Mean ± SD).

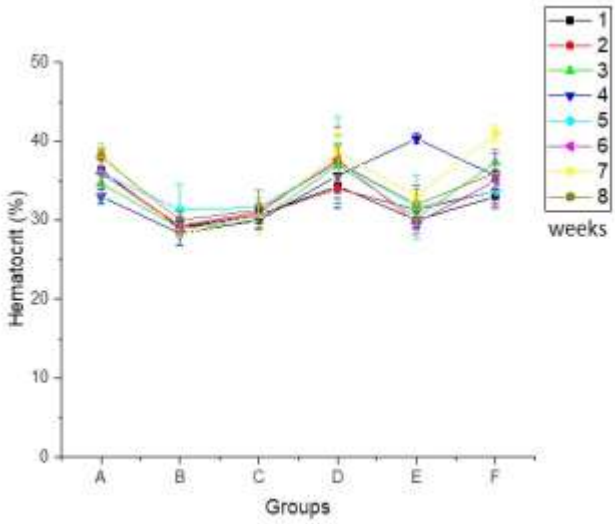

Fig. 2: Hematocrit values of white Leghorn male layers fed with different levels of Aflatoxin B1, *Moringa olifera* and Vitamin E (Mean ± SD).

Table 1: Experimental Design

Groups	No of birds	Treatments
A	20	Control –ve
B	20	AFB1 400ppb
C	20	AFB1 400ppb + *Moringa oleifera* 1 %
D	20	*Moringa oleifera* 1%
E	20	AFB1 400pbb + Vitamin E 100ppm
F	20	Vitamin E 100ppm

RESULTS

Hematobiochemical parameters: In the hematobiochemical parameters, total proteins, albumin, globulin, ALT, AST, LDH, Hb and hematocrit were determined. The level of total proteins and albumin were nonsignificantly different in all the groups compared with negative control A group and positive control B group at day 30 and 60 of experiment. The globulin level was significantly lower in contrast to control group A, C, D and F at day 30 while no difference at day 60 of experiment (Table 2 and 3). The ALT and AST were significantly increased in groups B at day 30 of experiment as compared with control group A and all other groups. While at day 60 of experiment the ALT value of group B was significantly higher as compared with group A, D and F as compared to other groups (Table 3). While AST value of group B was significantly higher in positive control (B group) in contrast to all other groups except group C where it was non significantly different (Table 3). The creatinine concentration of group B at day 30 of experimental trail was significantly higher as compared with group A (control) and D, while it was non significantly different from all other groups (Table 2). At day 60 of the experiment the level of was non significantly different among different groups. (Table 3). The LDH concentration of group B was significantly higher in contrast to control group A and D at day 30 and at day 60 was significantly higher in group B in contrast to all groups except group E (Table 2 and 3).

At 1st week of experiment, Hb conc. was significantly lower in B, C, E and F groups as compared to control A group while group D was nonsignificantly different from control (Fig. 1). Similarly, at 2nd, 3rd and 4th weeks of age Hb level was significantly reduced in B, C and E groups compared with control group A while group D and F were nonsignificantly different from control and significantly higher from group B. During week 5 nonsignificant difference was present between group A and B. At 6th, 7th, 8th weeks of age, group B, C and E was significantly lower in contrast to group A. While group D and F were nonsignificantly different from control A group. Hematocrit values in group B, C, E were significantly lower in comparison with control group A throughout the experiment except week 4. While group D and F were nonsignificantly different from control group A and significantly higher from group B. from week 1-8. The group D and F is showing normal pattern like control group A, while groups C and E were nonsignificantly higher from group B showing partial amelioration.

Histopathology

Kidneys: The parenchyma of kidney in control group was in the normal appearance. Nuclei of cells of tubular epithelium were normal in structure. The urinary space was clear in glomeruli (Fig. 3a). Renal parenchyma in group B was indicating that the urinary space was not clear. At fewer areas condensation of tubular epithelial nuclei was present. However, the lumen of tubule was patent. Congestion was seen throughout renal parenchyma. At few places, tubular areas with liquefactive necrosis were also seen (Fig. 3b). Moderate to severe necrotic changes throughout the renal parenchyma were seen in group C (3c). Slight to moderate level of tubular necrosis represented by the condensed and pyknotic nuclei of cells of tubular epithelium. The urinary space was not clear (Fig. 3c). The renal parenchyma showed nearly normal tubular epithelial cells. Urinary space was normal in group d (3d). At few places cells of tubular epithelium were separated from the basal membrane, it was an artifact in group D. Parenchyma of kidney was showing moderate to severe parenchymal congestion in group E (Fig. 3e). Slight to moderate tubular necrosis was also seen. Urinary space was not clear containing proteinaceous material (Fig. 3e). The parenchyma of renal tissues was showing pretty normal histology of cells of tubular epithelium and urinary space was normal in group F (Fig. 3f). At few places cells of tubular epithelium were detached from the basal membrane which was again an artifact (Fig. 3f).

Table 2: Serum biochemical parameters of white Leghorn male layers at day 30th of experiment fed with different levels of Aflatoxin B1 (Mean ± SD).

Groups	Total Protein	Albumin	Globulin	ALT	Creatinine	LDH	AST
A	3.455±0.59^{ab}	1.772±0.70^{a}	1.683±0.40^{ab}	32.333±7.02^{b}	0.167±0.12^{a}	1551.667±151.01^{c}	62.000±24.64^{c}
B	2.826±0.44^{b}	2.002±0.12^{a}	0.824±0.36^{c}	45.333±5.51^{a}	0.533±0.21^{a}	2393.000±65.64^{a}	157.333±12.58^{a}
C	3.898±0.57^{ab}	2.127±0.33^{a}	1.770±0.48^{ab}	33.000±4.36^{b}	0.400±0.10^{a}	1840.333±34.93^{b}	159.667±17.21^{a}
D	4.177±0.65^{a}	2.468±0.46^{a}	1.710±0.29^{ab}	21.000±2.65^{c}	0.167±0.12^{a}	1626.667±113.72^{c}	64.333±6.03^{c}
E	3.465±0.71^{ab}	2.262±0.56a	1.202±0.19^{bc}	27.667±2.08^{bc}	0.167±0.06^{a}	1829.333±30.50^{b}	107.33±6.43^{b}
F	4.280±0.36^{a}	2.177±0.33^{a}	2.103±0.24^{a}	24.000±1.00^{b}	0.133±0.06^{a}	1646.333±128.41^{c}	91.667±6.66^{b}

Values in each column followed by different letters are significantly different (p≤0.05): **A=**Control (kept on basal feed), **B=**AFB1 400 ppb, **C=**AFB1 400 ppb + *Moringa oleifera* 1 %, **D=** *Moringa oleifera* 1 %, **E=**AFB1 400 + Vit E (100 ppm), and **F**=Vit E 100 ppm

Table 3: Serum biochemical parameters of white Leghorn male layers at day 60th of experiment fed with different levels of Aflatoxin B1 (Mean ± SD).

Groups	Total Protein	Albumin	Globulin	ALT	Creatinine	LDH	AST
A	3.69 ± 0.27^{a}	2.035 ± 0.14^{ab}	1.663 ± 0.14^{bc}	18.333 ± 3.21^{b}	0.17 ± 0.12^{bc}	1954.7 ± 259.66^{b}	90.67 ± 14.01^{c}
B	3.36 ± 0.31^{a}	1.86 ± 0.09^{b}	1.501±0.25^{cd}	34±10.54^{a}	0.433±0.21^{a}	2839±63.69^{a}	184.33±10.07^{a}
C	4.01±0.08^{a}	2.66±0.31^{a}	1.36±0.27^{cd}	25.33±1.53^{ab}	0.23±0.06^{abc}	1753.67±5.51^{bc}	152.67±14.19^{b}
D	4.81± 0.38^{a}	2.62±0.60^{ab}	2.2±0.22a	20±1.00^{b}	0.13±0.06^{c}	1614±48.22^{c}	66.33±6.51^{d}
E	3.116±0.05^{a}	2.14±0.07^{ab}	0.97±0.11^{d}	27±2.65^{ab}	0.40±0.10^{ab}	1823.3±14.64^{bc}	139.3±15.82^{b}
F	4.42±0.23^{a}	2.26±0.68^{ab}	2.15±0.51^{ab}	23.67±2.52^{b}	0.23±0.15^{abc}	1643.3±106.98^{c}	84.66±5.03^{cd}

Values in each column followed by different letters are significantly different (p≤0.05): **A=**Control -ve, **B=**AFB1 400 ppb, **C=**AFB1 400 ppb + *Moringa oleifera* 1 %, **D=** *Moringa oleifera* 1 %, **E=**AFB1 400 + Vit E 100 ppm and **F**=Vit E 100 ppm

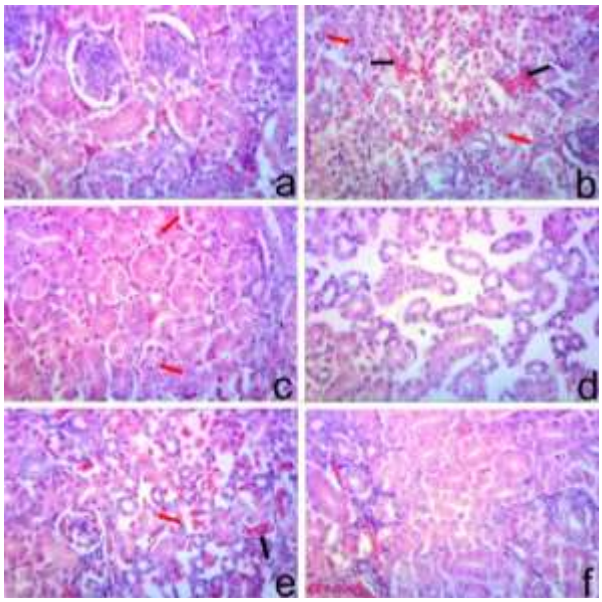

Fig. 3: Photomicrograph of kideny in group A representing normal renal parenchyma (3a), while in group Bwas showing tubular nerosis indicated by red arrows and congestion with black arrows (3b), group C is indicating mild necrotic changes indicated by red arrows (3c) and normal renal parenchyma in group D (3d), group E indating mild congestion (black arrows) and necrosis (red arrows) shown in figure3 e), while group F was showing normal renal parenchyma in fig. 3f. (H & E Staining 400X).

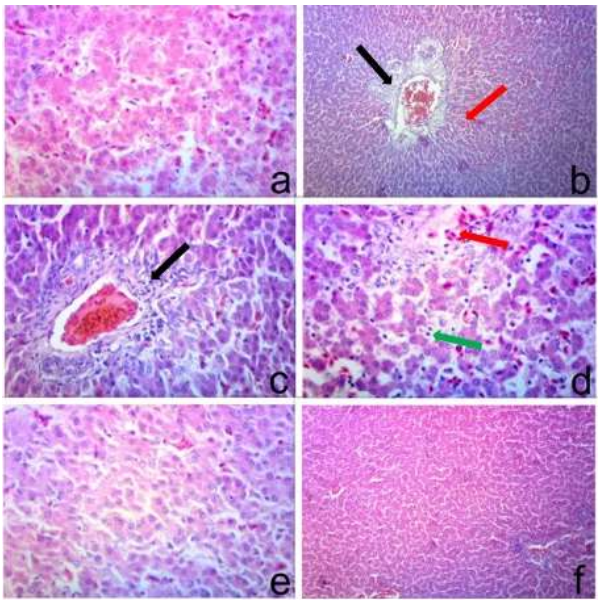

Fig. 4: Photomicrograph of liver in control (group A) showing normal hepatic parenchyma and hepatocytes, arranged properly in fine hepatic cords (4a), while 4b & 4c indicating cellular infiltration, perivascular cuffing and congestion shown by black and red arrows. Fig. 4d hepatic parenchyma in group C is showing individual cell necrosis (green arrow), cellular infiltration & congestion (red arrow). Fig. 4e showing almost normal parenchyma in group D and 4f indicating normal hepatic parenchyma in group F (H & E Staining 100X and 400X).

Liver: The parenchyma of hepatic cells was of normal appearance in control group A. The hepatocytes were arranged in hepatic cords. Cytoplasm had pinkish appearance. The nuclei had chromatin filled nucleoli and normal appearance. The hepatic triad was normal in appearance having hepatic artery, portal vein and bile duct (Fig. 4a). Hepatic parenchyma in group B showed perivascular cuffing and necrotic changes around the blood vessels and there was fibroblast proliferation. Cellular infiltrate was also seen. Mild to moderate level of congestion in hepatic parenchyma was also seen (Fig. 4b and c). In group C parenchyma of hepatic cells was showing mild to moderate level of congestion at few areas. Necrotic changes were also seen, represented by the condensed and pyknotic nucleus of hepatic cells. These condensed nuclei were representing initiation of necrosis of individual cell. Perivascular cuffing was also present. Cell swelling was responsible for the diminishing of sinusoidal spaces. Blood vessels were engorged indicating passive hyperemia. At fewer areas cellular aggregates were found. The hepatic parenchyma was showing the normal outlooks of hepatocytes in group D (Fig. 4e).. Parenchyma of hepatocytes was representing moderate level of changes in group E. Mild to moderate congestion was seen in the entire parenchyma. Hepatic parenchyma showed the normal structure of cells. Sinusoidal spaces were normal. Presence of nucleolus and chromatin material represented the normal texture of hepatic nuclei (Fig. 4f). Sinusoidal areas were normal in group F (Fig. 4f).

DISCUSSION

Poultry farming is one of the most vibrant industries of Pakistan and it shares major role in the GDP. Poultry feed is the fuel of birds and it is adversely affected by the

mycotoxins including aflatoxin B1, B2, G1 and G2. Mycotoxins lead to severe economic losses to poultry industry after the high prices of feed. In 2011 in Pakistan, about 61% samples were confirmed positive for mycotoxins which indicated the presence of toxins especially aflatoxin in the feed (Khan *et al*., 2011). In USA form 2000-2007, there were 1.5 billion losses in poultry industry due to mycotoxins contamination of grains (Wu, 2007). Different approaches have been adopted at household level to detoxify the mycotoxins (Tsiouris *et al*., 2021). Results of present study showed a partial improvement of hematobiochemical alterations and reversion of liver and kidney toxicity by vitamin E and *M. oleifera* in AFB1 exposed white Leghorn layer male birds. Total proteins and albumin values were nonsignificantly different in all the groups compared with negative control and positive control at day 30 and 60 of experiment. The globulin level was significantly lower in comparison to control A, C, D and F group at day 30 while no difference at day 60 of experiment. Similar results were presented by Rastogi *et al.* (2001) and Corcuera *et al*. (2015) in male wistar rats and chicken respectively. Naseem *et al*. (2018a) also reported lower serum total protein, albumen and globulin concentrations in AFB1 intoxicated birds these were same as reported in our findings. The findings of Gowda *et al*. (2008) and Zhao *et al*. (2010) were in line with our results. However, serum enzymes including ALT and AST were significantly increased in groups B at day 30 of experiment as compared with all groups. While at day 60 of experiment the ALT value of group B was significantly higher as compared with group A, D and F as compared to other groups. Similar results of higher ALT and AST levels in AFB1 intoxicated broiler birds were reported by Naseem *et al*. (2018a). Bhatti *et al*. (2020) reported nonsignificant difference of OTA intoxication on ALT, AST and creatinine in broilers, while AST value was significantly higher in group B in comparison to other groups except group C where, it was nonsignificantly different. The creatinine concentration of group B at day 30 of trail was significantly higher as compared with control group A and D, while it was nonsignificantly different from all other groups. Similar results were presented by Rastogi *et al.* (2001), Allameh *et al.* (2005), and Naseem *et al*. (2018a). The LDH concentration of group B was significantly higher as compared with control group A and D at day 30 and days 60. In group D and F enzymes were also normal due to supplementation of ameliorative agents. Group B showed increase in LDH, AST, ALT and creatinine due to toxic effects of aflatoxin. These scavenging effects may be due to ameliorative activities of *M. oleifera* (Mansour *et al*., 2014; Sheikh *et al.*, 2014; Elkloub *et al.*, 2015). Awais *et al*. (2022) also reported amelioration of serum biochemical parameters with distillery sludge and local bentonites clay in broilers. Similar results of amelioration of these parameters with vitamin E were presented by Khan *et al.* (2014), Ulaiwi (2018) and Zubair *et al.* (2018) in white Leghorn birds and male bucks.

At 1st week of experiment, Hb concentration was significantly lower in groups B, C, E and F as compared with control group A while group D was nonsignificantly different from control (Fig. 1). Similarly, at 2nd, 3rd and 4th weeks of age Hb level was significantly reduced in group B, C and E compared with control group A while group D and F were nonsignificantly different from control and significantly higher from group B. It is an indication of partial amelioration with *M. oleifera* and vitamin E. During week 5 nonsignificant difference was present between group A and B. From 6-8 week, group B, C and E was significantly lower as compared with group A. While group D and F were nonsignificantly different from control group A. Similar to current experimental results. Khan *et al*. (2017) reported lower values of total erythrocyte count, total leukocyte count, hemoglobin concentration and hematocrit values in white Leghorn layers given feed containing AFB1 and OTA and its amelioration with graded doses of distillery Hematocrit values in group B, C, E were significantly lower in comparison with control group A throughout the experiment except week 4. While group D and F were nonsignificantly different from control group A and significantly higher from group B. The group D and F was showing normal pattern like control group A, while groups C and E were nonsignificantly higher from group B showing partial amelioration. Same results were presented by Fapohunda *et al.* (2008). Ahmed *et al*. (2012b) reported decrease in hemoglobin concentration and hematocrit values in OTA treated white Leghorn layer birds and its amelioration with silymarin.

In histopathological lesions of the tissue's parenchyma of kidney in control group A was in the normal appearance. Nuclei of cells of tubular epithelium were normal in appearance and urinary space was clear. While renal parenchyma in group B was indicating that the urinary space was not clear and in some areas condensation of tubular nuclei was present. However, the lumen of tubule was patent. The congestion was seen throughout renal parenchyma in group B in comparison to control group A. At few places, tubular area was showing necrotic changes as similar lesions were presented by Quezada *et al.* (2000), Sineque *et al.* (2017), and Kurniasih and Prokoso (2019) in broiler chicken. Hussain *et al.* (2016) also reported significantly increased scores of gross lesions with increasing dietary levels of AFB1 in broilers. Moderate to severe necrotic changes throughout the renal parenchyma were seen in group C. Slight to moderate level of tubular necrosis represented by the condense and pyknotic nuclei of cells of tubular epithelium. The renal parenchyma was indicating almost normal tubular epithelial cells and urinary space was normal in group D. At few places cells of tubular epithelium were detached from the basal membrane was an artifact in group D. Parenchyma of kidney was showing moderate to severe parenchymal congestion in group E. Slight to moderate tubular necrosis was also seen. Urinary space was not clear containing proteinaceous material. The parenchyma of renal tissues was showing pretty normal histology of cells of tubular epithelium and urinary space was normal in group F. The parenchyma of hepatic cells was of normal appearance in control group A. Ahmed *et al.* (2012b) reported amelioration of nephrotoxic effects of OTA in birds with addition of silymarin. Hepatic parenchyma in group B was indicating perivascular cuffing and necrotic changes around the blood vessels and there was fibroblast proliferation. Cellular infiltrate was also seen. Mild to moderate level of congestion in hepatic parenchyma was also seen. Similar to our results, Naseem *et al.* (2018b) also reported necrotic changes, congestion in broilers

exposed to AFB1 and FAdV-4. In group C hepatic parenchyma was showing similar changes but at lower intensity. The hepatic parenchyma was showing the normal appearance in group D. Parenchyma of hepatocytes was representing moderate level of changes in group E. Mild to moderate congestion throughout parenchyma was present while mostly hepatic parenchyma was indicating the normal appearance. Cells were settled in a hepatic cord and sinusoidal spaces were normal in appearance. Presence of nucleolus and chromatin material was representing the normal appearance of hepatic nuclei in group F. Khan *et al*. (2017) reported moldy feed containing AFB1 and OTA caused macro and microscopic changes such as enlargement, vacuolar degeneration, cellular infiltration, ecchymosis hemorrhages on the surface and congestion, etc. in liver, kidney and spleen respectively however addition of 2% DYS in moldy feed resulted in normal compared to control group. Awais *et al*. (2022) also reported amelioration of toxicopathological effects of OTA in liver and kidneys with dietary supplementation of distillery sludge and local bentonite clay.

It may be concluded that aflatoxin in feed had negatively affected the juvenile male birds. Aflatoxins contaminated feed was responsible for the change of many parameters including serum enzymes, hematological values and histological alterations. When *M. oleifera* and Vitamin E were added to aflatoxin contaminated feed, these mitigating agents were responsible for amelioration of toxic effect of aflatoxin and may be added in the feed to prevent the toxic effects of AFB1 in birds.

Authors contribution: MKS, AK, MZ, XY, XL, MI, FM, FA, FJ, AB designed experiment, analyzed results and manuscript preparation, AR, MJ, MKR, IA, KZ, SAB, MB involved in execution of experiment and data analysis, manuscript preparation.

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